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MULTIPLE ORIGINS PROMOTE THE ECOLOGICAL AMPLITUDE OF ALLOPOLYPLOID *AEGILOPS* (POACEAE)[1]

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Polyploidy has been ubiquitous in plant evolution and is thought to be an important engine of biodiversity that facilitates speciation, adaptation, and range expansion. Polyploid species can exhibit higher ecological tolerance than their progenitor species. For allotetraploid species, this higher tolerance is often attributed to the existence of heterosis resulting from entire genome duplication. However, multiple origins of allopolyploid species may further promote their ecological success by providing genetic variability in ecological traits underlying local adaptation and range expansion. Here we show in a group of allopolyploid species in the genus *Aegilops* that range size and abundance are correlated with the number of inferred origins. We found that allopolyploid *Aegilops* spp. contain multiple chloroplast haplotypes, each identical to haplotypes of the diploid progenitor species, indicating multiple origins as the major source of variation. The number of inferred origins in each allopolyploid species was correlated to the total area occupied by the allopolyploid and the tendency for the species to be common. Additionally, we found differences in ecological tolerance among independent origins in *Aegilops triuncialis*. These results strongly support the hypothesis that the introduction of genetic variability by multiple origins can increase the ecological amplitude and evolutionary success of allopolyploid species.

Key words: *Aegilops*; allopolyploidy; ecological success; multiple origin; Poaceae; wild wheat.

Polyploidization is an important evolutionary process (Stebbins, 1940) and can be considered the norm in the plant kingdom (Grant, 1981). Probably all angiosperms have polyploid histories (Masterson, 1994; Soltis et al., 2009), and polyploidy played a major role during crop domestication and biological invasions (Soltis and Soltis, 1999). The rate of speciation involving polyploidization is estimated to be between 4% (Otto and Whitton, 2000) and 34% (Stebbins, 1971). According to a recent review, the higher estimate is assumed to be more likely, considering the large number of morphologically defined plant species that include several ploidy levels, each of which might be considered as cryptic species (Rieseberg and Willis, 2007; Soltis et al., 2007).

We focus on allopolyploid species, which originate either after nonreduction prior to hybridization or genome duplication after hybridization, resulting in offspring that contain the merged entire diploid genomes of both parental progenitor species. In many cases, the increase in chromosome number results in a large degree of reproductive isolation between a new allopolyploid and its diploid progenitors and thus abrupt speciation. Allopolyploidy has clearly been a driving force in the diversification of angiosperms, but was largely ignored in evolutionary thinking about speciation and the modern synthesis (but see Stebbins, 1940) in part because allopolyploidy is not easily described in tractable mathematical models of speciation. Until recently, allopolyploid speciation has also been largely ignored in phylogenetics, which instead has focused on cladogenesis.

With allopolyploidy, having two entire genomes results in a sort of fixed heterozygosity that may increase the ecological amplitude of the allopolyploid relative to its diploid progenitors (Ramsey and Schemske, 1998, 2002; Wang et al., 2006). Such fixed heterozygosity should also mask deleterious recessives, thus facilitating inbreeding and selfing. In addition, genetic variability in allopolyploid species could be enhanced through modifications of newly formed polyploid genomes. As shown mainly with artificial hybrids, initially, all loci are represented as fixed heterozygotes in an allopolyploid hybrid. The lineage then enters a hypothesized "revolutionary phase" in which genetic and epigenetic adjustments stabilize the newly integrated genomes (Levy and Feldman, 2002). These rearrangements can lead to the creation of variation also in lines that were initially identical (Kashkush et al., 2002, 2003; Pires et al., 2004; Gaeta et al., 2007) and often includes a substantial nonrandom downsizing of one or more of the genomes (Leitch and Bennett, 2004; Adams and Wendel, 2005; Chantret et al., 2005), subfunctionalization or neofunctionalization of the duplicated loci (Force et al., 1999) followed or mediated by epigenetic changes (Levy and Feldman, 2004). Such mechanisms could create genetic variation also in naturally occurring allopolyploids, as demonstrated in *Tragopogon*, for which differential gene silencing and genome rearrangements have been confirmed (Tate et al., 2006). Together these factors may promote range expansion in the allopolyploid and foster the evolutionary success of polyploids (Liu and Wendel, 2003; Paterson, 2005).

Nevertheless, a single origin of an allopolyploid species involves only one individual of each diploid progenitor species and would necessarily result in a severe decrease in genetic variation. As a result of this extreme genetic bottleneck, differential

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expression of duplicated loci, gene silencing, or rearrangement (including homeologous recombination) would seem to represent the primary, if not sole, source of genetic variation in the newly formed allopolyploid species.

Recurrent multiple origins of polyploids, each independently originating from unique pairwise combinations of genotypes from the diploid progenitors, would be an additional source for genetic variation in allopolyploid species. It has been argued that multiple origins are the rule rather than the exception, based on many examples of multiple origins of allopolyploid (and autopolyploid) species (Doyle et al., 1990; Soltis and Soltis, 1991; Ashton and Abbott, 1992; Soltis and Soltis, 1999; Soltis et al., 2003; Comai, 2005). Genetic diversity resulting from multiple origins might be further enhanced by both homologous and homeologous recombination and epigenetic changes (Chen and Pikaard, 1997; Udall and Wendel, 2006). Thus, multiple origins could be a significant source of genetic variation in allopolyploid species that would help to explain their evolutionary success (Soltis and Soltis, 1999; Soltis et al., 2003).

An ideal opportunity for a comparative study of allopolyploid species is provided within the genus *Aegilops* (Poaceae). The genus represents a "hotspot" of allopolyploid speciation consisting of 10 diploid and 11 allopolyploid species (Fig. 1; van Slageren, 1994). *Aegilops* is closely related to bread wheat and thus has an extensive history of research on the evolution of the diploid and polyploid genomes (Wang et al., 1997). In particular, we can benefit from existing hypotheses and experimental data on the diploid progenitors of each allopolyploid species. There is also published evidence of multiple independent origins for some of the allotetraploid species (Vanichanon et al., 2003, Gandhi et al., 2005)

An open question is whether the increase of genetic variation in allopolyploids resulting from multiple origins significantly enhances the capacity for range expansion or adaptation to variable environments. To address this question, we tested whether the number of lineages resulting from independent origins per allopolyploid species correlates with both the geographic range as well as the abundance of that allopolyploid species. We looked for the presence of shared, identical chloroplast DNA (cpDNA) haplotypes between the allopolyploid species in *Aegilops* and their diploid progenitors to obtain evidence for multiple origins as a source of genetic variation. We then evaluated the impact of this variation on the evolutionary success of the species expressed as range size, population abundance, and adaptive phenotypic variability (Doyle et al., 2004). We also examined whether multiple origins confer differences in ecological tolerance in *Ae. triuncialis*, an allopolyploid that currently is invading California rangelands (Meimberg et al., 2006).

MATERIALS AND METHODS

Plant material—Accessions were obtained via USDA-GRIN (Germplasm Resources Information Network; http://www.ars-grin.gov) as seeds and are summarized in Table 1 (a complete list is provided in Appendix S1; see Supplemental Data with the online version of this article). For most allopolyploid species between nine and 15 accessions were included, except *Ae. juvenalis* for which only two accessions were available. One individual per accession was used for DNA extraction, and accessions were chosen according to available locality information to select a stratified sample across the whole range of a species when possible (Table 1). As an outgroup, several accessions from *Triticum* (wheat), *Aegilops speltoides*, and *Secale* (rye) were included, as well as *Ambylopyrum muticum,* a close relative of *Aegilops* (Van Slageren, 1994). We also examined one allopolyploid species in detail, using 58 accessions of *Ae. triuncialis* to compare phenotypic variation among multiple origins.

