

**Natural hybridization between two allopolyploid  
wheatgrasses *Elytrigia intermedia* and  
*E. repens* (Poaceae, Triticeae)**

Ph. D. Thesis

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2007

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Hereby I declare that I have not submitted or presented any part of this thesis for any other degree or diploma.

Václav Mahelka

Průhonice, November 2006

## **Acknowledgements**

Hereby I would like to express cordial thanks to all people who helped me to complete this work.

In particular, my thanks go to my supervisor František Krahulec. Five years ago he introduced me to scientific work and consequently significantly contributed to my professional growth. He was always patient and willing to help me, not only intellectually but also with fieldwork. I appreciate him not only for his scientific knowledge in the field of botany but also as a man of wide scope.

Judith Fehrer was a supervisor specialist of my thesis. She played a significant role throughout my work, since she introduced me to molecular biology. I greatly appreciate her interest and scientific contribution to the whole project and her thorough and patient approach. Her ability to comprehend the scientific background of projects from various fields of botany is excellent.

I thank Bohumil Mandák for reading the manuscripts of the papers and for his helpful comments throughout my work. Fred Rooks is appreciated for English editing. Mária Loncová, Marie Stará, Ludmila Tereková, Petr Jurkovský, and Petra Wildová are appreciated for technical assistance in the DNA laboratory. Vlasta Jarolímová performed all karyological analyses, Pavel Trávníček, Jan Suda, and Anna Krahulcová kindly assisted me in flow cytometric analyses. I also thank Eva Ibermajerová, Eva Morávková and Eva Slívová for their help in the experimental garden.

This work was supported by the Ministry of Agriculture of the Czech Republic (grant no. QC 1362), Czech Science Foundation (grants nos. 206/05/0778 and 206/03/H137) and the Grant Agency of the Academy of Sciences of the Czech Republic (AV0Z60050516).

## Aims of the Thesis

The thesis is part of an ongoing project focused on hybridization in the wheat (*Triticum aestivum*)–*Elytrigia intermedia*–*E. repens* species complex. This complex represents a real model of crop–weed (wild relative) system with potential gene flow. The aim of the project is to detect and possibly to evaluate the frequency of gene flow from wheat into its wild relative, *E. intermedia*. Such study could provide a crucial background for potential risk assessment of the release of genetically modified wheat into the environment. Wheat is often crossed with *E. intermedia* in order to transfer some desirable traits of the wild grass into the wheat genome. Such hybridization is routinely performed under laboratory conditions; however, it has not yet been observed under natural conditions. Since *E. intermedia* crosses with *E. repens*, it may serve as a bridge species for gene flow from wheat into the weedy *E. repens*. Knowledge of the frequency of hybridization between both *Elytrigia* congeners with its consequences is thus of high interest and is the main objective of the thesis. The aims of the thesis can be summarized as follows: (1) to evaluate cytological variability of *Elytrigia repens* and *E. intermedia* in natural populations in the Czech Republic; (2) to establish reliable diagnostic markers for species and hybrid identification; (3) to evaluate the frequency of hybridization between *E. repens* and *E. intermedia* in natural populations; (4) to evaluate ecological and evolutionary consequences of natural hybridization.

## Study system

### Triticeae tribe

Tribe Triticeae is a large group of genera and species from the family Poaceae. It is well known for the worldwide economic importance of its three major crops: wheat, barley, and rye; and thus, among others, it comprises plants which were extremely important for the development of human civilization in the past and still are for food production at present. Many members of the tribe have also been used in crop improvement, since they possess many valuable traits that are transferred into crop genomes (e.g., Fedak 1999). The taxonomy and phylogeny of members of Triticeae and the relationships among them have been the object of a long-term dispute and remain in need of further investigation. In particular, it has long been a crucial question how to treat particular genera. Two extreme attitudes were suggested by Stebbins and Löve. Stebbins (1956) pointed out in his comment that, strictly genetically taken, all the species should be merged into one comprehensive genus. However, Stebbins himself was aware of the difficulty of the situation and especially of the necessity to find reliable morphological markers that would be in congruence with cytogenetic data. Therefore, until such evidence had been obtained, he had accepted the then conventional treatment while recognizing it as completely artificial. In the middle of the nineteen-eighties, Douglas R. Dewey and Áskel Löve had the same idea to treat genera on the basis of their genome constitution (Dewey 1984; Löve 1984). While Löve treated all the taxa of the tribe, Dewey restricted his treatment to perennial genera. With one exception (Löve's *Lophopyrum* Dewey treated as *Thinopyrum*), both authors reached the same conclusion and proposed the same classification. However, this approach was then unusual and the immediate response of most systematists was negative (Barkworth & Bothmer 2005). An obvious disadvantage of the genomic classification is that it is difficult to apply in the field. Another one is that the

