Restriction Fragment Length Polymorphism and Divergence in the Genomic Regions of High and Low Recombination in Self-Fertilizing and Cross-Fertilizing Aegilops Species

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ABSTRACT

RFLP was investigated at 52 single-copy gene loci among six species of Aegilops, including both cross-fertilizing and self-fertilizing species. Average gene diversity (H) was found to correlate with the level of outcrossing. No relationship was found between H and the phylogenetic status of a species. In all six species, the level of RFLP at a locus was a function of the position of the locus on the chromosome and the recombination rate in the neighborhood of the locus. Loci in the proximal chromosome regions, which show greatly reduced recombination rates relative to the distal regions, were significantly less variable than loci in the distal chromosome regions in all six species. Variation in recombination rates was also reflected in the haplotype divergence between closely related species; loci in the chromosome regions with low recombination rates were found to be diverged less than those in the chromosome regions with high recombination rates. This relationship was not found among the more distantly related species.

THE probability of meiotic crossing over is not a uniform function of the physical length of a chromosome region in many plants and animals. Some chromosome regions show a high density of meiotic exchanges, whereas other regions show a low density or are entirely devoid of them. In the *Drosophila melanogaster* females, meiotic crossing over is virtually absent from the pericentromeric heterochromatin (Roberts 1965). In the euchromatin, crossing over frequency per unit of chromosome length generally increases in the distal direction and then declines in the vicinity of the arm terminus (Lindsley and Sandler 1977). A similar pattern exists in wheat (*Triticum L.*) and closely related goatgrasses (*Aegilops L.*). The existence of reduced recombination rates in the proximal regions relative to the distal regions in the chromosomes of these species was first reported for chromosome arm *6BS* in which a region spanning the proximal two thirds of the length of the metaphase chromosome arm was found to account for >1 centimorgan (cM) on the linkage map; virtually all recombination was found to be localized in the distal third of the arm (Dvořák and Chen 1984; Dvořák and Appels 1986). Crossing over reductions of similar magnitudes have since been reported for the pericentromeric region of every wheat or Aegilops chromosome in which linkage maps and metaphase chromosome maps could be compared (Jampates and Dvořák 1986; Curtis and Lukaszewsky 1991; Werner *et al.* 1992; Lukaszewski and Curtis 1993; Hohmann *et al.* 1994; Delaney *et al.* 1995a,b; Mickelson-Young *et al.* 1995; Gill *et al.* 1993, 1996a,b).

Gene loci in chromosome regions with low recombination rates were shown to have reduced levels of DNA polymorphism relative to chromosome regions with high recombination rates in several species of Drosophila (Aguade *et al.* 1989; Begun and Aquadro 1991; Berry *et al.* 1991; Martin-Campos *et al.* 1992; Stephan and Mitchel 1992; Langley *et al.* 1993). In *D. melanogaster*, polymorphism levels were shown to correlate with regional levels of recombination (Begun and Aquadro 1992; Aguade and Langley 1994). This relationship was initially attributed to "hitchhiking" of neutral polymorphisms with favorably selected alleles (Aguade *et al.* 1989; Begun and Aquadro 1992; Wiehe and Stephan 1993). The hitchhiking model is based on the idea that a linkage disequilibrium between selectively favorable and neutral alleles may persist in regions of low or no recombination, and the fixation of the favorable allele may result in the fixation of hitchhiking neutral alleles, thus sweeping neutral polymorphism out of the region (Maynard Smith and Haigh 1974; Kaplan *et al.* 1989). In regions of high recombination, neutral polymorphism remains in a population because of recombination between the selected and neutral alleles. However, empirical observations in Drosophila agree poorly with some of the predictions based on the hitchhiking model (Braverman *et al.* 1995).

Alternatively, the relationship between the levels of nucleotide variation and recombination rates across Drosophila chromosomes may be caused by reduction

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of neutral variation in regions of low recombination resulting from "background selection" (Charlesworth *et al.* 1993; Charlesworth 1994; Nordborg *et al.* 1996). In this process, selection against deleterious mutations reduces the effective population size and thus reduces linked neutral variation in a population. Levels of nucleotide variation across Drosophila chromosomes predicted by background selection models fit reasonably well with the observed levels of nucleotide diversity across Drosophila chromosomes (Charlesworth 1996).

The relationship between genetic variation and recombination rates across chromosomes has been investigated only in Drosophila. A relevant question is whether an analogous relationship exists in other organisms, particularly in those differing from Drosophila in the mating system, such as self-fertilizing plants in which there is a genomewide reduction of recombination. To investigate this question, the levels of RFLP in chromosome regions with low recombination rates (centromeric regions) were compared with RFLP levels in the rest of the chromosomes in five species of Aegilops that are considered self-fertilizing, *Ae. searsii* Feld. et Kislev (genome S^{se}), *Ae. bicornis* (Forssk.) Jaub. et Spach. (genome S^{b}), *Ae. sharonensis* (genome S^{sh}), *Ae. longissima* Schweinf. et Muschl. (genome S^{l}), and *Ae. tauschii* Coss. (genome *D*), and one cross-fertilizing species, *Ae. speltoides* (Tausch) Gren. (genome *S*). In addition to their breeding habit, the six species vary in their relative phylogenetic age, as suggested by their position in the phylogenetic tree of the genus Aegilops (Figure 1). According to the hypothetical phylogeny, *Ae. tauschii* is the oldest of the six species. It also has the largest geographic distribution of all diploid Aegilops species, ranging from Eastern Turkey to China and Pakistan. The second oldest species is *Ae. speltoides*. This species has the second largest geographic distribution, ranging from Israel to Turkey and Western Iraq. The remaining species evolved more recently and have limited geographic distributions. *Ae. longissima* is found in Israel, southern Lebanon, and western Jordan; *Ae. bicornis* in southern Israel, northwestern Sanai, northern Egypt, and Cyprus; *Ae. searsii* in Syria, Jordan, and Israel; and *Ae. sharonensis* is endemic to coastal areas of Israel (Waines *et al.* 1982).

