

## SHORT COMMUNICATION

## Genome-specific introgression between wheat and its wild relative *Aegilops triuncialis*

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### Abstract

Introgression of sequences from crop species in wild relatives is of fundamental and practical concern. Here, we address gene flow between cultivated wheat and its widespread polyploid relative, *Aegilops triuncialis*, using 12 EST-SSR markers mapped on wheat chromosomes. The presence of wheat diagnostic alleles in natural populations of the barbed goatgrass growing in proximity to cultivated fields highlights that substantial gene flow occurred when both species coexisted. Furthermore, loci from the A subgenome of wheat were significantly less introgressed than sequences from other subgenomes, indicating differential introgression into *Ae. triuncialis*. Gene flow between such species sharing nonhomeologous chromosomes addresses the evolutionary outcomes of hybridization and may be important for efficient gene containment.

### Introduction

Reproduction between genetically distinct taxa, producing offspring of mixed ancestry (i.e. hybridization), plays a crucial role in evolution (Arnold, 2006). However, interspecific gene flow has been generally overlooked, and the factors determining the outcome of hybridization remain poorly understood (Abbott *et al.*, in press). In particular, the impact of spontaneous hybridization followed by backcrosses leading to introgression of genetic material into related species deserves further consideration. Introgression of loci from domesticated plants to wild relatives is of fundamental, practical and economic interest (Ellstrand *et al.*, 1999) and therefore represents a convenient model to explore the evolutionary significance of hybridization. The majority of cultivated plants indeed potentially hybridize at low level with wild species, and at least 27 cases of crop-to-wild gene transfer

have been reported in European agro-ecosystems (Felber *et al.*, 2007). With the advent of genetically modified crops, the consequences of introgression on local biota are receiving growing attention (Chapman & Burke, 2006; Kwit *et al.*, 2011).

The *Triticum/Aegilops* species complex represents an outstanding model to evaluate crop-to-wild gene flow. The cultivated durum wheat (*Triticum turgidum*;  $2n = 4x = 28$ ; genome BA) is an allotetraploid species with subgenomes originating from *T. uratu* (A genome) and *Ae. speltoides* (B genomes), whereas the bread wheat (*T. aestivum*;  $2n = x = 42$ ; genome BAD) is an allohexaploid species with an additional subgenome (D genome) originating from *Ae. tauschii* (Feldman & Levy, 2009). In addition to those species that participated in wheat evolution, the genus *Aegilops* presents a great diversity of diploid and polyploid species that have been classified in three natural clusters (i.e. the U, D and A genome clusters) based on conserved karyotypes (Zohary & Feldman, 1962; Kilian *et al.*, 2011). Cultivated wheat and several wild relatives are crossable, resulting in hybrids that occasionally produce viable seeds (reviewed in Kilian *et al.*, 2011). Most of the tetraploid *Aegilops* grow in geographical proximity and present phenological overlap with wheat, sustaining occasional hybridization. Spontaneous hybridization was indeed reported for most of the species from the cluster U

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(*Ae. columnaris*, *Ae. geniculata*, *Ae. neglecta* and *Ae. triuncialis*) and the cluster D (*Ae. cylindrica*, *Ae. ventricosa*; reviewed in Zaharieva & Monneveux, 2006), and wheat is considered as a 'moderate risk crop' for transgene escape (Stewart *et al.*, 2003). However, empirical studies conclusively documenting the magnitude of introgression in natural *Aegilops* populations are scarce, precluding formal risk assessment.

A recent molecular survey of European populations of three common relatives belonging to the cluster U using anonymous markers provided indirect evidence of gene flow from wheat (Arrigo *et al.*, 2011). In particular, the tetraploid barbed goatgrass (*Ae. triuncialis*; genomes UC) showed signs of introgression from cultivated wheats. These species only share homeologous chromosomes, which expectedly hampers pairing and recombination in resulting hybrids (Sears, 1976), and would be predicted to show limited introgression. As targeting transgenes to specific subgenomes not shared by wild relatives was suggested as a strategy to limit their escape, the molecular mechanisms underlying the unpredicted introgression reported in *Ae. triuncialis* need to be further addressed. The present work thus uses suitable interspecific genetic markers mapped on wheat chromosomes to: (i) demonstrate and quantify introgression of cultivated wheat sequences into the barbed goatgrass genome in Europe, but also in the United States where the species is presently invasive and (ii) document genome-specific patterns of introgression. The presence of wheat diagnostic alleles in natural populations of *Ae. triuncialis* offers conclusive evidence of substantial gene flow in the course of species coexistence and reveals that wheat subgenomes introgress with differential effectiveness.

