Sequence Evidence for Sporadic Intergeneric DNA Introgression from Wheat into a Wild Aegilops Species

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Introgressive hybridization has played a crucial role in the evolution of many plant species, especially polyploids. The duplicated genetic material and wide geographical distribution facilitate hybridization and introgression among polyploid species having either homologous or homoeologous genomes. Such introgression may lead to the production of recombinant genomes that are more difficult to form at the diploid level. Crop genomes that have introgressed into wild relatives can increase the capability of the wild relatives to adapt to agricultural environments and compete with crops or to compete with other wild species. Although the transfer of genes from crops into their conspecific immediate wild progenitors has been reported, little is known about spontaneous gene movement from crops to more distantly related species. We describe recent spontaneous DNA introgression from domesticated polyploid wheat into distantly related, wild tetraploid Aegilops peregrina (syn. Aegilops variabilis) and the stabilization of this sequence in wild populations despite not having homologous chromosomes. Our results show that DNA can spontaneously introgress between homoeologous genomes of species of the tribe Triticeae and, in the case of crop-wild relatives, possibly enrich the wild population. These results also emphasize the need for fail-safe mechanisms in transgenic crops to prevent gene flow where there may be ecological risks.

Introduction

Introgression, the incorporation of DNA from one species into the gene pool of another species, has played a crucial role in the evolution of many plant species (Anderson and Hubricht 1938; Anderson 1949; Stiebbs 1971; Arnold 1997). Introgression can greatly enrich the gene pool of the recipient species, increase its evolutionary potential, and in extreme cases lead to speciation (Rieseberg 1991). Introgression of genes from crops into their wild relatives may increase the capability of the wild species to adapt to agricultural environments and compete with the cultivated forms or to replace other wild species in native habitats (Ellstrand 2003). The introduction of cultivars from afar might also introduce new alleles into wild relatives and thus increase their competitive ability. Transgenes may introgress from engineered crops into adjacent wild or weedy relatives, with the expanding use of genetic engineering, potentially affecting biodiversity and producing more competitive weeds. Although several recent reports have demonstrated the occurrence of gene flow from crops to their biologically conspecific immediate wild progenitors (Linder et al. 1998; Chevre et al. 2003; Ellstrand 2003; Chen et al. 2004; Darmency 2005), little is known about spontaneous gene movement and subsequent introgression from crops to more distant relatives in other genera.

Common (bread) wheat (Triticum aestivum) is a case of a major crop that was most probably formed by hybridization in farmers’ fields. It is a hexaploid (2n = 6x = 42; genome BBAADD) that is thought to be produced by two sequential hybridization events (Feldman 2001). The first hybridization was probably between an unknown diploid donor of the B genome and diploid wheat Triticum urartu (2n = 2x = 14; genome AA) giving rise to tetraploid wheat Triticum turgidum (2n = 4x = 28; genome BBAA). Then, a domesticated form of T. turgidum that migrated through domestication to the habitat of wild diploid Aegilops tauschii (2n = 2x = 14; genome DD) is presumed to have hybridized with the surrounding wild population to create hexaploid T. aestivum (fig. 1). There are no known species presently extant having a B genome at the diploid level, and the SS genome of Aegilops speltoides has the greatest known homology to the B genome. Domestic wheat is grown on >200 million ha worldwide (http://faostat.fao.org) where it comes in contact with various wild members of the tribe Triticeae, especially Aegilops spp. that grow in or near wheat fields and may feasibly exchange genes with them. Introgressions from various genera of the tribe Triticeae including Aegilops spp. into wheat have been artificially demonstrated as part of breeding programs (Heun and Friebe 1990; Thomas, Chen, and Talbert 1998; De Pace et al. 2001; Martin-Sanchez et al. 2003; Li et al. 2004). Despite the potential hazards from gene flow from wheat into wild species, there is little published evidence that genes from domesticated wheat can or have introgressed into related wild species. For example, Wang et al. (2000), Guadagnuolo, Savova-Bianchi, and Felber (2001), and Kroiss et al. (2004) demonstrated that DNA segments of wheat can introgress into Aegilops cylindrica (2n = 4x = 28; genome CCDD), a tetraploid species having one of its two genomes homologous to the D genome of common wheat. There is even less evidence for natural homoeologous gene flow and stabilization in the field (Guadagnuolo et al. 2001).