Of the six genomes involved in this study, only the *D* genome has been genetically mapped (Gill *et al.* 1991, 1992; Lagudah *et al.* 1991; Gale *et al.* 1995; Nelson *et al.* 1995a,b; Van Deynze *et al.* 1995). The genomes of the remaining species are related to the *B* genome of bread wheat, *Triticum aestivum* L., $2n = 6x = 42$, genomes *AABBDD* (Sarkar and Stebbins 1956; Dvořák and Zhang 1990), which has been mapped (Gale *et al.* 1995; Nelson *et al.* 1995a,b; Van Deynze *et al.* 1995). Comparative mapping has so far provided no evidence that the wheat *B* and *D* genomes differ structurally, except for changes that occurred at the polyploid level. Cytogenetic investigations suggested that the genomes of the remaining species of the Aegilops sect. Sitopsis (Figure 1) are structurally similar although not identi-

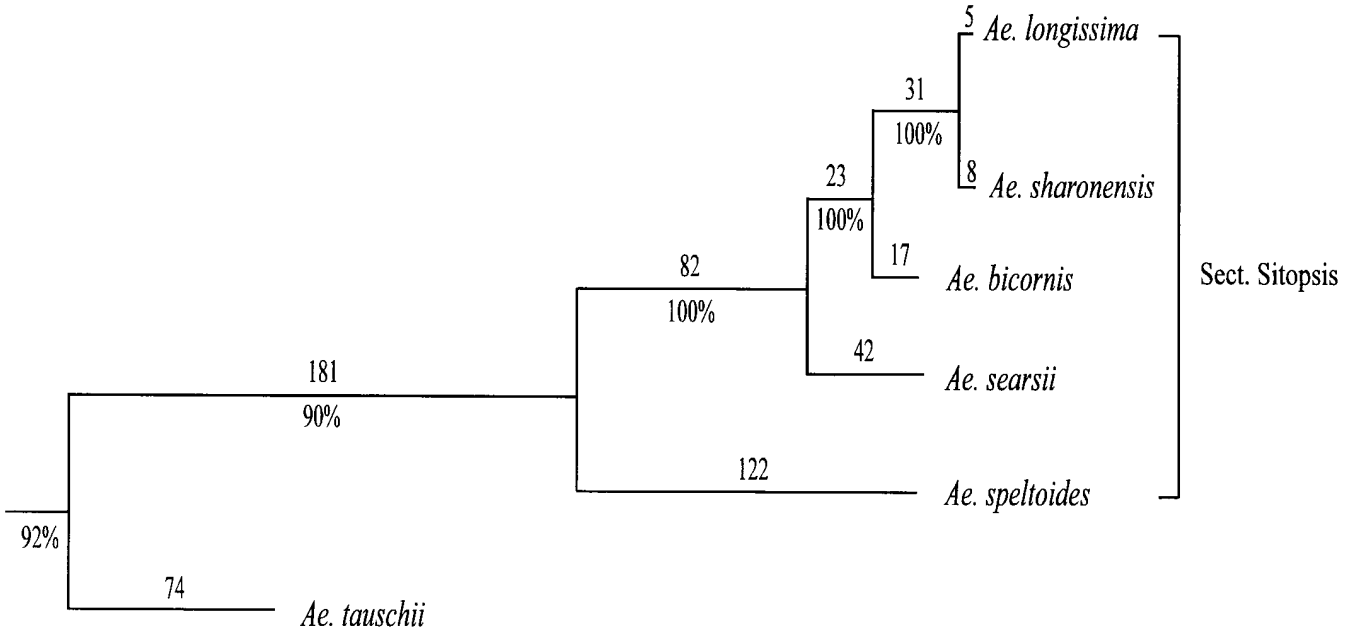


Figure 1.—A hypothetical phylogenetic tree of the species involved in this study reconstructed with Wagner parsimony method from variation in restriction fragments of nuclear repeated nucleotide sequences (Dvořák and Zhang 1992). The length of each branch is proportionate to the number of character changes (above the branches) that have occurred since a preceding node. The confidence of each node is indicated below a branch, and it is a percentage of bootstrap trials yielding that node. Species belonging to the section Sitopsis of the genus Aegilops are indicated.