Genetic analyses—DNA was isolated from freeze-dried plant tissue using the Plant Charge-Switch kit (Fa Invitrogen, California, USA). We analyzed six independently amplified noncoding loci of chloroplast DNA (cpDNA), in total about 4000 bp (Table 2); including the spacers *ndhf-rpl32* and *rpl32-trnL*(CUA), the *trnT-trnL* region, and the *trnK* intron.

The PCR was performed in 25-μL reactions, containing 125 μM of each dNTP, 0.25 μM of each primer, and 0.5 U *Taq* polymerase (New England Biolabs, Ipswich, USA). The 0.5-μL DNA solution used as template typically contained 20–50 ng DNA. Amplification was carried out according to the following temperature profile: (1) 94°C for 2 min; (2) 35 cycles at 94°C for 30 s, 57°C for 50 s (55°C for the loci *rpl32-trnL*(CUA) and *trnK*-intron 5′-noncoding region, Table 2), 72°C for 1 min; and (3) a terminal extension phase at 72°C for 3 min. PCR products were cleaned and subsequently sequenced using the ABI (Applied Biosystems, Foster City, California, USA) BigDye Terminator kit 3.1 according to the manufacturers protocol.

Phylogenetic analyses and number of origins estimate—Our basic method is to identify the major chloroplast haplotypes in the diploid species and look in the allotetraplolids for the presence of haplotypes identical (shared haplotypes) to those in the diploids. All sequences have been deposited in GenBank (accessions EU012508–EU013929). Indels were included in the matrix as simple characters (Simmons and Ochoterena, 2000), excluding single nucleotide repeats to prevent overweighting of these positions. Exclusion of these positions did not change the number of different haplotypes in the data set. Bayesian analysis (Ronquist and Huelsenbeck, 2003) was performed using two analyses of 10 000 000 generations, sampling trees every 1000 generations, and a "burn-in" of 25%. The 50% majority rule consensus tree is shown in Fig. 2. In addition, we used maximum parsimony (MP) for phylogenetic analysis as described earlier (Meimberg et al., 2001). The resulting topologies from both analyses were congruent. However, the maximum parsimony analysis was less resolved, so only the Bayesian analysis is shown in Fig. 2. The major clades that had been used for the inference of origination events were also highly supported using MP.

To illustrate the patterns of identical haplotypes that are found in more than one species (shared haplotypes) within the genus *Aegilops*, we used the program Arlequin to construct a minimum spanning tree using pairwise differences (Schneider et al., 2000). AMOVA (analysis of molecular variance), as implemented in Arlequin, was performed to describe the structure of variation within and among allotetraploid and diploid species. The hierarchical model for population structure includes variation within species, variation among species nested within ploidy levels, and variation among ploidy levels. We expect little variation between diploid vs. tetraploid groups, because of shared or similar haplotypes between the polyploid and diploid species from which they arose.

We estimated the minimum number of origins as the number of unique haplotypes identical between the polyploid and each of their diploid parental species. We also report the number of haplotypes in the allopolyploid species that were closely related to haplotypes of either progenitor species (i.e., when they belonged to well-supported clades containing either of the respective diploid progenitors). We then correlated the minimum number of origins for each allotetraploid (the number haplotypes identical between allopolyploid and diploid progentiors), with the range size for that allotetraploid. We also correlated the number of haplotypes in each allotetraploid (the maximal possible number of origins indicated in our dataset) with the geographic range size of each allotetraploid.

Mean genetic diversity (Arlequin; Schneider et al., 2000) was estimated as an additional indicator for multiple origins and used in the regression of the amount of sequence polymorphism among the haplotypes against species range size and abundance. This value constitutes an average over the number of samples included per species and thus able to correct for differences in sample size.

Range analyses—The geographic range size of each species was determined using several methods. (1) Records including coordinates were downloaded for all species from the Global Biodiversity Information Facility (GBIF; University of Copenhagen, Copenhagen, Denmark, website http://www.gbif.org) and coordinates were imported into the program DIVA-GIS 5.0 (Hijmans et al., 2001). In this method, the range was defined as number of one-degree-grid cells with as at least one record, and range size was estimated as the area of all these grid cells or the area covered by the locations as determined using the

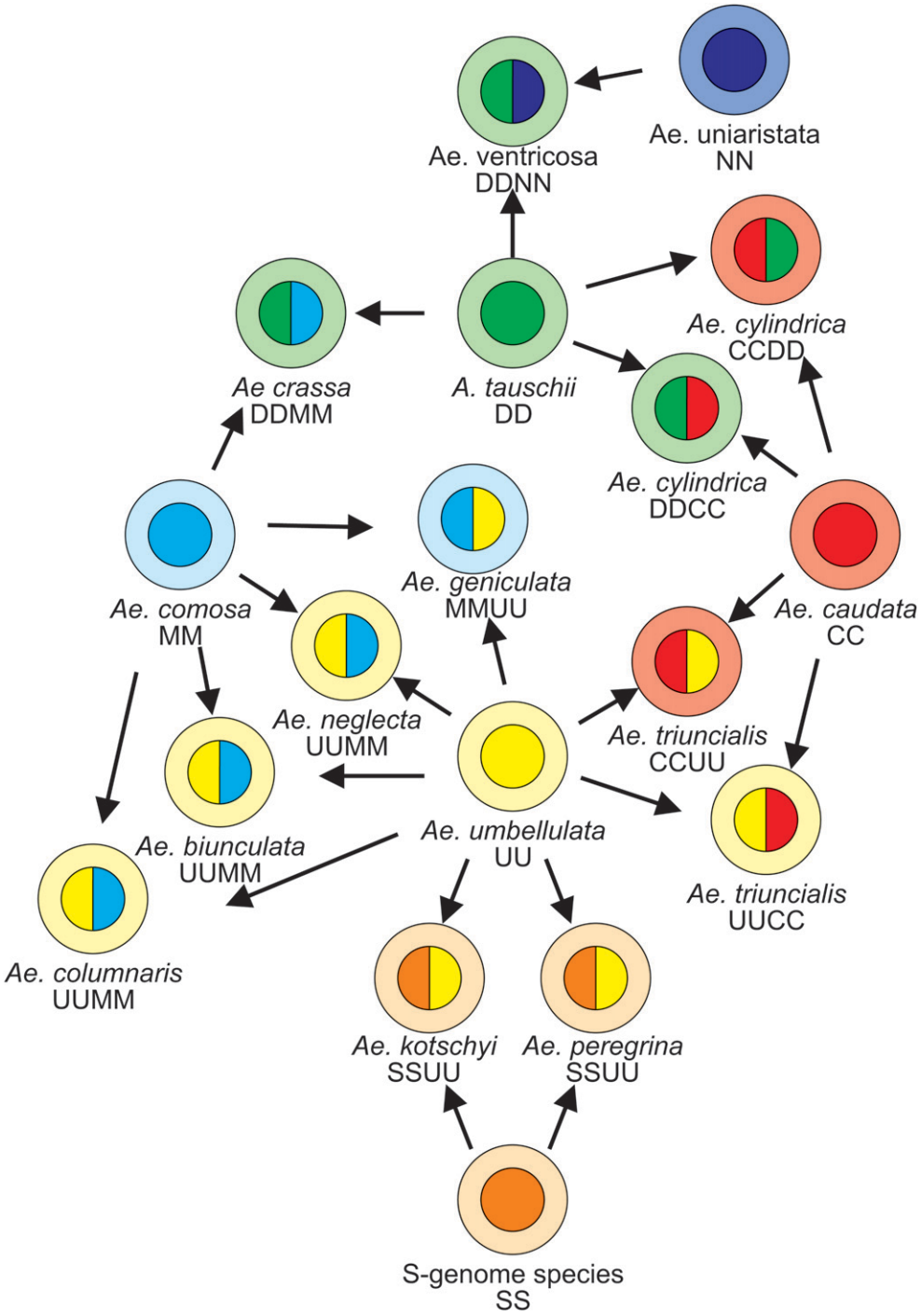

Fig. 1. Hypothesized evolutionary history of allopolyploid speciation in the genus *Aegilops*. For each species, the outer ring represents the chloroplast genome, and the inner circle represents the nuclear genome. The genome constitution is noted as female parent × male parent. Note that for *Ae. triuncialis* and *Ae. cylindrica*, evidence for multiple origins has already been described as these allotetraploids have chloroplast genomes from both diploid progenitors (van Slageren, 1994; Gandhi et al., 2005).