Aegilops peregrina and Aegilops kotschyi are two wild tetraploid species (genome S’S’UU) belonging to the Triticeae. They are thought to be formed by hybridization between diploid Aegilops longissima (genome S’S) and diploid Aegilops umbellulata (genome UU) (Kihara 1954). Aegilops peregrina is a Mediterranean species occupying the southwestern part of the distribution of the genus, around the Mediterranean and into Jordan and Syria. It grows on a large variety of soils and in stable and disturbed habitats including pastures, abandoned fields, edges of cultivation, disturbed areas, and roadsides (Kimber and

Key words: introgression, wheat, Triticum aestivum, Aegilops peregrina, polyploidy, transgenic crops.

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Feldman 1987) but not as a competitive weed in fields. *Aegilops kotschyi* is an Irano-Turanian species penetrating into desert (Saharo-Arabian) regions, widely distributed in Transcaucasia, the republics of central Asia through to Afghanistan and Pakistan, north to Turkey and south to Kuwait, and lower Egypt and around the Mediterranean to Tunisia. It grows on a variety of soils in stable and disturbed habitats (Kimber and Feldman 1987), growing on warmer steppes than *Ae. peregrina*. Experimental hybrids between *Ae. peregrina* or *Ae. kotschyi* and *T. aestivum* have been described (Jewell 1979; Fernandez-Calvin and Orellana 1991; Ozkan and Feldman 2001). They reported a low level of pairing in F1 hybrids between hexaploid or tetraploid wheat and *Ae. peregrina*, with 0–5.0 bivalents per cell, depending on the accession of *Ae. peregrina*. This amount of pairing produced some viable backcross seed, transferring genes from *Ae. peregrina* into wheat by repeated backcrossing to *T. aestivum*. Powdery mildew resistance (Spetsov et al. 1997) and resistance to root-knot nematodes, *Meloidogyne naasi* (Yu, Person-Dedryver, and Jahier 1990), were introgressed into bread wheat in this way. Conversely, no reports could be found about natural hybridization between *T. aestivum* and *Ae. peregrina* or about gene flow from *T. aestivum* into *Ae. peregrina*.

We describe recent natural, sporadic spontaneous, introgression and stabilization of a DNA sequence from domesticated polyploid wheat into related wild *Ae. peregrina* (2n = 4x = 28; genome S’S’UU) in and near agroecosystems, despite the wild species having no chromosomes that are homologous to wheat, and from *Aegilops searsii* into related nonhomologous *Ae. kotschyi*.

Materials and Methods

Plant Material

The accessions of domesticated and wild polyploid wheat and tetraploid *Ae. peregrina* and *Ae. kotschyi* as well as accessions of diploid *Aegilops* species used for this study are described in table 1 in Supplementary Material online. The wild accessions were sampled from various locations within the area of distribution of each species. All plants were grown in 3-l pots in a greenhouse with 16 h light per day at 20°C. All plant material is maintained as stored seeds in the laboratory of Moshe Feldman at the Weizmann Institute of Science.

The Source of the Sequence

A 120-bp fragment of the noncoding locus WPG118, specific to the long arm of chromosome 3B was isolated from a genomic library of the common wheat cultivar Chinese Spring (Liu et al. 1997). A preliminary Southern blot scan of several wheat-specific markers indicated that the marker WPG118 was present in one accession of *Ae. peregrina*. We thus chose this representative marker for a detailed investigation of the occurrence of spontaneous introgression from cultivated wheat into neighboring related wild species.

Initial Amplification

The initial screen for the presence of the 120-bp segment of WPG118 used the following polymerase chain reaction (PCR) primers: forward (5’-AGCAACCATAAATTGGACCTTTCA-3’), reverse (5’-CATGTTCTGTGCTTCTCG-3’). The expected product size of this reaction...
Table 1
The Incidence of Three PCR-Amplified Fragments of the Locus WPG118 in Species and Subspecies of
Aegilops and Triticum