TABLE 1

Sources and geographic origins of Aegilops accessions used in this study

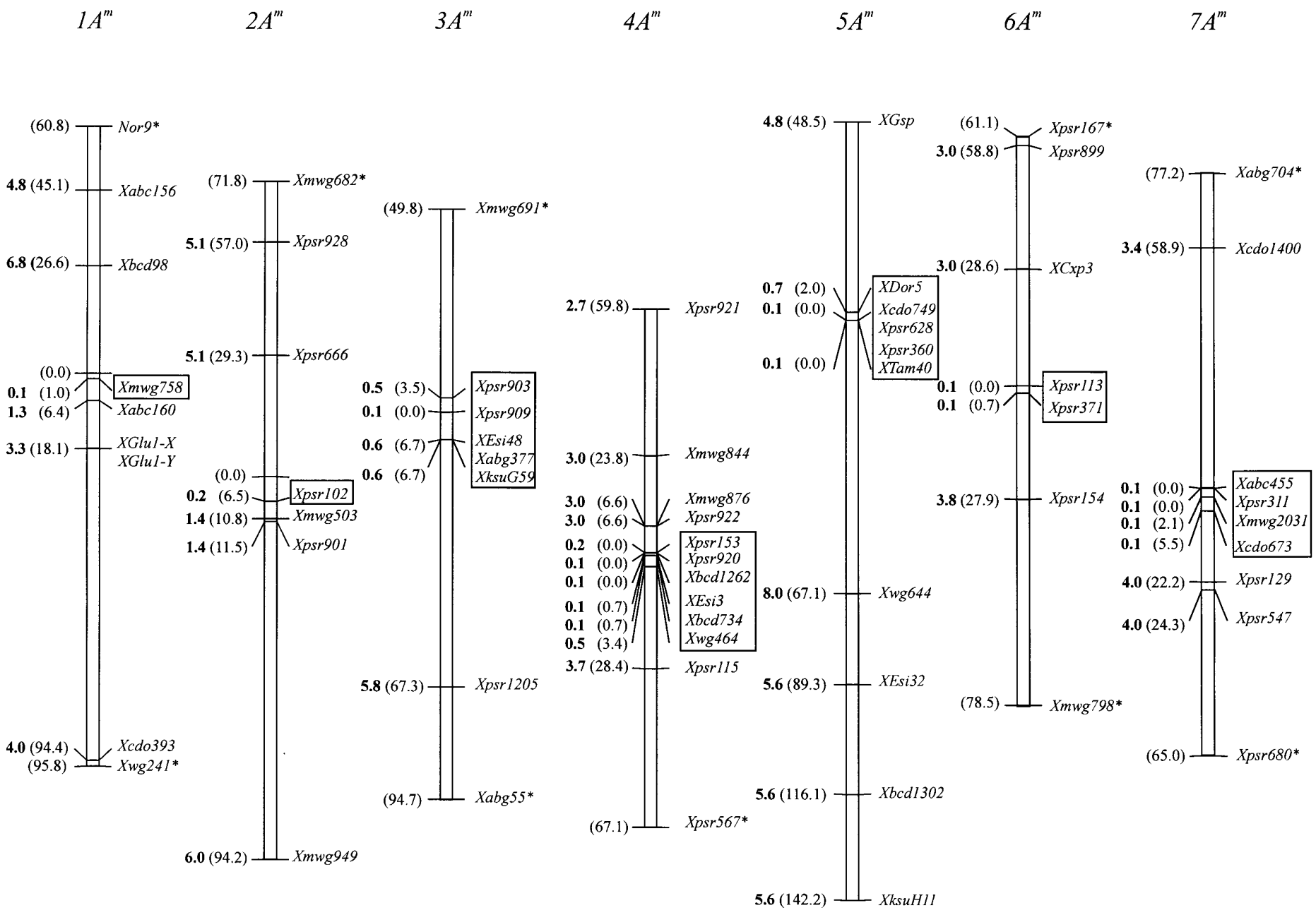
Species	No. accessions	Location	Accessions
Ae. bicornis	7	Egypt	G1420, G1420-1, G1420-2, G1421, G1423, G1425, G1426, G1427
	3	Israel	G594, TB8, TB11
	3	Unknown	G944, G756, TB2
Ae. longissima	13	Israel	G609, G1303, G1305, G1307, TL1, TL2, TL3, TL4, TL12, TL14, TL15, TL19, TL24
	1	Jordan	G1415
	1	Unknown	G945
Ae. searsii	12	Israel	G3068, G3525, TE5, TE7, TE8, TE10, TE20, TE21, TE22, TE23, TE24, TE25
	2	Syria	G1418, G1419
Ae. sharonensis	5	Israel	G614, G1315, G1321, TH4, TH12
Ae. speltoides	34	Turkey	D, F, G, H6, H9, G410, G711, G978, G1039, G1045, G1049, G1050, G1062, G1064, G1066, G1075, G1083, G1089, G1158, G1168, G1171, G1176, G1180, G1181, G1405, G1408, G1819, G1864, G1909, G1971, G1972, G2011, G2294, G1819
	7	Israel	G619, G1271, G1316, pop250, TS5, TS8, TS42
	3	Syria	San-1, San-2, San-3, G2148
	4	Iraq	G722, G724, G2502, G2815
	9	Unknown	G733, G762, G768, G852, G859, G861, G942, G943, G3071
Ae. tauschii	15	Afghanistan	KU20-4, KU2013, KU2022, KU2032, KU2035, KU2039, KU2044, KU2047, KU2048, KU2050, KU2056, KU2058, KU2059, KU2063, KU2066
	37	Armenia	AL11/80(4), AL7/80(4), AL8/78(3), AL9/78(9), KU2808, KU2810, KU2811, KU2814, KU2816, KU2821, KU2823, KU2824, TA2572, TA2573
	6	Azerbaijan	AL46/77(2), K1574/72(2), KU2804, KU2806
	12	China	82-Ae5, 82-Ae42, 82-Ae46, AS71, AS72, AS75, AS76, AS77, AS78, AS79, AS80, AS81, AS82
	1	Dagestan	TA2370
	7	Georgia	KU2826, KU2827, KU2828, KU2832, TA2580, KU289B, KU2835B
	58	Iran	AL370/77, RL5288-1, RL5288-2, TA2375, TA2376, TA2377, TA2378, KU2118, KU2145, KU2151, KU2159, KU2161, KU2123, KU2087, KU2088, KU2091, KU2095, KU2124, KU2115, KU2110, KU2111, KU2112, KU2122, KU2074, KU2076, KU2078, KU2079, KU2088, KU2090, KU2092, KU2093, KU2095, KU2096, KU2097, KU2098, KU2125, KU2126, KU2113, KU2114, KU2100, KU2101, KU2102, KU2103, KU2104, KU2105, KU2106, KU2108, KU2158, KU2160, KU2068, KU2069, KU2071, KU2086, KU2154, KU2155, KU2156, KU2080, KU2082, KU2083
	12	Nakhitchewan	AL10/80-1 to 4, KU2116, KU2117, KU2120, KU2121, KU2142, KU2143, KU2144, KU2148
	8	Turkey	KU2131, KU2132, KU2133, KU2134, KU2136, KU2138, KU2140, KU2141
	11	Turkmenia	AL20/79-1 to 4, AL25/79-1 to 3, AL7/79-1 to 4
	5	Unknown	RL5263, RL5496, RL5237, P74-31, PI330489

Accessions designated by a number preceded by G were collected by L. B. Johnson (University of California, Riverside, CA), those preceded by TB, TL, TE, TH and TS were collected by M. Feldman (Weizman Institute, Rehovot, Israel), those preceded by KU were collected by H. Kihara and Yamashita (Kyoto University, Kyoto, Japan), those preceded by AL or K were collected by V. Jaaska (Estonian University, Tartu, Estonia). Accessions D, F, G, H and San were collected by S. Jana (University of Saskatchewan, Saskatoon, Canada). Accessions designated TA were supplied by B. S. Gill (University of Kansas, Manhattan, KS). Accessions preceded by Ae were supplied by Dr. Y.-S. Dong (Chinese Academy of Agricultural Sciences, Beijing), those preceded by RL by E. Kerber (Agriculture Canada, Winnipeg, Canada), those preceded by AS by Prof. C. Yen (The Triticeae Research Institute, Sichuan Agricultural University, China), and the accession preceded by P was supplied by E. R. Sears (University of Missouri, Columbia, MO). The origins and sources of the remaining accessions are unknown.

cal to the genome of *Ae. speltoides* (for review see Waines *et al.* 1982). Comparative RFLP mapping showed that the *B* and *D* genomes are also structurally similar to the genome of the diploid wheat *T. monococcum* (genome A^m), from which they differ by the absence of a reciprocal translocation between chromosomes 4 and 5 (Dubcovsky *et al.* 1996).