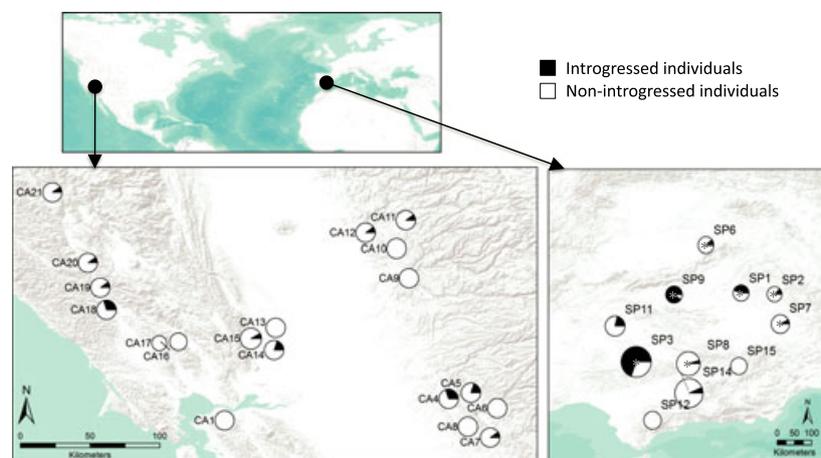
## Materials and methods

### Surveyed populations

Eleven and 19 populations of the barbed goatgrass (*Aegilops triuncialis*) were collected in Spain (Europe) and in California (United States of America; Fig. 1). The present study characterizes 351 individuals using wheat genome-specific markers (Table 1). The distance from the nearest cultivated field was recorded (irrespective of the crop currently grown), allowing to distinguish between populations collected 'close to wheat' and 'distant from wheat' (i.e. more than 50 m away from field). As pollen-mediated gene flow rarely exceeds 30 m in wheat (Zaharieva & Monneveux, 2006; Matus-Cadiz *et al.*, 2007), this partition is assumed conservative.

### Molecular survey

Naturally occurring individuals of *Ae. triuncialis* were genotyped with twelve EST-SSR loci mapped on wheat chromosomes using nulli-tetrasomic wheat lines (Zhang *et al.*, 2007). Such genic molecular markers are appropriate for introgression assessment and allow evaluating genome-specific gene flow. The effectiveness of the EST-SSR markers in assessing gene flow between wheat and *Ae. triuncialis* was evaluated by genotyping 25 cultivated wheat references (tetraploid and hexaploid accessions collected during field sampling and from the 'Agroscope Changins-Wädenswil' germplasm) and three accessions of pure *Ae. triuncialis*, *Ae. geniculata* and *Ae. cylindrica* chosen to represent much of the genetic diversity present in the species. A total of 37 EST-SSR were initially examined and showed almost no intraspecific allelic variation. Four EST-SSRs markers showing clearly



**Fig. 1** Gene flow from wheat in naturally occurring populations of its wild relatives *Aegilops triuncialis* in Spain (Europe) and in California (USA). The proportion of individuals showing introgression from wheat as detected with genome-specific EST-SSR markers is shown in black. The size of the pie chart is proportional to the number of individuals sampled, and populations collected 'close to wheat' are marked by a star.

**Table 1** Sampled populations of *Aegilops triuncialis* (Pop) with geographical coordinates (NDD: latitude; EDD: longitude) and introgression of cultivated wheat subgenomes (B, A or D).