<table>
<thead>
<tr>
<th>Species and Subspecies</th>
<th>Ploidy</th>
<th>Genome</th>
<th>120-bp Segment</th>
<th>663-bp Segment</th>
<th>Conserved Primers: Low-Then High-Stringency PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Level</td>
<td>Genome</td>
<td>Low-Stringency PCR</td>
<td>High-Stringency PCR</td>
<td>Number of Lines Screened</td>
</tr>
<tr>
<td>Triticum aestivum</td>
<td>6X</td>
<td>BBAADD</td>
<td>2</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>ssp. spelta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ssp. macha</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ssp. dicoccoides</td>
<td>4X</td>
<td>S’S'UU</td>
<td>84</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ssp. brachythera</td>
<td>4X</td>
<td>S’S'UU</td>
<td>13</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>ssp. durum</td>
<td>4X</td>
<td>BBAAA</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Aegilops peregrina</td>
<td>4X</td>
<td>S’S’UU</td>
<td>36</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ssp. peregrina</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ssp. brachythera</td>
<td>4X</td>
<td>S’S’UU</td>
<td>13</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Aegilops searsii</td>
<td>4X</td>
<td>S’S’UU</td>
<td>36</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Aegilops speltoides</td>
<td>2X</td>
<td>SS</td>
<td>63</td>
<td>29</td>
<td>2</td>
</tr>
<tr>
<td>Aegilops sordida</td>
<td>2X</td>
<td>S’S’</td>
<td>63</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td>Aegilops bicorpus</td>
<td>2X</td>
<td>S’S’</td>
<td>63</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td>Aegilops sharonensis</td>
<td>2X</td>
<td>S’S’</td>
<td>63</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td>Aegilops longissima</td>
<td>2X</td>
<td>S’S’</td>
<td>63</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td>Aegilops umbellulata</td>
<td>2X</td>
<td>SS</td>
<td>63</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td>Aegilops tauschii</td>
<td>2X</td>
<td>DD</td>
<td>63</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td>Aegilops crassa</td>
<td>4X</td>
<td>DDM’M’</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Aegilops uniaristata</td>
<td>2X</td>
<td>NN</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Aegilops caudata</td>
<td>2X</td>
<td>CC</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Aegilops comosa</td>
<td>2X</td>
<td>MM</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Amblyopyrum muticum</td>
<td>2X</td>
<td>TT</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Note.—Dashes denote “not determined.”

* The same accessions that contained the short (120 bp) amplified segment also contained the long (663 bp) fragment of the WPG118 sequence.

is 120 bp. The PCR reaction mixture contained 6 pM of each primer, 0.25 mM of each deoxynucleotide (Roche Molecular Biochemicals, Mannheim, Germany), 1 μl of 10× reaction buffer (Sigma supplied with the enzyme), 1 U of Taq polymerase (Sigma, St. Louis, Mo.), and 100–200 ng of template DNA, all in a volume of 10 μl. The amplification profile was 3 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 52°C, and 1 min at 72°C and then 10 min at 72°C. This initial screen was performed at a low stringency (annealing temperature of 52°C) to allow annealing of the primers to possible variations of this amplified segment from other species. Five microliters of the PCR reaction were run on a 1.5% agarose gel with 20 mM ethidium bromide, visualized under UV light, and photographed.

High-Stringency Amplification of a 663-bp Segment of WPG118

The original 120-bp sequence was enlarged using inverse PCR: 300 ng of genomic DNA of common wheat (cv. Chinese Spring) was digested for 4 h at 37°C by 20 U of HindIII (Roche Molecular Biochemicals) in a volume of 10 μl, according to the manufacturer’s instructions. Restriction digests were then heated to 65°C for 5 min to inactivate HindIII. The 10-μl restriction digests were self-circularized overnight at 16°C by 400 U of T4 DNA ligase (New England Biolabs, Beverly, Mass.) in a final volume of 100 μl. Thirty-three microliters of the self-ligated DNA were used as an inverse PCR template with 0.25 mM of each deoxynucleotide (Roche) with 6 pM each of the forward primer 5’ ATATCTCAGCCATAGTCCAAAGCAACACC 3’ and reverse primer 5’ ATATCCATTTAAGCATACTCCACT 3’ in an Expand High Fidelity PCR System (Roche, Indianapolis, Ind.). The PCR profile was as recommended by the manufacturer with an annealing temperature of 50°C. Fifty microliters of the PCR product were fractionated on 1% agarose gels with 20 mM ethidium bromide, visualized under UV light, and photographed. Amplified products were excised from the gels, cleaned with a QIAquig Gel Extraction Kit (Qiagen, Hilden, Germany), and sequenced by the sequencing unit of the Weizmann Institute of Science. Seven hundred base pairs upstream of the original 120-bp fragment of WPG118 were sequenced using the following primers designed to extract the enlarged sequence from the tested species: forward 5’-GAGACAAAGGTTTCTAGGCTTCGT-3’ and reverse
5’-AGGGGCCCATAAAATAAGGTCAATTG-3’. The amplification reaction and visualization of the products and sequencing were performed as described above.