MATERIALS AND METHODS

Plant materials: RFLP was investigated in 58 accessions of *Ae. speltoides*, 14 of *Ae. searsii*, 15 of *Ae. longissima*, 13 of *Ae. bicornis*, 5 of *Ae. sharonensis*, and 165 of *Ae. tauschii*. The geographic origins of the accessions are summarized in Table 1. Except for some of the *Ae. tauschii* and *Ae. speltoides* accessions, which were original seeds collected in nature, the remaining


1A^m
2A^m
3A^m
4A^m
5A^m
6A^m
7A^m
(60.8) Nor9*
4.8 (45.1) Xabc156
6.8 (26.6) Xbcd98
(0.0) Xmwg758
0.1 (1.0)
1.3 (6.4) Xabc160
3.3 (18.1) XGlu1-X
XGlu1-Y
4.0 (94.4) Xcdo393
(95.8) Xwg241*
(71.8) Xmwg682*
5.1 (57.0) Xpsr928
5.1 (29.3) Xpsr666
(0.0) Xpsr102
0.2 (6.5)
1.4 (10.8) Xmwg503
1.4 (11.5) Xpsr901
6.0 (94.2) Xmwg949
(49.8) Xmwg691*
0.5 (3.5) Xpsr903
0.1 (0.0) Xpsr909
0.6 (6.7) XEsi48
Xabg377
0.6 (6.7) XksuG59
5.8 (67.3) Xpsr1205
(94.7) Xabg55*
2.7 (59.8) Xpsr921
3.0 (23.8) Xmwg844
3.0 (6.6) Xmwg876
3.0 (6.6) Xpsr922
0.2 (0.0) Xpsr153
0.1 (0.0) Xpsr920
0.1 (0.0) Xbcd1262
0.1 (0.7) XEsi3
0.1 (0.7) Xbcd734
0.5 (3.4) Xwg464
3.7 (28.4) Xpsr115
(67.1) Xpsr567*
4.8 (48.5) XGsp
0.7 (2.0) XDor5
0.1 (0.0) Xcdo749
Xpsr628
Xpsr360
0.1 (0.0) XTam40
8.0 (67.1) Xwg644
5.6 (89.3) XEsi32
5.6 (116.1) Xbcd1302
5.6 (142.2) XksuH11
(61.1) Xpsr167*
3.0 (58.8) Xpsr899
3.0 (28.6) XCxp3
0.1 (0.0) Xpsr113
0.1 (0.7) Xpsr371
3.8 (27.9) Xpsr154
(78.5) Xmwg798*
(77.2) Xabg704*
3.4 (58.9) Xcdo1400
0.1 (0.0) Xabc455
0.1 (0.0) Xpsr311
0.1 (2.1) Xmwg2031
0.1 (5.5) Xcdo673
4.0 (22.2) Xpsr129
4.0 (24.3) Xpsr547
(65.0) Xpsr680*

were seeds from germplasm collections. DNA was isolated from a single plant per accession.

Recombination rates: Because all loci used in this study had been mapped in the *T. monococcum* genome (Dubcovsky *et al.* 1996), the gene order on the *T. monococcum* linkage maps was used as a reference and assumed to reflect the gene order in the genomes of the investigated species (Figure 2). Recombination rates in the neighborhoods of the investigated loci (Figure 2) were estimated from comparisons of the positions of the loci on linkage maps of *T. aestivum* and *T. monococcum* (Gale *et al.* 1995; Dvořák *et al.* 1995; Dubcovsky and Dvořák 1995; Dubcovsky *et al.* 1995, 1996) and *T. aestivum* deletion maps (Gill *et al.* 1993, 1996a,b; Hohmann *et al.* 1994; Delaney *et al.* 1995a,b; Mickelson-Young *et al.* 1995). For some loci, location relative to visual landmarks on chromosomes, C bands (Curtis and Lukaszewski 1991) or *Nor* loci (Dvořák and Chen 1984; Dubcovsky and Dvořák 1995), were used. The following approach was used to infer the recombination rate in the neighborhood of a locus: (1) The position of a locus on a deletion map was either found directly or was inferred from the position of a closely linked locus on a deletion map. (2) Loci mapped in deletions flanking a region in which an investigated locus resides were selected, and the linkage distance in cM between them was determined on a wheat linkage map; if more than one linkage map was used, the distances were averaged. The distance between the same loci in terms of percentage of metaphase chromosome was calculated from the distance between the centers of the deletions in which the loci resided relative to the total chromosome length; if more than one deletion map was used, the percentages were averaged. For loci residing in the terminal deletions, the distance from the center of a terminal deletion to the center of the neighboring deletion was used to estimate the length of the interval in percentage of metaphase chromosome. (3) The average distance between the loci in centimorgans was divided by the distance between them in percentage of metaphase chromosome length. The resulting fraction, centimorgans per percentage of metaphase chromosome length, was used as an estimate of recombination rate in the chromosome region in which an investigated locus resided (Figure 2). These fractions are related to the coefficients of exchange that have been computed for Drosophila chromosomes (Lindsley and Sandler 1977). Compared to the Drosophila estimates, however, the wheat estimates are imprecise because the density of wheat deletions is low, particularly in the distal regions of linkage groups. Hence, only an approximate position of a locus on a wheat metaphase chromosome could be inferred. This imprecision was more serious in the high recombination rate regions than in the low recombination rate regions.

RFLP: Nuclear DNAs were isolated from leaves (Dvořák *et al.* 1988) and digested with *Dra*I or *Xba*I. Restriction endonuclease–digested DNAs were electrophoretically fractionated in 1% agarose gels and transferred to Hybond N+ nylon membranes (Amersham, Arlington Heights, IL) by capillary transfer in 0.4 N NaOH overnight. The membranes were then rinsed in 2 × SSC for 5 min and then immediately prehybridized or stored wet. The membranes were never allowed to dry out completely. DNA inserts were isolated from plasmids either by restriction enzyme digestion and electroelution or by PCR amplification using plasmid primers. Probes were ^{32}P-labeled by the random hexamer primer method. Prehybridization and hybridization were performed in a rotary hybridization chamber (National Labnet Co., Woodbridge, NJ) at 65° as described earlier (Dubcovsky *et al.* 1994). The membranes were washed in 2 × SSC and 0.5% SDS from 30 min to 2 h at 60°, 1 × SSC, and 0.5% SDS for 30 min at 65°, and 0.5 × SSC and 0.5% SDS for 12 min at 65°.

Clones for this study were selected from a pool of clones used for the construction of an RFLP map of the diploid wheat *T. monococcum* (Dubcovsky *et al.* 1996). Since more than one third of loci are duplicated in the Triticeae genomes (Dubcovsky *et al.* 1996), it would have been impossible to ascertain which fragments were from orthologous loci and which were from paralogous loci among the six species if clones hybridizing with multiple restriction fragments had been used. Therefore, only clones that hybridized with one or a few DNA fragments that cosegregated in a *T. monococcum* mapping population (Dubcovsky *et al.* 1996) were used in this study. All loci had been mapped in the *T. monococcum* genome (Dubcovsky *et al.* 1996; Figure 2). The position of each locus in the *B* and *D* genomes was confirmed by synteny mapping using *T. aestivum* nullisomic-tetrasomic stocks and *T. aestivum* disomic substitutions lines harboring single chromosome pairs of *Lophopyrum elongatum* substituted individually for wheat homoelogous chromosome pairs. Only clones that hybridized with DNA fragments in homoelogous chromosome regions in the A^m genome, *B* genome, and *D* genome were used in this investigation. This procedure maximized the likelihood that orthologous loci were investigated among the six diploid species.