points to polygon function in DIVA. (2) Maps of locations given in van Slageren (1994) were digitized, and range size was determined by extrapolating a continuous area from the points either by connecting the most outlying points (maximal area) or using the shortest possible distance with which two points at the edge of the range could be connected. (3) Species presence data were copied by hand to planar projected maps, ranges drawn accordingly, and the ranges cut out and weighed on an analytical balance. The range size in square kilometers was calibrated using cut out areas of known size (countries). All estimates were done under inclusion and exclusion of the area covered by large water bodies. The area where a species is abundant was estimated in the same way using the part of the species range within the countries for which van Slageren (1994) gave the two highest abundance classes (common and widespread, common in restricted areas; table 11 in van Slageren, 1994). All methods of calculating range area led to comparable results, showing a significant positive correlation

TABLE 1. List of species studied. Indicated are number of samples, Genome type (in the allopolyploids female parent × male parent) according to Van Slageren (1994), range size as used for the analysis, number of origins, number of haplotypes, and a description of the range. *Abbreviations:* EM: East Mediterranean, WM: West Mediterranean, WA: Western Asiatic, M: Mediterranean, B: Circum Boreal.

Taxon	*N*	Genome	Range size (km^2)	Origins	Haplotypes	Range description
Allopolyploid species						
Aegilops biuncialis	14	UM	3 599 363	3	6	M-WA; Spain to Turkey and Caspian Sea
Ae. columnaris	11	UM	2 028 180	2	6	WA; Turkey to Central Iran
Ae. crassa	8	DM, DDM	3 099 648	1	5	WA; Syria to S Turkey and S Kazakhstan
Ae. cylindrica	14	DC, CD[a]	4 746 913	2	6	EM-WA-B; Balkan to Central Asia, Black Sea region
Ae. geniculata	15	MU	5 040 289	2	12	M-WA; Spain to Central Asia, Black Sea region
Ae. juvenalis	2	DMU	1 168 678	1	2	WA; N Syria and Iraq to Central Asia
Ae. kotschyi	10	SU	3 315 954	3	7	EM-WA; E North Africa to Central Asia
Ae. neglecta	14	UM	4 577 480	2	3	M-WA; Spain to Central Asia, Black Sea region
Ae. peregrina	14	SU	2 088 573	1	5	WA; S Turkey to E North Africa and Central Iran
Ae. triuncialis	58	UC, CU	7 452 817	4	16	M-WA; Spain to Central Asia, Black Sea region
Ae. ventricosa	6	DN	2 476 582	1	5	WM; Spain to Italy in North Africa east to Egypt
Diploid progenitor species						
Ae. caudata	18	C	1 655 104			WA; Greece and Turkey to Iran
Ae. comosa	4	M	646 346			EM; Greece and adjunct regions
Ae. tauschii	4	D	3 397 950			WA; Iran to Central Asia
Ae. umbellulata	14	U	1 885 631			WA; Turkey to Central Iran
Ae. uniaristata	4	N	783 409			EM; Costal Croatia to Greece and European Turkey
S-Genome species						
Ae. bicornis	2	Sb	929 736[b]			EM; Costal Aereas from Lybia to Syria
Ae. longissima	4	Sl	restricted			WA; Costal Egypt to NW Jordan
Ae. searsii	4	Ss	restricted			EM; W Jordan to Lebanon and Syria
Ae. sharonensis	2	Ss	restricted			EM; Coastal Israel to Lebanon
Ae. speltoides	2	S	1 098 135			WA; Turkey to W Iran, S to Jordan
Outgroup species						
Ambylopyrum muticum	1	TT				
Secale cereale subsp. *cereal*	1	n/a				
Triticum ispahanicum	1	AB				
T. monococcum subsp. *aegilopoides*	1	A				
T. monococcum subsp. *monococcum*	1	A				
T. timopheevii subsp. *armeniacum*	1	AG				
T. timopheevii subsp. *timopheevii*	1	AG				
T. turgidum subsp. *carthlicum*	1	AB				
T. turgidum subsp. *dicoccoides*	1	AB				
T. turgidum subsp. *Dicoccum*	1	AB				
T. turgidum subsp. *Durum*	1	AB				
T. turgidum subsp. *paleocolchicum*	1	AB				
T. turgidum subsp. *turanicum*	1	AB				
T. urartu	1	A				
T. zhukovskyi	1	AGA				
T. aestivum		ABD				

[a] Bidirectional origin according Gandhi et al. (2005)

[b] Range size is given for all S-genome species without *Ae. speltoides*.

with number of origins. Data shown are based on method 3, using species presence data from van Slageren (1994), excluding large bodies of water, to estimate range sizes in square kilometers.

The correlation between range sizes and number of origins, haplotypes, and genetic diversity was determined using Pearson's correlation coefficient, as implemented in the program JMP 5.0 module (version 7; SAS Institute, Cary, North Carolina, USA). Values of $P < 0.05$ were considered as statistically significant.

Phenotypic comparison of independently originated lines of Aegilops triuncialis—Ten replicate seeds of each of 58 *Ae. triuncialis* accessions were sown singly in 6.5 cm diameter pots, five in loam soil and five in serpentine soil, in a greenhouse at University of California-Davis. For each plant that reproduced, we measured flowering time, seed number per plant, total seed mass, and awn length.

The 58 accessions were grouped according to their cpDNA haplotypes. Five haplotypes comprised multiple accessions, and these were included in the statistical analysis. Results including the data for all replicates per accession were analyzed using analysis of variance and Students *t*-test. All statistical analyses were performed using the JMP 5.0 module.

RESULTS

Patterns of shared haplotypes, i.e., haplotypes that are identical between species, and phylogenetic reconstruction indicate that the chloroplast haplotypes of all except one allopolyploid species are assigned to multiple clades, so most allopolyploid species are indicated as polyphyletic in the analysis (Fig. 2). All species contained multiple haplotypes with a maximum of 18 in *Ae. triuncialis*.

Most haplotypes shared between different species were found between allopolyploid and diploid species or between two allopolyploid species. Only in two cases were haplotypes shared between diploid species: one haplotype was shared between *Ae. caudata* and *Ae. tauschii* and one between *Ae. sharonensis* and *Ae. longissima*. The latter species belong to a very closely related group of S-genome species with a restricted distribution in

TABLE 2. Sequence specifications of the loci studied and primers used for amplification and sequencing. Pimers marked with an asterisk are taken from Johnson and Soltis (1994).