Amplification of a 316-bp Sequence of WPG118 Using Primers from Conserved Areas of the 663-bp Segment

Primers from two well-conserved areas of the 663 bp of WPG118 were constructed to ascertain that WPG118 was not present in various Aegilops species that were not included in the initial screening (table 1): forward 5’-AGG-ACTGTCATTTGTCATCA-3’ and reverse 5’-GCAGAG-AAAAGGCAAAGCAGTG-3’. PCR conditions were as described above for the 120-bp fragment (low stringency) and 663-bp fragment (high stringency). The amplification products were fractionated and visualized as described above.

Sequence Analysis

Multiple sequence alignments were performed using the Pileup algorithm in GCG 10.3 package, Accelrys (Burlington, MA, USA). A genetic difference matrix was obtained using the Phylogeny Inference Package (PHYLIP) version 3.57c developed by Felsenstein (1995). Multidimensional scaling (MDS) analysis of the genetic distances between the analyzed sequences was performed using the open source of the R software (The R Project for Statistical Computing, http://www.r-project.org/, 1997). The number of mean substitutions per site was calculated using the two-parameter distance model (Kimura 1980) using MEGA 2.1 software (Kumar et al. 2001). We chose this model as it considers the differences in the rates of transitions and transversions. Standard errors (SE) were calculated using the bootstrap option with 5,000 replications.

Southern Hybridization

Ten micrograms of DNA from each sample were restricted overnight at 37°C by 10 U of HindIII (Roche). Samples were separated overnight on 0.8% agarose gel with 20 mM ethidium bromide, visualized under UV light, and photographed. Southern blot and hybridization analyses were performed as described by Kashkush, Feldman, and Levy (2002). We used the 663-bp fragment of WPG118 as a probe for hybridization.

Results

Detection of Spontaneous Gene Introgression

The polyploid wheat DNA locus WPG118 located on the long arm of chromosome 3B also occurs with some sequence variation in two diploid Aegilops species (Feldman et al. 1997; Liu et al. 1997, 2003) suggesting that it would be a good probe for discerning sporadic introgression. Thus, we used this sequence to study the possibility of spontaneous gene transfer from field-cultivated domesticated wheats into the distantly related wild tetraploid relatives including Ae. peregrina and Ae. kotschyi. Initially, 47 accessions of Triticum and 170 of diploid Aegilops species from many sources (table 1 in Supplementary Material online) were screened for the presence of a 120-bp–amplified segment, by high-stringency PCR analysis (table 1).

This 120-bp segment was present in all accessions of polyploid wheat containing the B genome that were studied, namely, tetraploid T. turgidum (domesticated and wild) and hexaploid T. aestivum (bread wheat) (table 1). The primers used indicated the presence of similarly related amplified segments in all accessions of Ae. searsii (genome S5S6) analyzed as well as in half of the accessions of Ae. speltoides (SS). The sequence was not detected in any of the 80 accessions (total) of Aegilops bicorinis (S5S6), Aegilops sharonensis (S5S6), Ae. longissima (S5S6), Ae. umbellulata (UU), or Ae. tauschii (DD).

We screened 97 accessions of the wild tetraploids Ae. peregrina (S5S6’U) and Ae. kotschyi (S5S’UU) growing near wheat fields for the presence of the 120-bp fragment of WPG118 (table 1). Aegilops peregrina is a highly polymorphic species (thus syn. Aegilops variabilis) and thus was an ideal candidate for gene introgression. WPG118 was found in accessions TKE11 and TKE51 of Ae. peregrina and in accession TKK33 of Ae. kotschyi and their progeny, but not in other accessions of these two species (fig. 1 and table 1). The sporadic presence of the sequence in Ae. peregrina and Ae. kotschyi was puzzling because the sequence was missing from all 41 accessions of Ae. longissima and Ae. umbellulata analyzed, which are thought to be the donors of the S’ and the U genomes to these tetraploid wild species, respectively (Kihara 1954) (fig. 1).