A total of 37 cDNA and 14 *Pst*I genomic clones were selected on the basis of the above criteria. The clones and their origins have been described earlier (Dubcovsky *et al.* 1996). A total of 52 loci were detected with the 51 clones (Figure 2). Six clones hybridized with several fragments in one or more species, suggesting a locus duplication. Such clones were used only for estimating genetic diversity and only in species devoid of a duplication. Only a single enzyme x probe combination was used per locus; 48 and 4 loci were detected in *Dra*I- and *Xba*I-digested DNAs, respectively. For statistical purposes, the loci were divided into proximal and distal groups according to the coefficient of exchange of a chromosome region in which a locus resided. Since most coefficients of exchange were either smaller than 1.0 or markedly greater than 1.0 (Figure 2), a coefficient of exchange of 1.0 was used as a threshold to allocate loci to the proximal and distal groups. Analyses of correlation between gene diversity and coefficient of exchange (below) further justified this arbitrary allocation of loci. Nevertheless, because of the arbitrariness of this allocation of loci, computations and statistical tests were also made for loci grouped by a coefficient of exchange threshold of 0.5.

The loci covered all seven chromosomes and both arms of each chromosome (Figure 2). If the gene order in a genome would differ from the consensus position of these loci because of an inversion, the use of a large number of loci in the study would minimize the impact of such a structural change on the results.

Data analyses: The lengths of restriction fragments were

Figure 2.—The location of the loci used in this study on the linkage maps of the *T. monococcum* chromosomes (Dubcovsky *et al.* 1996). Centromeres are at the 0.0 points on the maps. The distance of each locus from the centromere in cM is indicated in parentheses to the left of a locus. Also to the left of each locus is the coefficient of exchange (centimorgans per percentage of metaphase chromosome length) of the chromosome region in which a locus resides. Loci in chromosome regions with coefficients of exchange <1.0 are boxed. In each chromosome arm, the most distal locus mapped in *T. monococcum* is shown for a reference. If the locus was not used in this study, it is marked by an asterisk.

used as the basis of estimating polymorphism. An allelic designation was assigned to each RFLP (haplotype), and the frequency of each allele was calculated. The allelic frequencies were used to calculate gene diversity h at a locus (Nei and Roychoudhury 1974).

$$h = \frac{2n}{2n-1}\left(1 - \sum_{i=1}^{m} x_i^2\right),$$

where n is the number of plants, x_i is the frequency of the ith allele, and m is the number of alleles at the locus. For self-fertilizing species, expression $2n/2n - 1$ was replaced by expression $n/n - 1$ (Nei 1987). The h values were used as variables in the calculation of average H over all loci and over the proximal and distal groups of loci, H_p and H_d, respectively, in each species:

$$H = \frac{\sum_{k=1}^{r} h_k}{r},$$

where h_k is gene diversity of the kth locus, and r is the number of loci.

Variation in H among species was analyzed with analyses of variance for a nested experimental design and Tukey's test. The statistical significance of the difference between H_p and H_d was tested individually in each species by t test using the h values as variables. The variables were transformed with arcsine transformation for these analyses. All statistical analyses were performed with SAS (1989) statistical programs.

The magnitude of divergence between species using all loci or those in the proximal and distal groups was computed as Nei's genetic distance D (Nei 1978):

$$D = -\log_e I,$$

where I is a normalized genetic identity.

$$I = \frac{\sum_{k=1}^{r} x_{ki}y_{ki}}{\sqrt{\sum_{k=1}^{r} x_{ki}^2 y_{ki}^2}},$$

where x_i and y_i are frequencies of the ith allele at the kth locus in species x and y, and r is the number of loci. The D values were calculated using the GDA program (Lewis and Zaykin 1997). A UPGMA phenogram was constructed with the GDA program. Variances of the D estimates, $V(D)$, were estimated according to the method of Nei (1987).

$$V(D) = \frac{1-I}{Ir},$$

where r is the number of loci. The variances were pooled, and the statistical significance of the difference between the divergence of the proximal loci and the divergence of the distal loci for each species pair was tested by t test.

The mean observed proportion of heterozygous loci (H_e) was computed as

$$H_e = \frac{\sum_{k=1}^{r} \frac{1}{n_k}\sum_{i \neq j} x_{ij}}{r},$$

where $\sum x_{ij}$ is the number of heterozygous pairs of alleles at the kth locus in a population of n plants, and r is the number of loci.

RESULTS

RFLP: Average gene diversity H of loci hybridizing with genomic clones across all species ($H = 0.39$) was similar to that of loci hybridizing with cDNA clones ($H = 0.37$). Average gene diversity was the highest in the cross-fertilizing *Ae. speltoides* and the lowest in the self-fertilizing *Ae. searsii* (Table 2). No heterozygous loci were encountered in *Ae. bicornis*, but were frequent in *Ae. sharonensis* and *Ae. longissima*, suggesting that the outcrossing rates vary among the five self-fertilizing species. Variation in the observed proportion of heterozygous loci (H_e) highly correlated with variation in H among the six species ($r = 0.9$, $P = 0.004$).

To investigate the relationship between H and the phylogenetic status of a species, the species were ranked according to their position on the phylogenetic tree shown in Figure 1. The H values were also ranked, and Spearman's coefficient of rank correlation (r_s) was calculated (Steel and Torrie 1960). Since *Ae. sharonensis* and *Ae. longissima* received the same phylogenetic rank, either *Ae. sharonensis* or *Ae. longissima* was used in the correlation analysis. In either case, r_s was 0.1 (NS),

TABLE 2

Phylogenetic ranks, mean observed proportion of heterozygous loci (H_e), average gene diversity H, and linear correlation coefficients (r) between the coefficient of exchange and gene diversity h in Aegilops species

Species	No. accessions	No. loci	Phylogenetic rank	H_e	H^a	r
Ae. searsii	14	43	3	0.01	0.14a	0.40 ($P < 0.01$)
Ae. bicornis	13	43	4	0.00	0.27b	0.66 ($P < 0.01$)
Ae. tauschii	165	51	1	0.01	0.27b	0.34 ($P < 0.01$)
Ae. longissima	13	42	5	0.03	0.39c	0.59 ($P < 0.01$)
Ae. sharonensis	5	40	5	0.06	0.49d	0.55 ($P < 0.01$)
Ae. speltoides	57	39	2	0.26	0.72e	0.26 ($P < 0.12$)

[a] Values sharing a common letter are not significantly different at the 5% probability level (Tukey's test).