Pop	NDD	EDD	Dist*	N†	N <sub>intro</sub> †	Genome-specific introgression‡			
						B	A	D	
<i>Spain, Europe</i>									
SP1	40.01	-2.96	C	9	4	1	3	4	
SP2	39.98	-2.09	C	8	1	1	0	0	
SP3	38.26	-5.7	C	33	23	23	0	0	
SP6	41.22	-3.88	C	9	1	0	0	1	
SP7	39.22	-1.94	C	12	1	0	0	1	
SP8	37.54	-4.34	C	20	1	0	0	1	
SP9	39.97	-4.71	C	10	9	9	0	0	
SP11	39.17	-6.25	D	14	3	2	0	2	
SP12	36.77	-5.27	D	11	0	0	0	0	
SP14	37.46	-4.32	D	29	2	0	1	1	
SP15	38.15	-3.02	D	10	0	0	0	0	
<i>California, USA</i>									
CA1	37.97	-122.20	D	10	0	0	0	0	
CA4	38.11	-120.66	D	10	3	1	1	1	
CA5	38.15	-120.61	D	10	2	0	0	2	
CA6	38.05	-120.44	D	10	0	0	0	0	
CA7	37.86	-120.51	D	10	1	0	0	1	
CA8	37.93	-120.63	D	10	0	0	0	0	
CA9	38.89	-121.01	D	10	0	0	0	0	
CA10	39.08	-121.09	D	10	0	0	0	0	
CA11	39.26	-121.03	D	10	1	1	0	0	
CA12	39.18	-121.29	D	10	1	0	0	1	
CA13	38.57	-121.87	D	10	0	0	0	0	
CA14	38.53	-121.97	D	10	2	0	0	2	
CA15	38.50	-122.03	D	12	1	0	0	1	
CA16	38.48	-122.61	D	8	0	0	0	0	
CA17	38.47	-122.62	D	6	0	0	0	0	
CA18	38.77	-122.96	D	10	3	1	0	2	
CA19	38.83	-123.00	D	10	1	0	0	1	
CA20	38.99	-123.08	D	10	1	0	0	1	
CA21	39.44	-123.31	D	10	1	1	0	0	
				Total	351	62	40	5	22
						(17.7%)	(64.5%)	(8.0%)	(35.5%)

\*Distance from nearest cultivated field: close to wheat (C) and distant from wheat (D; i.e. more than 50 m away from field).

†Number of individuals sampled (N) and individuals showing introgression (N<sub>intro</sub>).

‡Number of wheat diagnostic alleles (mapped EST-SSR) amplified in addition to *Ae. triuncialis* alleles.

distinguishable alleles in wheat and *Ae. triuncialis* were selected for each of the wheat subgenomes. Those twelve genome-specific markers are presented with species-specific allele sizes in Table 2. Selected markers also presented alleles distinguishing *Ae. triuncialis* from other common *Aegilops* species to ensure that wheat diagnostic alleles amplified in the barbed goatgrass did introgress from wheat (data not shown). Allele additivity was confirmed in experimental F1 hybrids between the barbed goatgrass and wheat. Accordingly, naturally occurring individuals were considered introgressed when they presented both *Ae. triuncialis* and wheat alleles.

PCR amplifications were performed on 5 ng of DNA in 10 µL, with 5× GoTaq reaction buffer, 0.2 mM dNTPs, 0.5 mM of each primer (one primer was

fluorescently marked with FAM, YYE or AT550) and 0.5 U of GoTaq DNA Polymerase (Promega, Dübendorf, Switzerland). Amplification was performed as follows: 120 s at 94 °C + 30x (30 s, 94 °C; 30 s, 60 °C; 30 s, 72 °C) + 8x (30 s, 94 °C; 30 s, 56 °C; 30 s, 72 °C) + 300 s at 72 °C. Resulting products were diluted five times, separated on a capillary 3500 Genetic Analyser (Applied Biosystems) and scored using GeneMapper 4.1.

## Data analyses

The proportions of individuals presenting wheat diagnostic alleles in populations of *Ae. triuncialis* located in Spain (Europe) and in California (USA) were compared taking the distance to nearest cultivated field into account,

**Table 2** Discriminating EST-SSR alleles considered for assessing introgression of genomic regions from cultivated wheat in *Aegilops triuncialis*.

Marker	Localization*	Band sizes in cultivated wheat (bp)	Band sizes in <i>Ae. triuncialis</i> (bp)
cfe25	1B	106	113
cfe126	2B	445	448
cfe188	4B	234	255
cfe288	6B	311	304
cfe189	1A	193	220
cfe195	3A	369	377
cfe208	5A	247	268
cfe132	6A	143	137
cfe66	2D	222	229
cfe159	5D	166	177
cfe277	6D	203	179
cfe20	7D	125	112

\*Marker localization on chromosomes (1–7) of the different wheat subgenomes (B, A and D) following Zhang et al., 2007.

using Generalized Linear Mixed Models (GLMM) fit by the Laplace approximation to the deviance and with binomial error distribution. Populations were treated as random effect.