Determination of the Putative Origins of WPG118 in Ae. peregrina and Ae. kotschyi

We sequenced an enlarged 663-bp–extended fragment of WPG118 to ascertain its probable origins in the two accessions of Ae. peregrina and one of Ae. kotschyi. The presence of the enlarged amplified segment was confirmed by high-stringency PCR in accessions of each species containing the 120-bp fragment (table 1). Of the accessions containing the 120-bp fragment of WPG118, 19 accessions of T. aestivum, 28 of T. turgidum, 2 of Ae. peregrina, 1 of Ae. kotschyi, 14 of Ae. speltoides, and 6 of Ae. searsii were sequenced and their 663-bp fragments were analyzed. A representative sample of these sequences is presented in figure 1 in Supplementary Material online.

The sequence analysis of the 663-bp–amplified sequences of WPG118 analogs within each species shows that the sequences found in tetraploid and hexaploid wheat differ considerably from those of diploid Ae. searsii and Ae. speltoides. The large variations (mean substitutions per site) between polyploid wheat and diploids Ae. searsii (T. aestivum–Ae. searsii = 25 and T. turgidum–Ae. searsii = 42) and Ae. speltoides (T. aestivum–Ae. speltoides = 38 and T. turgidum–Ae. speltoides = 29) were three to five times higher than the intraspecific variation within each species (T. aestivum = 5, T. turgidum = 13, Ae. searsii = 8, and Ae. speltoides = 8) (table 2). The minor variation of the amplified segment between polyploid wheat and the two accessions of Ae. peregrina is within the intraspecific range (T. aestivum–Ae. peregrina = 4 and T. turgidum–Ae. peregrina = 9). This strongly supports a hypothesis that the sequence introgressed into these two accessions of Ae. peregrina from wheat. There is a minute difference between the two Ae. peregrina accessions (differences in four
Table 2
Sequence Variation of the 663-bp Fragment of WPG118 Within and Between Species That Contain It

<table>
<thead>
<tr>
<th>Number of plants</th>
<th>Triticum aestivum</th>
<th>Triticum turgidum</th>
<th>Aegilops speltoides</th>
<th>Aegilops searsii</th>
<th>Aegilops peregrina</th>
<th>Aegilops kotschyi</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. aestivum</td>
<td>5 ± 4 (0)</td>
<td>12</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>T. turgidum</td>
<td>10 ± 3 (0–1)</td>
<td>13 ± 6 (0–1)</td>
<td>8 ± 6 (1–2)</td>
<td>8 ± 6 (2–3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ae. speltoides</td>
<td>38 ± 15 (9–11)</td>
<td>29 ± 4 (9–12)</td>
<td>39 ± 16 (8–9)</td>
<td>23 ± 11 (4–8)</td>
<td>0.008 (0)</td>
<td></td>
</tr>
<tr>
<td>Ae. searsii</td>
<td>25 ± 11 (4–7)</td>
<td>42 ± 14 (5–8)</td>
<td>36 ± 14 (9–11)</td>
<td>9 ± 11 (0–4)</td>
<td>19 ± 12 (6)</td>
<td></td>
</tr>
<tr>
<td>Ae. peregrina</td>
<td>4 ± 4 (0)</td>
<td>9 ± 4 (1)</td>
<td>33 ± 14 (4–5)</td>
<td>9 ± 11 (0–4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ae. kotschyi</td>
<td>21 ± 11 (6)</td>
<td>24 ± 11 (6–7)</td>
<td>14 (9–11)</td>
<td>14 (4–5)</td>
<td>0.008 (0)</td>
<td></td>
</tr>
</tbody>
</table>

Note.—a = as there was only one plant found with WPG118, the variation could not be measured. Note the low variation between hexaploid and tetraploid wheat and Ae. peregrina. Mean substitutions per site ± 1.96 SE (number of indels [insertions/deletions] in parenthesis) were calculated using the two-parameter distance model (Kimura 1980) using MEGA 2.1 software (Kumar et al. 2001). SEs were calculated using the bootstrap option with 5,000 replications.

The 663-bp–amplified segment of the single accession of Ae. kotschyi is almost identical to that of Ae. searsii (Ae. kotschyi–Ae. searsii = 9), while it differs from that of Ae. speltoides (interspecific variation of Ae. kotschyi–Ae. speltoides = 33) and the polyploid wheats (interspecific variation of T. aestivum–Ae. kotschyi = 21 and of T. turgidum–Ae. kotschyi = 24). Additionally, the 663-bp–amplified segment of the diploids and that of Ae. kotschyi contain 1–9 indels (insertions/deletions) compared with polyploid wheat (Ae. searsii–T. aestivum = 6 indels, Ae. searsii–T. turgidum = 6, Ae. speltoides–T. aestivum = 9, Ae. speltoides–T. turgidum = 9, and Ae. kotschyi–T. aestivum = 6, Ae. kotschyi–T. turgidum = 6). The sequences of polyploid wheat and the two accessions of Ae. peregrina lack indels relative to one another (fig. 1 in Supplementary Material online). This result further emphasizes the close relatedness of the sequences from the two accessions of Ae. peregrina to those of wheat and of the sequence from Ae. kotschyi to the sequences of the diploid species.