Locus name	Length alignment	Length range	Variable positions (%)	No. indels	PolyA/T regions	Inversions	Forward primer	Reverse primer
rpl32-trnL(CUA)	883	779–820	5,1	10	1	1	ATCCGCATTAGACAAAATG AAG	GTGTTTCTATTGGGCAAA GCA
ndhf-rpl32	670	607–641	4,5	4	1	1	ACACTAGGAAAAGCCCAT ATG	GCAATAGATGTCTTTCACA TAC
trnK-intron 5′ noncoding region	309	246–284	1,9	6	1	0	GGGGTTGCTAACTCAA CGG*	AAGCAAGAAGA TTGTTTACGAAG
trnK-intron 3′ part	1167	1157–1158	3,3	3	0	0	GGTTCAACTCCTTCAATA CCG	AACTAGTCGGATGGAGTAG*
trnT-trnL	585	278–327	2,1	10	0	0	AATGCGATGCTCTAACCT CTG	TTCCATTGAGTCTCTGCACC
trnL-trnF	1015	855–991	3,3	13	2	0	TACACAAGGAATCCTGGT CTC	TACCAACTGAGCTATCCT GAC

the Near East. The high number of shared haplotypes between allopolyploids and diploids was also supported in an analysis of molecular variance (AMOVA; Excoffier et al., 1992). Variation was mainly explained by differences between species within ploidy levels (allotetraploid or diploid; 70.35%, Vb = 5.62, sum of squares = 1093, df = 18). The remaining variation was found within species (37.42%, Vc = 2.99, sum of squares = 610, df = 204). Variation was not explained by differences among the diploid species and the polyploid species (−7.7%, Va = −0.62, sum of squares = 36, df =1); thus, polyploid species are not significantly differentiated from their progenitors. Phylogenetic reconstruction of the haplotypes corresponded with previously published cpDNA phylogenies (i.e., Wang et al., 1997; Yamane and Kawahara, 2005) and the classification of cpDNA types (Wang et al., 1997). Several distinct clades containing all sampled diploid species of one genome and plastome type (C, D, N, S, and U; Wang et al., 1997) also included haplotypes of allopolyploid species that are derived from these diplolids (Fig. 2).

For four species, *Ae. crassa* (DM), *Ae. juvenalis* (DMU), *Ae. peregrina* (SU), and *Ae. ventricosa* (DN), a single origin can be assumed. All haplotypes of these species are members of a terminal clade and shared with not more than one haplotype of a diploid species. For several allopolyploid species, bidirectional origin was indicated by the placement of haplotypes within both major clades containing the diploid progenitors. This placement suggests that chloroplast DNA had been transmitted at least once from each diploid species as maternal progenitor. Two origins can be inferred for three species that are showing bidirectional origin: *Ae. cylindrica* (CD) shares haplotypes with *Ae. tauschii* (D) and with *Ae. caudata* (C). *Aegilops geniculata* (MU) and *Ae. columnaris* (UM) are sharing one haplotype each with *Ae. umbellulata* (U), other haplotypes are assigned to a different clade (M^o). This clade does not contain a haplotype from a diploid, but its neighbor group position to the U-clade indicates that the haplotypes of these species were derived from two differentiated progenitors. Multiple unidirectional origins were indicated by multiple haplotypes shared between the polyploid species and one diploid progenitor. For *Ae. neglecta* (UM), two unidirectional origins are indicated by sharing two different haplotypes with *Ae. umbellulata* (U). Three origins are indicated for *Ae. biuncialis* (UM), by sharing three different haplotypes with *Ae. umbellulata* (U), and for *Ae. kotschyi* (SU) by sharing two haplotypes with S-genome diploids and one with *Ae. umbellulata*. Four origins can be inferred for *Ae. triuncialis* (CU, UC) sharing two haplotypes with *Ae. umbellulata* (U) and two with *Ae. caudata* (C) (Figs. 2 and 3).

The geographic range size of allopolyploid species was significantly correlated with the estimated number of origins (Fig. 4). This relationship exists using either the number of haplotypes (i.e., where multiple origins were assumed to be the only source of cpDNA variation within the allopolyploids; $r = 0.80$, $P < 0.005$; Fig. 4A), or the number of origins estimated by the number of haplotypes shared with progenitors ($r = 0.73$, $P < 0.01$; Fig. 4B) or mean genetic diversity and is thus corrected for differences in sampling size ($r = 0.72$, $P < 0.05$). These relationships remain significant when only the part of the geographic range where a species is common was considered (number of haplotypes, Fig. 4C: $r = 0.89$, $P < 0.0005$; number of origins, Fig. 4D: $r = 0.64$, $P < 0.05$; mean genetic diversity: $r = 0.64$, $P < 0.005$). In addition, a significant correlation exists between the number of haplotypes ($r = 0.82$, $P < 0.005$; Fig. 4E) or mean genetic diversity ($r = 0.67$, $P < 0.05$) and the proportion of the total geographic range where a species is common. After exclusion of *Ae. juvenalis*, which may have been under sampled, most of these correlations are also significant (Range size, [1] number of haplotypes: $r = 0.76$, $P < 0.05$; [2] number of orgins: $r = 0.71$, $P < 0.05$; [3] mean genetic diversity: $r = 0.72$, $P < 0.05$. Size of range where species is common, [1] $r = 0.88$, $P < 0.005$; [2] nonsignificant, $r = 0.58$, $P > 0.05$; [3] $r = 0.79$, $P < 0.01$; Part of total geographic range where a species is common, [1] $r = 0.79$, $P < 0.01$; [2] nonsignificant, $r = 0.36$, $P > 0.1$; [3] $r = 0.67$, $P < 0.05$).

Comparison with geographic range sizes of the respective diploid parental species shows that the size of the area where both progenitors can be found, thus the area where new polyploids can be formed is significantly correlated with haplotype diversity ($r = 0.78$, $P < 0.01$; Fig. 5A), number of origins ($r = 0.81$, $P < 0.001$) and geographic range size in the polyploids ($r = 0.71$, $P < 0.03$). In contrast, the respective parameters are not correlated to the size of the geographic range where at least one of the progenitors occurs ([1] $r = 0.25$, $P > 0.5$, Fig. 5B; [2] $r = 0.43$, $P > 0.2$, [3] $r = 0.07$, $P > 0.8$) or the overlap between the geographic range of progenitors and polyploids ([1] $r = 0.4$, $P > 0.2$, Fig. 5C; [2] $r = 0.43$, $P > 0.2$; [3] $r = 0.55$, $P > 0.1$; Fig. 5).

The positive correlation of polyploid *Aegilops* abundance and geographic range size with cpDNA haplotype diversity indicates a positive effect of multiple origins or introgressions on the ecological success of a species. We tested whether different allopolyploid origins of *Ae. triuncialis* expressed phenotypic differences in phenological and adaptive traits. If these differences exist,