Differential introgression of wheat subgenomes (B, A and D) was tested by comparing the proportions of individuals within Spanish populations showing signs of introgression with wheat B, A or D diagnostic alleles using GLMM (Laplace approximation; binomial error distribution) with populations as random effect. Statistical analyses were performed with R 2.11, using the lme4 package. This analysis of the patterns of genome-specific introgression into the barbed goatgrass focused on populations from Spain to avoid bias due to founder effects after the introduction of the species in the New World.

## Results

Among the 351 individuals of *Ae. triuncialis* from 30 natural populations surveyed here with 12 EST-SSR marking the different wheat subgenomes, 62 individuals (17.7%) from 20 populations (66.6%) presented wheat diagnostic alleles in addition to their own and thus showed patent signs of introgression from wheat (Fig. 1; Table 1). Eleven Californian populations of 19 (57.9%) present introgressed individuals as compared with 9 Spanish populations of 11 (81.8%). GLMM showed that populations of the barbed goatgrass collected in California (United States of America) presented significantly lower proportions of introgressed individuals than in Spain (Europe;  $z_{1,29} = -2.114$ ,  $P = 0.034$ ). Across all populations, fewer introgressed individuals were detected in sites located more than 50 m away from any crop field than 'close to wheat' ( $z_{1,29} = -3.317$ ,  $P < 0.001$ ). A similar trend was also observed when only considering the Spanish populations where both

situations (i.e. 'close to wheat' and 'distant from wheat') commonly occurred ( $z_{1,10} = -1.873$ ,  $P = 0.061$ ). In Spain, three of the seven populations growing close to cultivation fields (i.e. SP1, SP3 and SP9) indeed show high proportions of introgressed individuals. Noticeably, only six introgressed individuals, all collected in Spain, presented wheat diagnostic fragments from multiple loci (i.e. 2, 1, 1, 2 individuals with 5, 4, 3, 2 wheat alleles, respectively). These individuals were mostly collected in the population SP1 that was located within a wheat field.

Focusing on Spanish populations, where wheat and the barbed goatgrass coexist for a long time and where introgressed individuals were common, diagnostic alleles from the distinct wheat subgenomes were reported in different proportions in *Ae. triuncialis*. Alleles from the B subgenome occurred frequently, in four Spanish populations, representing 77.7% of the introgressed individuals. Individuals with alleles from the D subgenome represented 24.4% of the introgressed individuals and occurred in six populations. Two remote populations showed alleles from the A subgenome, accounting for 8.9% of the introgressed individuals. Taking nonindependence within populations into account, GLMM confirmed that the proportions of individuals showing signs of introgression were significantly lower for the A subgenome alleles than for the B or D subgenomes of wheat (Table 3).

## Discussion

### Extensive gene flow from cultivated wheat to the barbed goatgrass

Spontaneous introgression of genetic material from crop species to wild relatives is of great concern for further understanding the consequences of hybridization on species evolution, but also for assessing the risk of spreading genetically modified organisms and developing efficient strategies of transgene containment. The present results firmly conclude that specific wheat genomic regions commonly introgress and segregate within natural populations of the barbed goatgrass.

The presence of wheat diagnostic alleles in 17.7% of individuals of *Ae. triuncialis* indicates that introgression is a frequent process having potential consequences for

**Table 3** Generalized linear mixed models (with binomial error distribution and populations as random effect) of the effect of wheat subgenomes B, A and D on the proportion of introgressed individuals of *Aegilops triuncialis*.

Explanatory variables*	Effect size	SE	z	P
B	0.404	0.372	1.084	0.278
A	-1.555	0.336	-4.626	<0.001
D	0.394	0.372	1.061	0.289

\*Cultivated wheat subgenomes.

the species evolution. Noticeably, different wheat sequences were amplified in distinct populations of the barbed goatgrass in Spain, suggesting that multiple hybridization events have succeeded in producing introgressed individuals. Crosses between *Ae. triuncialis* and cultivated wheat have indeed been shown to regularly produce hybrids with relatively high fertility (Claesson *et al.*, 1990). As 98.3% of the introgressed individuals showed only one wheat diagnostic allele, our results indicate successive backcrossing with *Ae. triuncialis*. Accordingly, introgression of wheat loci in *Ae. triuncialis* is an effective process with possible long-term impact on natural populations.