Cluster analysis of the sequences indicates three completely distinct groups based on the interspecies variation (fig. 2); (1) polyploid wheat and the two accessions of Ae. peregrina that contain WPG118, (2) Ae. speltoides, and (3) all accessions of Ae. searsii and the accession of Ae. kotschyi containing WPG118.

Verification of the Stable Introggression of WPG118 into Ae. peregrina and Ae. kotschyi Sequences

We verified that WPG118 sequence introgressed into the genomes of the accessions by Southern blotting to exclude the possibility that WPG118 in the Ae. peregrina and Ae. kotschyi accessions is due to PCR contamination. The banding pattern in the two accessions of Ae. peregrina is the same as that of polyploid wheat (two bands) and differs from that of diploids Ae. searsii and Ae. speltoides (one band) (fig. 3). This result further supports the contention that Ae. peregrina received the DNA from wheat.

It was necessary to exclude the possibility that WPG118 in Ae. peregrina and Ae. kotschyi came from another Aegilops species. We designed a pair of consensus primers from two highly conserved areas of the 663-bp fragment and used them to probe 14 accessions by low- and then high-stringency PCR. Five Aegilops species with genomes not previously tested (Aegilops crassa, Aegilops umiaristata, Aegilops caudata, Aegilops comosa ssp. comosa and ssp. subventricosa, as well as Amblyopyrum muticum) were included. All these accessions lacked the conserved sequence (table 1). These results strongly suggest that WPG118 did not originate from a source other than the B genome of wheat or the S genome of Ae. searsii or Ae. speltoides. The latter is far less probable due to the much greater differences in sequence homology.
Stabilization of WPG118 in a Wild Population of Ae. peregrina

We returned to the field where the original TKE11 spike had been collected 26 years earlier to ascertain whether the sequence remained in the population. Spikes from nine different plants were collected from various locations in the one section of abandoned field remaining at the site. Several seeds from each spike were planted, and the plants were tested for the presence of the 120 bp of WPG118 using high-stringency PCR. Two plants originating from one spike contained the sequence, but none of the 30 seeds from the other eight spikes contained the sequence. These sequences were identical to those of the originally tested plants of TKE11 collected over two decades earlier from the same location.

Discussion

Sporadic Intergeneric Introgression in Nature

The three accessions of the wild species containing WPG118 were from different habitats. Aegilops peregrina TKE11 was collected near where hexaploid bread wheat has been cultivated for the last 80 years and tetraploid T. turgidum ssp. durum cultivated for the previous centuries, but 20 km from the habitats of wild tetraploid wheat, T. turgidum ssp. dicoccoides. Aegilops peregrina TKE51 shared a habitat with T. turgidum ssp. dicoccoides as well as cultivated tetraploid and hexaploid wheat. Ae. kotschyi TKK33 was collected near populations of Ae. searsii.

A sequence shared by a crop and a wild relative can derive either from hybridization or by inheritance from a common ancestor (Doebly et al. 1990). Common ancestry is excluded by the sporadic nature of the introgressed sequence in the cases described above. The accumulated evidence of slight sequence polymorphism suggests that WPG118 independently introgressed from polyploid wheat into each of the two accessions of Ae. peregrina. The sequence similarity suggests that Ae. kotschyi accession TKK33 received the sequence from Ae. searsii (fig. 1). These conclusions in both cases are supported by (1) their ecosystem proximity, (2) the sequence data (fig. 1 in Supplementary Material online), (3) the common clustering (fig. 2), (4) Southern blot analysis (fig. 3), (5) the rarity of the sequence within the two recipient tetraploid species (table 1), and (6) the fact that WPG118 is absent from all analyzed accessions of the two diploid progenitors of Ae. peregrina and Ae. kotschyi (table 1). These intergeneric introgression events could only have occurred naturally in the field, sporadically, yet remain fixed in the population for many generations.