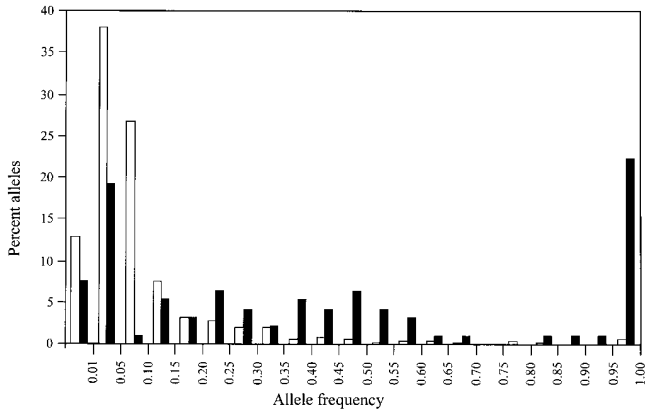


Figure 3.—Percentages of alleles with indicated frequencies in *Ae. speltoides* (opened bars) and *Ae. tauschii* (solid bars).

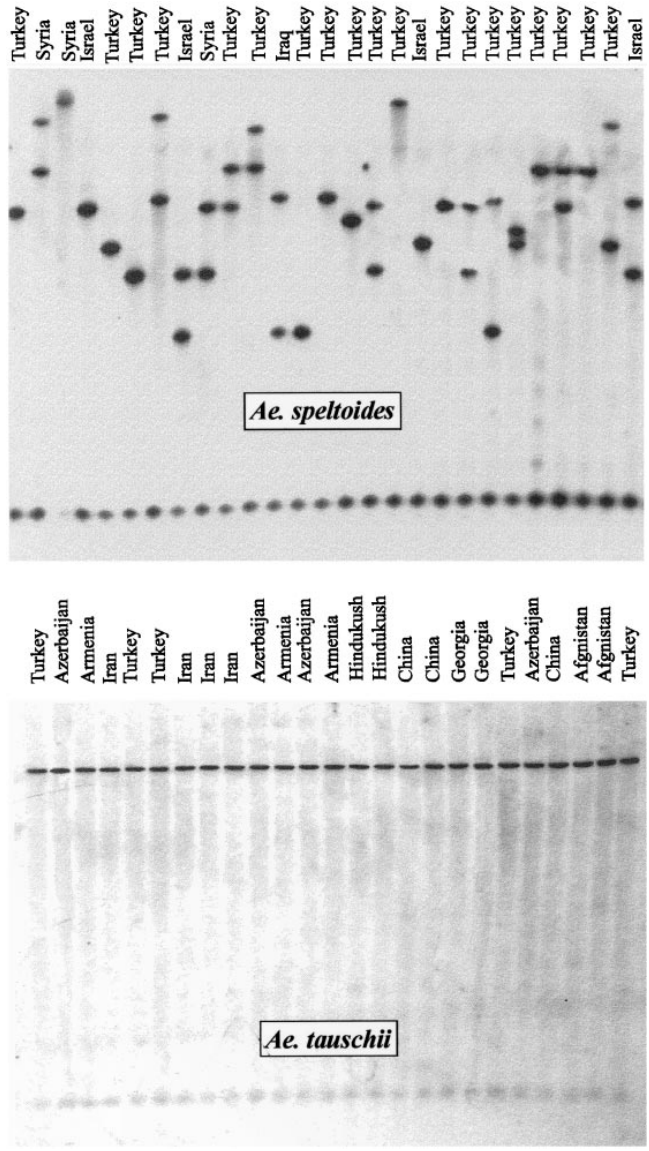


Figure 4.—Restriction fragment length polymorphism among *Ae. speltoides* DNAs (top) and monomorphism among *Ae. tauschii* DNAs (bottom) at the *Xpsr922* locus. The geographic origins of the accessions are indicated above the lanes. DNAs were digested with *Dra*I restriction endonuclease.

indicating no appreciable relationship between H and the phylogenetic status of a species.

There was a conspicuous difference in the allelic frequencies between the self-fertilizing *Ae. tauschii* and the cross-fertilizing *Ae. speltoides* (Figure 3; for an example see Figure 4); allelic frequencies in the remaining four species could not be meaningfully compared because of insufficient numbers of investigated plants. In *Ae. speltoides*, 66% of alleles had frequencies of 0.1 or less. Alleles with intermediate frequencies were rare, and only 1 of 541 alleles was fixed (*XGsp*, *Dra*I). In *Ae. tauschii*, the distribution of alleles classified according to their frequencies in the species was trimodal (Figure 3). Twenty-seven percent of alleles had frequencies <0.05. Alleles with these frequencies formed a distinct group separated from those with frequencies >0.1. Twenty-two percent of alleles were fixed or nearly fixed. The remaining 51% alleles had intermediate frequencies.

Relationship between RFLP and recombination: Gene diversity h correlated with the coefficient of exchange of the chromosome region in which a locus resided in all species except for *Ae. speltoides* (Table 2). In *Ae. speltoides*, values of h rapidly increased with increasing coefficient of exchange and then asymptotically approached the upper extreme in a scatter plot of h values relative to coefficient of exchange (not shown), suggesting that the relationship between these variables was not linear. For all species, with the sole exception of *Ae. bicornis*, the correlation between h and the coefficient of exchange considering only the loci in chromosome regions with the coefficient of exchange >1.0 were low, ranging from $r = 0.04$ (*Ae. speltoides*) to $r = 0.35$ (*Ae. longissima*), and were not statistically significant. In *Ae. bicornis*, this correlation was significant ($r = 0.58$, $P = 0.007$). These facts suggest that the correlations between h and the coefficients of exchange observed when all loci were considered were overwhelmingly caused by a difference between loci with the coefficients of exchange <1.0 and >1.0. This provides additional rationale for grouping loci by the coefficient of exchange thresholds of 1.0 and 0.5 to test statistically the relationship between gene diversity and the position of a locus on a chromosome (see materials and methods and below).