Although wheat sequences can be recovered from individuals currently growing in isolation from any cultivation due, for instance, to long distance dispersal of seeds, the proportion of introgressed individuals is significantly associated with the geographical distance to the nearest cultivated field (also see Arrigo *et al.*, 2011). This suggests significant pollen-mediated gene flow when the species grow in sympatry, stimulating the recurrent hybridization and introgression of wheat sequences into the barbed goatgrass. Accordingly, introgressed individuals were more frequently reported in Spain than in California (27.3% vs. 9.14%, respectively), probably as a consequence of the repeatedly encountered sympatry between species in the Old World. American populations of *Ae. triuncialis* were indeed never observed along wheat fields in this work, and the proportion of introgressed individuals collected away from cultivation is not different in Spain and in California, suggesting that microgeographical isolation generally limits gene flow from wheat. Given that the barbed goatgrass has been invading the United States for the last century (Zaharieva & Monneveux, 2006), it could be that some of the originally introduced individuals in the New World were already introgressed. Accordingly, only a subsample of the wheat loci frequently observed in Spanish populations of *Ae. triuncialis* are segregating in Californian populations. Would the barbed goatgrass and wheat regularly occur in sympatry in the United States, massive introgression, as otherwise observed in Europe, should be further monitored.

### Genome-specific introgression into the barbed goatgrass

Among introgressed individuals from Spain, wheat loci originating from the A subgenome occurred significantly less frequently in barbed goatgrass populations than loci from either the B or the D subgenomes. Pairing of *Ae. triuncialis* and wheat chromosomes is common in hybrids and results in remarkably high number of chiasmata (Claesson *et al.*, 1990). Accordingly, intergenomic rearrangements involving the recombination of wheat subchromosomal regions in the *Aegilops* genome likely

underlies the long-term maintenance of wheat alleles in *Ae. triuncialis* (Dvorak, 2009). In addition, multiple EST-SSR from the same wheat chromosome did not consistently amplify (data not shown), further suggesting introgression of chromosomal fragments rather than retention of wheat chromosomes in the barbed goatgrass.

The A subgenome may introgress less frequently because it originates from a *Triticum* species and may thus present lower recombination with *Ae. triuncialis* chromosomes than the B and D subgenomes coming from *Aegilops* species. While the A subgenome shows higher density of homeologous recombination events per physical units than the B subgenome (Peng *et al.*, 2000), expectedly buffering hybrid fertility and supporting introgression of foreign sequences from taxa sharing homeologous chromosomes (e.g. wheat and *Ae. cylindrica*; Schoenenberger *et al.*, 2005), it may hardly recombine and thus stably introgress sequences from structurally divergent genomes. Challenging such interpretation of uneven introgression based on specific failure of chromosome pairing, it should be noticed that the A subgenome shows considerable structural stability over evolutionary timescale (Zohary & Feldman, 1962) and high colinearity with other Triticeae genomes (Devos & Gale, 2000). In particular, the barbed goatgrass genomes show limited affinity with any of the wheat progenitors (*Aegilops* section *Sitopsis*, genome S, close to genome B; *Triticum monococcum*, genome A; *Aegilops tauschii*, genome D), suggesting that none of the wheat subgenomes presents a considerably higher probability of pairing and recombining with *Ae. triuncialis* (summarized in Kilian *et al.*, 2011). Accordingly, the causes of the observed unequal, genome-specific introgression may deserve further explanation. As the A subgenome maintained a complete set of functional genes over long-term evolutionary scales and dominates the phenotype of all polyploid wheats of the A genome cluster (Peng *et al.*, 2003), it may give reason for deficient maintenance of introgressed sequences in the *A. triuncialis* genetic background in the long term. To what extent loci of the A subgenome fail to stably introgress or show limited retention within *Ae. triuncialis* as compared with those from the wheat B or the D subgenomes remains an open question, and the mechanisms underlying differential introgression of distinct genomes deserve further attention.

### Conclusions for genetically modified wheat

Presently, field tests with genetically modified wheat are under process but no transgenic variety is commercialized. Documenting extensive introgression, our work anticipates wheat as a worldwide risky crop for transgene escape. It further questions strategies for efficient transgene containment. In particular, we conclude that placing a transgene on the A subgenome might reduce,

but will not be sufficient to exclude its escape in wild populations of widespread relatives. The evolutionary outcomes of hybridization between species remain hardly predictable yet and the environmental risk associated with transgene escape could only be minimized with the advent innovative containment strategies precluding introgression in nontarget organisms.

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