The transfer of the sequence between nonhomologous yet homoeologous chromosomes indicates that gene flow and establishment can occur among species with homoeologous genomes of the tribe Triticeae. Gene transfer between polyploid Triticum species and Ae. peregrina is difficult due to the low level of pairing in F1 hybrids between hexaploid or tetraploid wheat and Ae. peregrina (Jewell 1979; Fernandez-Calvin and Orellana 1991; Ozkan and Feldman 2001). Such F1 hybrids are functionally male-sterile but have low female fertility and may produce a few seeds upon backcrossing to either parent. Further generations of progeny regain fertility after two to three backcrosses with the wild species as the recurrent pollen parent (unpublished data). Fertile BC3 (backcross to Ae. peregrina) plants morphologically resemble the wild parent but may contain introgressed crop genes as a result of rare recombination between wheat and Aegilops chromosomes in the hybrid and in backcross progeny. As described in the introduction, useful genes have been bred from Ae. peregrina into wheat by repeated backcrossing to T. aestivum despite the low pairing in the hybrids. In contrast, our results demonstrate that introgression and establishment can occur spontaneously in the other direction, from wheat into wild populations of Ae. peregrina.

Introgression has played a greater role in the evolution of polyploid species than in diploid evolution. The duplicated genetic material in the polyploids facilitates hybridization and introgression among different polyploid species leading to the production of recombinant genomes that rarely can be formed at the diploid level (Zohary and Feldman 1962). Polyploids can tolerate genetic and chromosomal disharmony caused by hybridization to a greater extent than diploids, due to the chromosomal redundancy of the polyploids (Sears 1954; Low, Snape, and Worland 1987). Diploids are usually more specialized in their ecological requirements and are often reproductively well isolated from one another by strong sterility barriers (Kihara 1954; Zohary and Feldman 1962). Polyploidy may...
allow a greater possibility for interspecific and intergeneric gene flow due to less intersterility. Consequently, large genetic pools, which cannot be recombined at the diploid level, become available at the polyploid level through introgression. Such introgression may be a source of readily available genetic variation, which could be adaptive in cases of rapid occupation of newly opened habitats. The loose interspecific or even intergeneric connections probably provide polyploids with such genetic flexibility.

The Impact of Genes Introgressed from Cultivated Crops into Wild Populations

Introgression of genes from crops into their distantly related relatives is clearly infrequent. Still, it can impact wild populations if the introgressed gene has an adaptive value, increasing variation and, consequently, accelerating evolution while changing the balance among species in the wild ecosystem (Stebbins 1971), and with weeds, increasing competition with crops. For example, the crop foxtail millet, Setaria italica was domesticated by human selection for crop traits from its conspecific progenitor, the pernicious weed Setaria viridis (green foxtail). Later hybrids between the crop and its progenitor led to the evolution of an even more problematic weed S. viridis ssp. major (giant green foxtail) (Darmency 2005). In extreme cases, as with wheat and Ae. cylindrica in the USA, this might pose a threat to crop production and yield (Morrison, Cremieux, and Mallory-Smith 2002) and can be exacerbated when transgenes having selective advantages escape from crops.

Our results demonstrate the sporadic establishment and persistence of presumably natural introgressed noncoding sequence, with no known selective advantage. Similar cases with coding genes may enrich the gene pool of the wild population and increase the potential for competition with the crop. This may necessitate implementing fail-safe mechanisms to contain and mitigate flow of transgenes encoding advantageous traits. Methods such as transgenic mitigation i.e., insertion of a tandem construct containing a gene that is deleterious to the wild or weedy species in addition to the primary transgene may be highly effective in mitigating such gene flow (Gressel 1999, 2002; Ellstrand 2003; Al-Ahmad, Galili, and Gressel 2004, 2005; Gressel and Al-Ahmad 2005). For example, a gene for nonbrittle spike that suppresses premature seed dispersal will result in weed seeds being harvested with the crops, and not replenishing themselves in the field, mitigating the selective advantages of the primary transgene to the weed. The same mitigating gene will also prevent the dispersal of seeds from weeds growing in the wild population surrounding the cultivated field, thus preventing transgene establishment there. Thus, even though crop genes have and will flow to their wild or weedy relatives, methods and genes are available to contain and mitigate such flow.

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Literature Cited


Supplementary Material

Supplementary figure 1 and table 1 are available at Molecular Biology and Evolution online (http://www.mbe.oxfordjournals.org/).

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