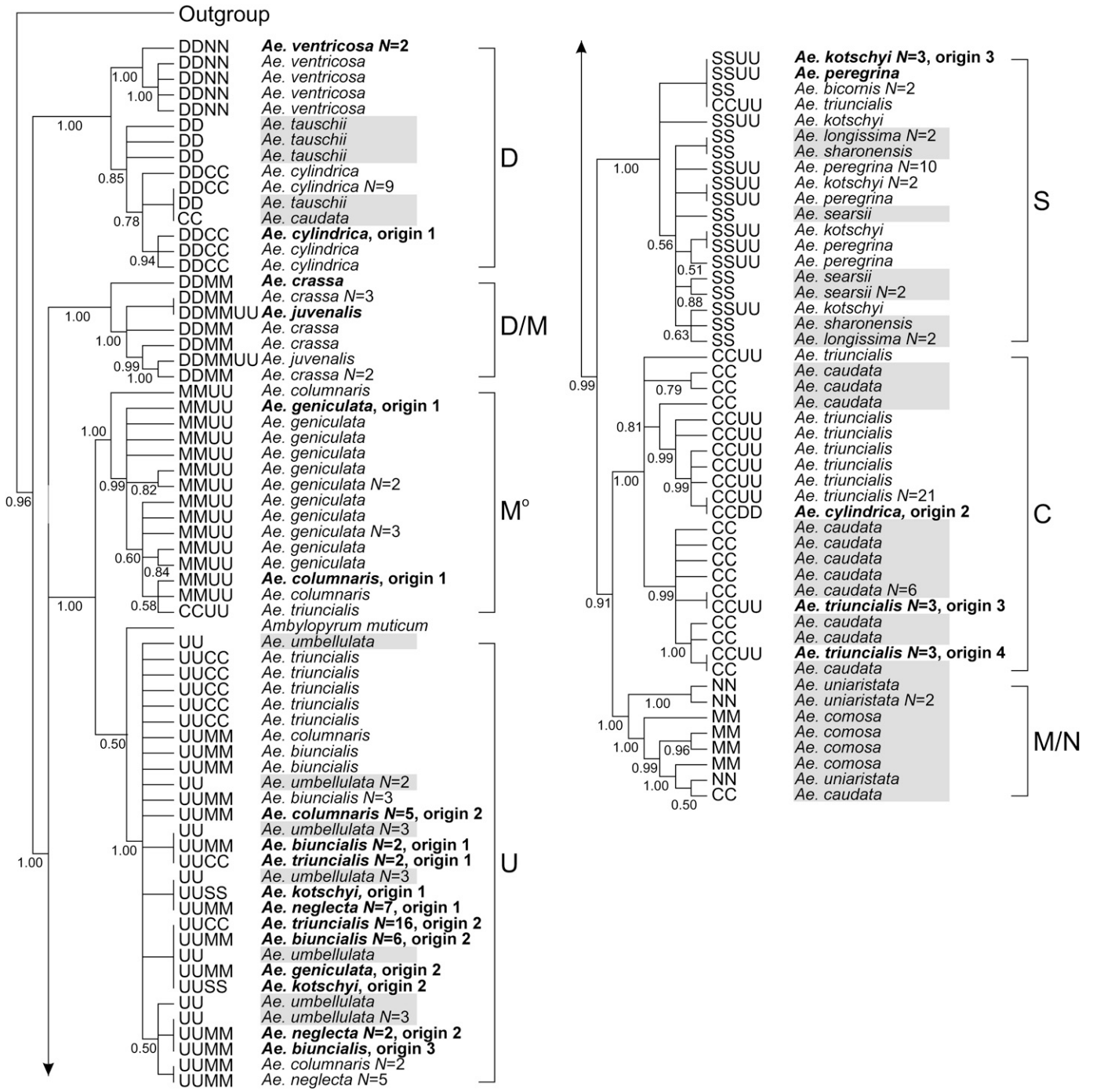

Fig. 2. Phylogenetic analysis of unique haplotypes within the genus *Aegilops* represented as a cladogram from a Bayesian analysis. From left to right: genome type (van Slageren, 1994; Wang et al., 1997) of species, indicating female × male parent in accordance with our analysis, species name, and groupings of the diploid (shaded gray) plastome types (van Slageren, 1994). Haplotypes found in more than one species are shown as terminal groups. The number of individuals of one species with a given haplotype is indicated by *N*. Clades are shown if supported by posterior probability values >50, also on the tree. Clades were assumed credible if the probability was >90. With the exception of *Ae. ventricosa*, all allopolyploid species are indicated as polyphyletic; highly supported in 9 of 20 cases. Three allotetraploid species (*Ae. kotschyi*, *Ae. cylindrical*, and *Ae. triuncialis*) had shared haplotypes with both diploid progenitors. Two allotetraploid species (*Ae. geniculata* and *Ae. columnaris*) were placed within two major clades, one containing the diploid *Ae. umbellulata* (U-clade). The other clade (Mº) contained the majority of the samples of tetraploid *Ae. geniculata* and probably reflects the placement of *Ae. geniculata* in previous studies (Wang et al., 1997).

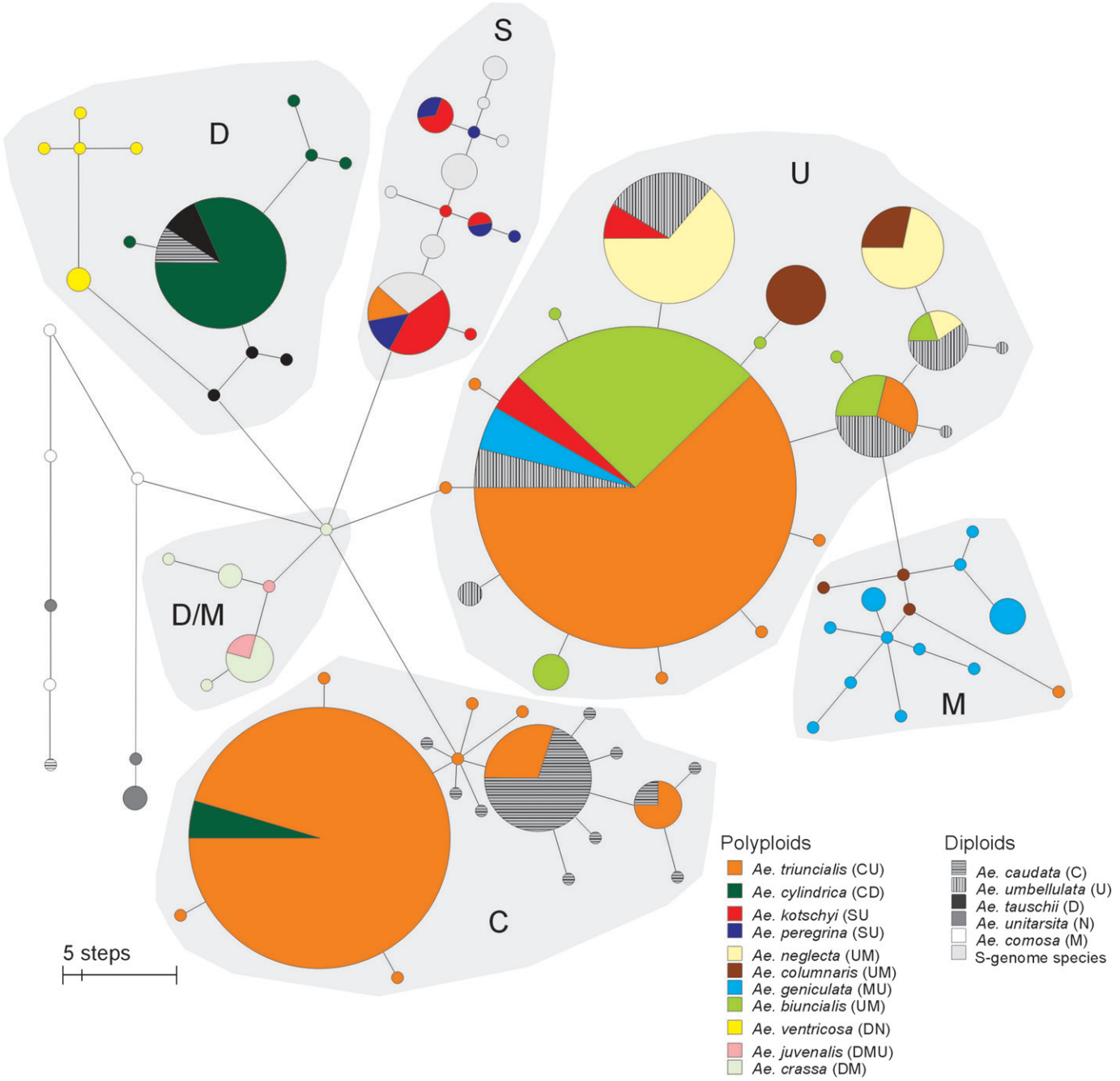

Fig. 3. Minimum spanning tree (produced with Arlequin) from pairwise differences among haplotypes. The area of the circles indicates the number of samples comprising the respective haplotype. The smallest circles represent haplotypes found only in a single individual and are therefore not used in the estimation of the number of origins of the allopolyploid species. Larger circles represent common haplotypes and reveal many cases where tetraploids have haplotypes identical to diploid progenitors. Colors indicate the species; the diploids are in solid or hatched gray. The four diploid species with the S-genome (*Ae. bicornis*, *Ae. longissima*, *Ae. searsi*, and *Ae. sharonensis*) are in the same shade of gray. Clusters are named in correspondence to the Bayesian analysis and are outlined in gray.