Average gene diversities were computed for the proximal loci as a group (H_p) and the distal loci as a group (H_d) in each species. In all six species, the proximal loci were significantly less polymorphic than the distal loci when a coefficient of exchange threshold of 0.5 was used to group the loci (Table 3). At a threshold of 1.0, the difference between H_p and H_d was significant in all species except for *Ae. speltoides* (Table 3). This lack

TABLE 3

Average gene diversities computed from loci in the proximal chromosome regions (H_p) with the coefficients of exchange (CE) ≤ 0.5 or < 1.0 and the distal chromosome regions (H_d) with CE ≥ 0.5 or > 1.0

Species	CE threshold	No. loci		H_p	H_d	H_d/H_p
		Proximal	Distal			
Ae. searsii	0.5	18	25	0.01	0.24**	24.0
	1.0	22	21	0.02	0.28**	14.0
Ae. bicornis	0.5	20	23	0.13	0.37**	2.9
	1.0	23	20	0.13	0.42**	3.2
Ae. tauschii	0.5	21	30	0.16	0.35**	2.2
	1.0	24	27	0.17	0.35**	2.1
Ae. longissima	0.5	18	24	0.13	0.58**	4.5
	1.0	22	20	0.18	0.61**	3.4
Ae. sharonensis	0.5	18	22	0.23	0.60**	2.3
	1.0	21	19	0.27	0.61**	2.3
Ae. speltoides	0.5	17	22	0.60	0.80*	1.3
	1.0	21	18	0.66	0.79 (NS)	1.2

* and ** indicate coresponding H_p and H_d values that differ from each other at the 5 and 1% probability levels, respectively (*t*-test).

of significance in *Ae. speltoides* between H_p and H_d was solely caused by increased *Hp* (Table 3). This reflected the fact that *h* was low only at loci with the lowest coefficients of exchange in *Ae. speltoides*, a fact revealed also by correlation analyses.

The H_d/H_p ratio was the highest in *Ae. searsii*, which had the lowest average gene diversity *H*, and was the lowest in *Ae. speltoides*, which had the highest *H* (Table 3). The H_d/H_p ratios were intermediate between these extremes in the remaining species (Table 3).

A relevant question is whether the relationship between gene diversity and the coefficient of exchange demonstrated for the entire genome holds for each chromosome. Data were available for comparisons of H_p with H_d in a total of 36 chromosomes across the six species. A coefficient of exchange threshold of 1.0 was used to group the loci to the proximal and distal groups. H_p was smaller than H_d in 32 chromosomes, and in 14 chromosomes, these differences were statistically significant at the 0.05 probability level (*t* tests). Thus, a number of chromosomes must contribute to the relationship between *h* and the coefficient of exchange in each genome.

Species divergence: Genetic distances were calculated between the five species of the section Sitopsis of the genus Aegilops using 39 loci (see Figure 1 for delineation of the section Sitopsis). *Ae. tauschii* was not included in these comparisons because the relationships between the *Ae. tauschii* alleles and those in the Sitopsis species were uncertain. The topology of the resulting UPGMA phenogram (Figure 5) was identical to the topology of the maximum parsimony tree constructed from variation in restriction fragments of repeated nucleotide sequences (Figure 1).

To assess the effect of the linkage map locus position on the rate of locus divergence between species, genetic distances *D* between species were calculated from allelic variation at either proximal loci (D_p) or distal loci (D_d) using the coefficients of exchange of 0.5 and 1.0 as thresholds (Table 4). D_p was significantly smaller than D_d in all comparisons among *Ae. longissima*, *Ae. sharonensis*, and *Ae. bicornis*. The remaining comparisons did not show any consistent trend.

DISCUSSION

In each of the six Aegilops species, loci that were near the centromere on the *T. monococcum* map were on average less polymorphic than the loci on the rest of the chromosome. This dichotomy in the level of RFLP in the proximal and distal chromosome region coincides with the low and high rates of recombination in the centromeric and distal chromosome regions in all wheat and Aegilops chromosomes examined to date. Correlations between locus diversity and the coefficient

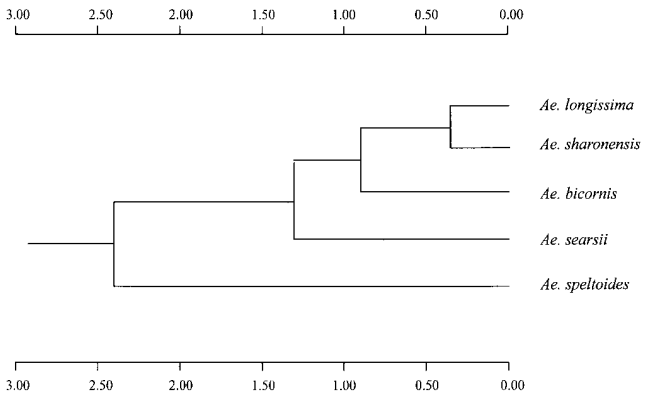


Figure 5.—A phenogram of the Sitopsis species constructed from the genetic distances (*D*) between species. The scale is a normalized genetic distance.

TABLE 4

Genetic distances D_p and D_d between the species of the section Sitopsis of the genus Aegilops

CE threshold	Species	*Ae. longissima*	*Ae. sharonensis*	*Ae. bicornis*	*Ae. searsii*	*Ae. speltoides*
0.5	*Ae. longissima*	—	0.21	1.00	1.86	2.17
	Ae. sharonensis	0.55**	—	1.09	2.50	2.13
	Ae. bicornis	1.70**	1.51**	—	2.74	4.05
	Ae. searsii	1.79	1.11**	1.41**	—	2.45
	Ae. speltoides	2.27	2.60	3.00**	3.40	—
1.0	*Ae. longissima*	—	0.26	1.11	1.93	2.29
	Ae. sharonensis	0.45**	—	0.93	1.82	2.21
	Ae. bicornis	1.48**	2.48**	—	1.51	4.19
	Ae. searsii	1.86	1.62	3.24**	—	2.59
	Ae. speltoides	2.22	2.60	2.89**	3.45	—

The D_p values (above the diagonal) were calculated from the allelic frequencies at loci in chromosome regions with CE $\leq$ 0.5 or $<$ 1.0, and D_d values (below the diagonal) were calculated from the allelic frequencies at loci in chromosome regions with CE $\geq$ 0.5 or $>$ 1.0. ** indicate corresponding values above and below the diagonals that differed from each other at the 1% probability level (*t*-test).

of exchange provide additional evidence that this dichotomy reflects variation in recombination rates. These correlations are, however, to a large extent caused by low *h* in the proximal loci and may largely be expressing the great difference in *h* between proximal and distal loci; their biological significance should, therefore, be treated with due caution. It should also not be neglected that other factors may affect the diversity of a specific locus (Hamblin and Aquadro 1997).