system is based on a single character, although genome constitution represents a very important character. Nowadays, there is prevailing agreement on generic delimitation within the Triticeae. At least, genomic constitution has become the key piece of information in species characteristics in the majority of papers dealing with cytology, hybridization, or systematics. According to Löve's treatment, about 500 taxa (i.e. species including subspecies) are divided into 37 genera. Apart from genera, which include diploid and autopolyploid species (e.g., *Pseudoroegneria*), whose treatment is largely unambiguous, there are many taxa of allopolyploid origin (e.g., *Elymus*), which evolved through hybridization of distinct species and whose treatment is more problematic. Up to dodecaploid species are known from the Triticeae.

The tribe's structure is highly reticulate, with distinct genomes/gene lineages occurring not only within some polyploid but also within some diploid species (Kellogg *et al.* 1996; Mason-Gamer 2004). The reticulate structure is likely a consequence of ancient hybridization events, introgression, lineage sorting of ancestral variation, multiple origins of particular species, or a combination of all these processes. They all resulted in a strong ecological, morphological, and genetic similarity of many Triticeae taxa (Stebbins 1956; Dewey 1984; Löve 1984). The ability of Triticeae taxa to hybridize with each other is so common that Stebbins (1956) noted: "So many hybrid combinations in one group is unparalleled in the higher plants." One consequence of a reticulate structure is that if subsequent hybridization between genetically related species occurs, fertility of the hybrids can be enhanced because their chromosomes may pair more readily, and polyploidization generally provides an effective way to escape from sterility (Stebbins 1940). If such hybridization occurs, the reticulation among Triticeae taxa becomes even more complicated. The complexity of the situation in the group can easily be demonstrated by the taxonomy of the two *Elytrigia* taxa studied: 149 and 142 synonyms were found for *E. intermedia* and *E. repens* (including subspecies), respectively (Clayton & Harman 2006).

The present-day situation in the Triticeae classification calls for development of a species-level treatment of the tribe, which would enable identifying species unambiguously (Barkworth & Bothmer 2005). In my thesis I focus on hybridization between *Elytrigia repens* and *E. intermedia*. Both species are rhizomatous perennial grasses that are out-crossing, wind pollinated, and reproduce by seeds and rhizomes. The predominantly hexaploid *E. repens* and *E. intermedia* are known to possess the ability to hybridize with many other species of the tribe, and also with one other (Dewey 1984 and references therein; Assadi & Runemark 1995). Moreover, *E. intermedia* is able to cross with wheat, whereby it is often used in wheat improvement (Sharma *et al.* 1995; Friebe *et al.* 1996; Fedak & Han 2005). However, in spite of this, hybrids between wheat and *E. intermedia* have not been found in the field yet. In this respect, hybridization of weedy *E. repens* with the comparably rare *E. intermedia*, combined with abundant production of at least partially fertile hybrids, might have a considerable impact on risk assessment of genetically modified wheat. Therefore, knowledge of the frequency of hybridization between *E. repens* and *E. intermedia* in nature is of general interest, especially with respect to the worldwide importance of *E. repens* as a problematic weed.

***Elytrigia repens* (L.) Nevski** [syn.: *Agropyron repens* (L.) P. Beauv., *Elymus repens* (L.) Gould] is a native Eurasian species that has become established in most temperate zones of the world. It is one of the most noxious weeds on cultivated land worldwide (Palmer & Sagar

1963). In the Czech Republic, it is widespread throughout the territory and ranges from lowlands to the mountain belt. It occupies all man-made habitats and arable ground, and also occurs on such natural habitats as steppic grasslands and wet meadows (Chytrý & Tichý 2003; author's observations). *E. repens* is an allopolyploid species with a surprisingly intricate genomic history. The genome constitution of hexaploid cytotypes ( $2n=6x=42$ ) was determined as StStH (where St and H designate *Pseudoroegneria* (Nevski) Á. Löve and *Hordeum* L. genomes respectively) (Assadi & Runemark 1995); nevertheless, a more complex genome pattern seems plausible (Mason-Gamer 2004; for detailed discussion see below).