then a contribution of multiple origins to phenotypic variation is suggested that could lead to an increased ecological amplitude of the respective species. For this comparison, five groups of accessions were considered, each defined by a different cpDNA haplotype. Among these five groups representing different origins, we found significant differences for seed mass, flowering time, and awn length (Fig. 6). Flowering time, for example, was significantly different among all three *caudata*-type haplotypes. One haplotype flowered significantly earlier than the other groups and had a significantly higher seed mass.

DISCUSSION

Our data suggest that an increase in genetic diversity by multiple origins may promote range expansion in polyploid

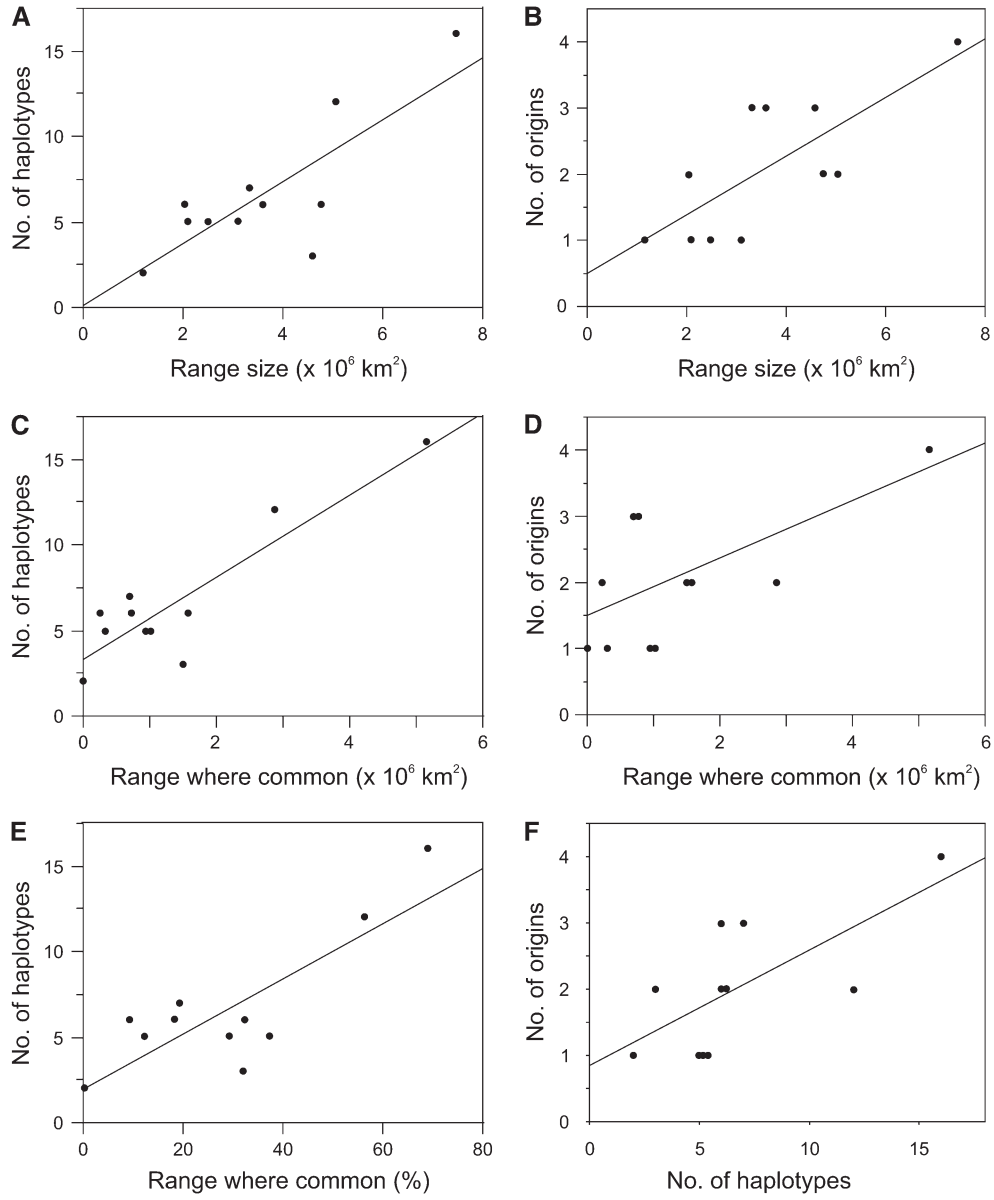

Fig. 4. Correlation of the total number of haplotypes (left column) and number of origins (right column) found in a species with geographic range size and abundance. Row 1: Correlation between range size and (A) number of haplotypes ($r = 0.80$, $P < 0.005$) or (B) number of origins ($r = 0.73$, $p < 0.05$). Row 2: Correlation between the part of the range where a species is common according to van Slageren (1994) and (C) number of haplotypes ($r = 0.89$, $P < 0.0005$) or (D) number of origins ($r = 0.64$, $P < 0.05$). Row 3: (E) Correlation between the area where a species is common relative to the total range size of the species and number of haplotypes ($r = 0.82$, $P < 0.005$), (F) correlation between estimated number of origins and number of haplotypes in each allopolyploid species ($r = 0.70$, $P < 0.05$).

Aegilops species, indicated by the significant correlation between range size or abundance and the number of origins. This genus-wide analysis was complemented by the finding of significant differences in ecological traits among accessions of *Ae. triuncialis* that were assigned to multiple independent origins.