Reduced polymorphism in the regions of low recombination in Aegilops agrees with similar findings made in Drosophila (Begun and Aquadro 1992; Aguade and Langley 1994). The relationship between the positions of loci on the chromosomes and polymorphism may also be true for the human genome, although a systematic investigation of this relationship has not been reported. Donis-Keller *et al.* (1987) found that loci located distally showed RFLP more often than those located proximally in a human mapping population, and they suggested that this difference was related to a higher rate of recombination in the distal regions of human chromosomes.

If there is a relationship between the level of RFLP and the regional rates of recombination within a genome, there should also be a relationship between the level of RFLP and the overall rate of recombination to which a genome is subjected, which among other factors is related to the mating system (Charlesworth *et al.* 1993; Clegg 1997). The mating system among the six Aegilops species investigated here ranged from cross-fertilizing to self-fertilizing. The variation in mating system among the species was reflected in observed proportion of heterozygous loci. The highest observed proportion of heterozygous loci was in *Ae. speltoides*, which is classified as a cross-fertilizing species. *Aegilops speltoides* was far more polymorphic than *Ae. tauschii*, *Ae. bicornis*, and *Ae. searsii*, which are classified as self-fertilizing species. The dramatic difference in the level of RFLP between these two groups is exemplified by the observation that almost a quarter of loci were monomorphic or nearly so in the inbreeding *Ae. tauschii* (for example see Figure 4) despite its great geographic distribution, whereas only 1 of 39 investigated loci was monomorphic in *Ae. speltoides*. *Ae. longissima* and *Ae. sharonensis* are usually considered self-fertilizing, but they must have a mixed mating system because heterozygosity was frequent in the investigated samples of the two species. In these two species, average gene diversity *H* was intermediate between those in *Ae. tauschii*, *Ae. bicornis*, and *Ae. searsii* on one hand and *Ae. speltoides* on the other hand.

The existence of great differences at the DNA level between closely related self-fertilizing and cross-fertilizing species is not unique to Aegilops. Differences in RFLP of magnitudes similar to those reported here have been found between the self-compatible and self-incompatible species of Lycopersicon (Miller and Tanksley 1990). The observation that DNA variation is greatly reduced in regions of low recombination within genomes, demonstrated here and in Drosophila, makes it very likely that low DNA variation in self-fertilizing plant species relative to their cross-fertilizing relatives is also caused by reduced recombination.

Interestingly, the relationship between recombination and genetic variation is less apparent in allozymes. An analysis of allozyme variation in more than 400 plant species revealed that the most important factor determining the level of allozyme variation in a species was the size of its geographic range; mating system played a secondary role (Hamrick and Godt 1989). Furthermore, allozyme gene diversity *H* differed relatively little between the cross-fertilizing and self-fertiliz-

ing species, which contrasts with the great differences observed in RFLP between the cross-fertilizing and self-fertilizing species in Aegilops and Lycopersicon. These observations suggest that allozyme variation may be less affected by variation in recombination rates than RFLP. This conspicuous contrast between variation in DNA and allozymes is echoed by the report that, while silent site variation in DNA correlates with variation in recombination across chromosome 3 in *D. melanogaster*, the allozyme variation does not (Aquadro *et al.*, 1994; Kindhal 1994 cited in Hamblin and Aquadro 1997). Possibly, the difference between allozyme variation and RFLP may be caused by different processes and rates with which the two types of polymorphism originate, and by different selection pressures operating on allozymes and DNA variation (Clegg 1997). It is also possible that some species were misclassified as to their breeding habit in the allozyme study, which would diminish the apparent significance of the mating system on allozyme polymorphism.

The H_d/H_p ratio was the highest in *Ae. searsii* and the lowest in *Ae. speltoides*. This is inversely related to the levels of overall gene diversity *H* in the two species, *Ae. speltoides* having the highest and *Ae. searsii* the lowest *H* among the six species. The remaining four species had *H* intermediate between *Ae. searsii* and *Ae. speltoides* and, concomitantly, they had H_d/H_p ratios intermediate between those of *Ae. searsii* and *Ae. speltoides*. In *Ae. speltoides*, only loci in chromosome regions with the lowest rates of recombination (those in chromosome regions with the coefficients of exchange <0.5) had significantly reduced diversity relative to the rest of the chromosome. These observations suggest that the impact of reduced recombination on DNA variation in the centromeric region is disproportionately greater in species with reduced overall recombination, such as in self-fertilizing species, than in species with high overall recombination, such as in cross-fertilizing species.

The central property of both background selection and selection sweeps caused by hitchhiking is that both reduce existing variation; recombination merely moderates their effects. Theoretically, new haplotypes could, however, be generated by recombination. The emergence of a new haplotype (restriction fragment) via recombination between two existing haplotypes was observed at the *Xwg983* locus in the wheat chromosome pair $1A^m/1A$ (J. Dubcovsky and J. Dvořák, unpublished results). In that study, 82 loci were mapped in a population of 96 backcross plants, hence 7,870 haplotypes were surveyed (Dubcovsky *et al.* 1995). The origin of the new haplotype at *Xwg983* was accompanied by recombination of flanking markers on the chromosome. A similar observation was reported in maize in which new haplotypes (restriction fragments) were observed to emerge with a frequency of 3×10^{-3} (Timmermans *et al.* 1996). If recombination would solely prevent the loss of neutral variation, no relationship would be expected to exist between the levels of intraspecific polymorphism for selectively neutral nucleotide sequences and the rates of nucleotide sequence divergence between species (Birky and Walsh 1988; Martin-Campos *et al.* 1992; Aguade and Langley 1994). In agreement, no correlation was observed between variation in recombination rates across a chromosome and nucleotide sequence divergence at nine loci between *D. melanogaster* and its sibling species, *D. simulans* (Begun and Aquadro 1992). In contrast, a positive relationship between recombination and interspecific haplotype divergence was observed here, but only between closely related species, *Ae. longissima*, *Ae. bicornis*, and *Ae. sharonensis*. Rearrangements of a fixed number of mutations in a specific nucleotide sequence by recombination generate new haplotypes, but they do not change the magnitude of average nucleotide sequence difference between populations. There may, therefore, be no discrepancy between the observations made here and those made in Drosophila because the present data are based on haplotypes, whereas Drosophila estimates are based on nucleotide differences between nucleotide sequences. The failure to observe the relationship between recombination rates and haplotype divergence between more diverged species, such as in comparisons involving *Ae. searsii* and *Ae. speltoides*, could be caused by too many haplotype changes in their evolutionary history.

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