The distribution of cpDNA haplotypes in *Aegilops* indicates a high amount of recurrent origin in almost all allopolyploid species within the genus. Because the inheritance of chloroplasts in wheat is maternal (Reboud and Zeyl, 1994), each allopolyploid origin should sample one haplotype from the maternal diploid progenitor. With a single origin, any additional genetic variation would have to be accumulated through mutations after this initial origin. As a result, the diploids should be paraphyletic to their allopolyploid descendants, or they should be the descendants' neighbor group. In contrast, we found that most allopolyploid species were polyphyletic within a clade containing the haplotypes of one progenitor or within both clades containing haplotypes of the progenitors. Only the M^o-clade, containing the samples of *Ae. columnaris* (UM) and the majority of samples of *Ae. geniculata* (MU), did not contain a haplotype of a potential diploid progenitor. Here either the diploid progenitor or the respective haplotype has gone extinct, or it was not present in our sample of the diploid accessions. Earlier studies classified this haplotype as related to the M-type of *Ae. comosa*, but specific to

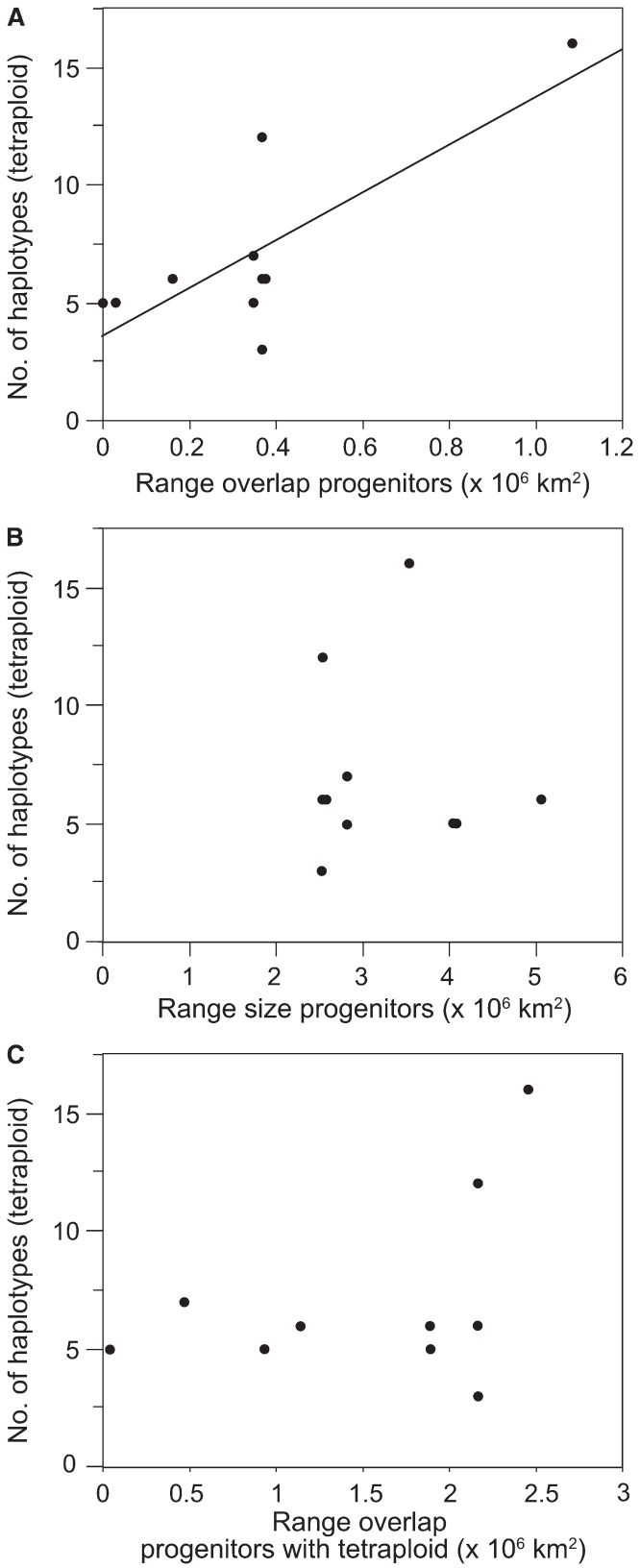

Fig. 5. Correlation of cpDNA haplotype diversity in polyploid *Aegilops* with species range parameters of the diploid parental species. (A) Size of geographic range where both diploid parental species are sympatric (i.e., size of area where formation of an allopolyploid could occur) by number of haplotypes in the tetraploid ($r = 0.78$, $P < 0.01$). (B) Size of the range where at least one of the progenitors occurs by number of haplotypes in the tetraploid (nonsignificant: $r = 0.25$, $P > 0.5$). (C) Size of range where the polyploid species overlaps with at least one of the diploids (nonsignificant: $r = 0.4$, $P > 0.2$).

Ae. geniculata (formerly *Ae. ovata*). However, these two allopolyploid species contained additional haplotypes assigned to the clade containing *Ae. umbellulata* (U).

A close relation of haplotypes of the allopolyploids to both progenitor species strongly supports independent, bidirectional origins with both progenitor species as maternal parents; bidirectional origins also have been shown in the genus *Aegilops* for *Ae. triuncialis* and *Ae. cylindrica* using nuclear markers (Vanichanon et al., 2003; Gandhi et al., 2005). Polyphyletic patterns of haplotypes within one major clade indicate multiple but unidirectional origins (Doyle et al., 2004). In addition to multiple origins, patterns of shared haplotypes between species may result from introgression by accidental paternal leakage or after hybridization and backcrossing of the hybrid with one species as the pollen donor (Tsitrone et al., 2003).

In *Aegilops*, introgression between diploid and polyploid levels is thought to be low. The transmission of cpDNA haplotypes from allopolyploid to diploid species is greatly limited by sterility of triploid intermediates (van Slageren, 1994). Chloroplast DNA is maternally inherited, so introgression from cpDNA haplotypes to the polyploid is mediated by hybridization with the polyploid as the pollen donor. Therefore, the direct transmission of cpDNA haplotype from a diploid to the polyploids and vice versa is likely to be rare (Feldman and Levy, 2005). Although hybridization between 4*n* and 6*n* species (in particular between 4*n Aegilops* species and 6*n Triticum aestivum*), can be fairly common (van Slageren, 1994), natural hybrids between diploid and tetraploid species are rare. Van Slageren (1994) only describes hybrids between *Ae. crassa* (4*n*) and *Ae. tauschii* (2*n*) in this respect. If gene flow between ploidy levels underlies the observed patterns, we predict that haplotype diversity should increase with increasing area of overlap between polyploid and progenitor. This prediction was not supported by our analysis. In contrast, the number of haplotypes in each allopolyploid species was significantly correlated to the area of overlap of both progentiors, i.e., the area in which allopolyploid hybridization can occur. This result is consistent with multiple origins rather than gene flow between ploidy levels.

On the other hand, allotetraploid species in *Aegilops* commonly form interspecific hybrids that may also facilitate recombination of genetic information from different diploid genomes (Wang et al., 1997; Feldman and Levy, 2005). According to van Slageren (1994), of 55 possible combinations between allopolyploid *Aegilops* species, 17 have been described as natural hybrids. Even though, we cannot out rule the possibility that gene flow between tetraploid species created the observed pattern, our analysis supports multiple origins as a major source of genetic variation in the allopolyploid *Aegilops* by the following findings: Nearly all haplotypes of allopolyploids cluster together with one or both of their progenitors. If introgression between tetraploids had primarily occurred, we would expect to find also cpDNA haplotypes that are shared between tetraploid species with different diploid progenitors. For example, *Ae. triuncialis* (CU) is able to form interspecific hybrids with all except one of the other seven tetraploid species comprising the

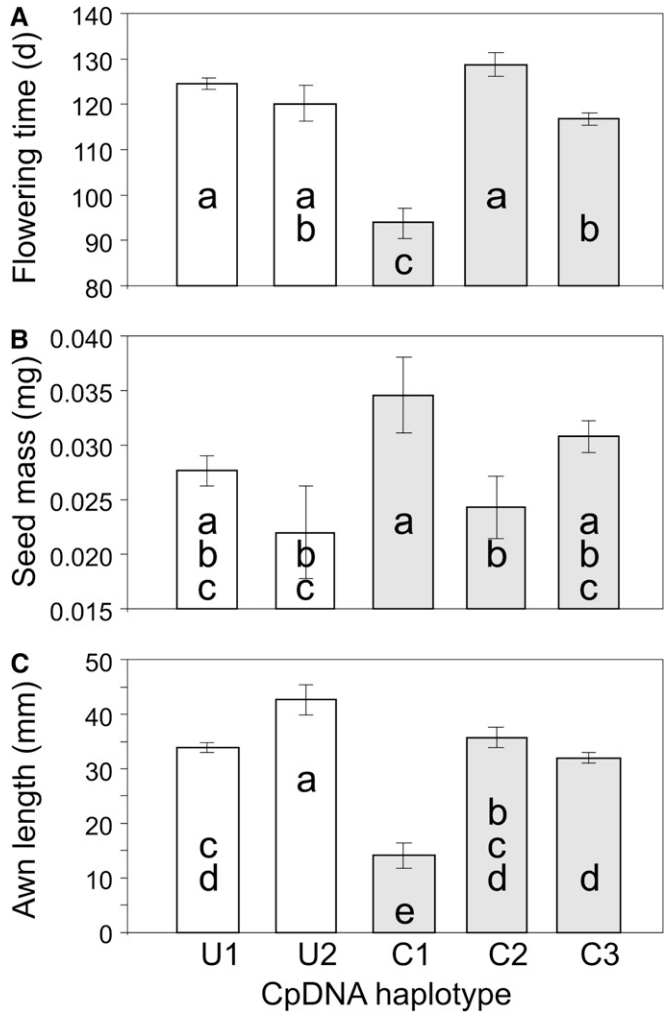

Fig. 6. Comparison between multiple samples of *Ae. triuncialis* sharing one haplotype and (A) flowering time, (B) seed mass, and (C) awn length in a greenhouse experiment on loam soil. Levels of significance (α = 0.05) are given within bars. Levels not connected by the same letter are significantly different. Error bars indicate standard errors. Number of accessions included per group: U1, N = 16; U2, N = 2; C1, N = 3; C2, N = 3; C3, N = 17. Five plants were measured for each accession.

U-genome. Hybrids between *Ae. cylindrica* (CD) and three of these species have described. Nevertheless, we did not find evidence for introgression of a C- or D-type chloroplast haplotype into any of these U-genome species (in total 78 samples). In 58 *Ae. triuncialis* samples (23 U-type and 33 C-type haplotypes), we found only two samples that fulfill the criteria to assume introgression: one sample comprising a cpDNA haplotype of the S-type and another sample assigned to the M^{o}-clade. Otherwise, shared haplotypes between allopolyploids were only detected within the clades containing their respective progenitors. However, we cannot exclude the possibility that reproductive isolation between polyploid species with the same progenitor species is lower than between species with different progenitors. Although no data are available that would support such a biased ability for hybridization within *Aegilops*, this case would represent an alternative way for the inclusion of different cpDNA lineages of the progenitors into the gene pool of a polyploid species. Nevertheless, the effect of introgressive hybridization on genetic variability in the allopolyploids (Feldman and Levy, 2005; Mallet, 2007) would be comparable to the effect of multiple origins. Both mechanisms could lead to increased genetic variation in allopolyploid species (Soltis et al., 2003).

The ability of a species to reach high population densities over a large area that encompasses a variety of different ecological conditions is an expression of the ecological amplitude of a species (i.e., the number of ecological niches a species can occupy without substantial decreases in fitness). In *Aegilops*, the ecological amplitude can be illustrated by the annual rainfall regime in which the respective species are growing (Van Slageren, 1994): the widely distributed species *Ae. triuncialis* and *Ae. geniculata* span from very dry regions with annual rainfall of around 100 mm to more humid areas with annual rainfall of 1400–1100 mm, respectively. In contrast, species with smaller ranges are restricted to either dry conditions like *Ae. kotschyi* (100–450 mm) or *Ae. crassa* (100–350 mm) or wetter environments like *Ae. columnaris* (450–1250 mm). The increase in *Aegilops* polyploid species abundance and range size with cpDNA haplotype diversity indicates a positive effect of the inclusion of several lineages of the progenitors on the ecological amplitude of *Aegilops* polyploids. The potential adaptive importance of multiple origins is further supported by the finding that different origination events, as indicated by shared haplotypes with the progenitors, contribute to phenotypic differences in *Ae. triuncialis*. *Aegilops triuncialis* is able to grow on serpentine soil where early flowering represents an important adaptation because these xeric, low fertility soils dry more rapidly during the spring growing season (Kruckeberg, 1984). Accordingly, *Ae. triuncialis* showed a significant negative correlation of fitness to flowering date on serpentine soil in field experiments (data not shown), indicating that early flowering time is important for adaptation to serpentine soil in *Ae. triuncialis*. We found that offspring of different origins can differ significantly in their flowering time and that this phenological difference is accompanied by a significantly higher seed mass for the line with the earliest flowering under greenhouse conditions. Thus, multiple origins could represent one source of genotypic variability that is expressed in differentially adapted lines, increasing the overall ecological amplitude in a polyploid species. In addition, independent origins could face different ecological conditions and selection regimes, assuming that it is likely that they originate in different parts of the range of the diploid progenitors. Different selection regimes could lead to selective divergence between origins. If multiple origins occur in succession, then density-dependent selection may favor each additional origin to be ecologically distinct. In addition, recombination among these multiple origins may further facilitate the expansion of the ecological niche of allotetraploids. These effects could be amplified by genome rearrangements in the independently originated lines. In artificial allopolyploids, it has been shown that initially identical lines can undergo differential gene rearrangement that leads to phenotypic variation (e.g., Gaeta et al., 2007). This variation can form the base for local adaptation in newly formed allopolyploids (Pires et al., 2004), and certain variants will be favored by selection. In case of multiple origins, such rearrangements will be very likely different between the independently originated, initially geographically isolated lines. Thus, genomic rearrangements could facilitate the development of several locally adapted genotypes or ecotypes. This mechanism could constitute one pathway by which multiple origins could lead to a broad ecological amplitude in allopolyploid species.

We cannot exclude that the likelihood of multiple origination increases with demographic factors such as abundance or similarity of habitat of progenitors that confound our results. Our approach is conservative and thus likely to underestimate the number of origins because only origins between individuals with detectable sequence divergence in their cpDNA are counted. Therefore, only a subsample of originations can be sampled, and population dynamics can lead to a homogenization of cpDNA after multiple origins that reduce the amount of detectable origination events. However, under the assumption that all these effects should be comparable between the species, our data provide first evidence that multiple origins could play a prominent role to explain the ecological success of polyploid species.

Fixed heterozygosity is well documented as a potent source of genetic variation and phenotypic variability in polyploids (Feldman and Levy, 2005), and fixed heterozygosity can contribute to a higher ecological tolerance of polyploids compared to their progenitors (Briggs and Walters, 1984). There are examples of obviously singly originated polyploid species, some of which are highly successful (e.g., *Spartina anglica*; Ainouche et al., 2004). However, our results indicate that multiple origins of polyploids represent an important additional dimension in the evolutionary dynamics of polyploid speciation. The positive effect that multiple origins have on the ecological success of the *Aegilops* species complex supports the view that this phenomenon may occur widely in many polyploid taxa. Our results suggest that multiple origins can expand the ecological amplitude of a species in its home range and also, in the case of *Ae. triuncialis* (Meimberg et al., 2006), facilitate the invasive spread of a species into entirely novel habitats. Although polyploidy as a factor promoting the success of invasive plants has been suggested (Ellstrand and Schierenbeck, 2000), our study indicates that multiple origins of polyploids could also be a significant component of biological invasions. In light of the growing number of examples of allopolyploid species with multiple origins, our results suggest that multiple origins may represent an important evolutionary phenomenon affecting patterns of genetic diversity and adaptive response in plants.

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