***Elytrigia intermedia* (Host) Nevski** [syn.: *Agropyron intermedium* (Host) P. Beauv., *Thinopyrum intermedium* (Host) Barkworth et D.R. Dewey] has more limited distribution. It occurs from France in the west to the Volga river region in the east, with further distribution forming a belt from Turkey and the Caucasus to Iran, Afghanistan, Pakistan, and the Pamir and Altai Mts. in Central Asia (Conert 1997). The species has also been introduced to North America, where it is grown as a forage grass. In the Czech Republic, its distribution strongly reflects the occurrence of steppic habitats. It colonizes dry and warm habitats like steppes and base-rich rocks and also pine forests on sandy ground, vineyards, orchards, and field margins in warm regions of the Czech Republic (Chytrý & Tichý 2003; author's observation). It is an allohexaploid with genome constitution determined as  $E^cE^cSt$  (Liu & Wang 1993) or  $E^cE^bSt$  (Chen *et al.* 1998), where  $E^c$  and  $E^b$  designate the closely related *Thinopyrum elongatum* (Host) D.R. Dewey and *Th. bessarabicum* (Savul. et Rayss) Á. Löve genomes. The St genome was donated by *Pseudoroegneria*. Recently, new insights into the genome composition of *E. intermedia* became available (Kishii *et al.* 2005), suggesting that not all potential genome donors have yet been identified.

In places where natural or semi-natural habitats with *E. intermedia* come in contact with agricultural land use, both species co-occur and may hybridize. The hybrid was originally described from the area of the present Czech Republic as *Agropyron*  $\times$ *mucronatum* Opiz (Berchtold & Opiz 1836) [= *Elytrigia mucronata* (Opiz) Prokudin]. The morphology of hybrids is intermediate between that of the parental species, but it sometimes overlaps with one or the other parent. The principal morphological characters that distinguish between the two species are as follows: (1) leaf sheath margins – hairy in *E. intermedia* vs. glabrous in *E. repens* (Kubát *et al.* 2002), and (2) glume shape – truncate or very shortly mucronate (never awn-tipped or gradually tapering) in *E. intermedia* vs. awn-tipped or gradually tapering (at least some of each inflorescence) in *E. repens* (Barkworth & Dewey 1985). Both species are morphologically variable (Mizianty & Szczepaniak 1997; Assadi 1998; Mizianty *et al.* 2001). Constancy of the morphological characters, their taxonomic significance, correlation with ecological preferences or with genetic variation, remain unexplored. Although there are references on putative natural hybrids in literature (Prokudin & Druleva 1971; Melderis 1980; Assadi & Runemark 1995), hybridization between *E. repens* and *E. intermedia* has attracted little attention and thus little proven evidence of either natural hybridization or of its frequency and ecological consequences is currently available.

## Species and hybrid identification

For the purpose of my thesis, it was necessary to develop reliable markers for species and hybrid identification. By flow cytometric analyses performed on samples from the Czech Republic (**paper #1**), we estimated absolute genome sizes for hexaploid *Elytrigia repens* and *E. intermedia*. The genome size difference between the species was estimated as 16% – sufficient to differentiate both species as well as hybrids. It turned out that the scarcity of reliable morphological characters that discriminate between the species sometimes caused species misidentification. In particular, this was due to large morphological variation of parental species and frequent overlapping of character values. Flow cytometric study showed that we had morphologically misidentified about 10% out of 238 samples. Most often, true hybrids were mistaken for *E. intermedia* in this respect. We thus established genome size measurement as a more reliable marker for species identification than morphology.

While genome size is most effective in detecting F<sub>1</sub> hybrids in my study species, we presumed that later-generation hybrids in cases of backcrossing (and consequent introgression) might be more problematic to detect, because the hybrids' genome size will approach that of one or the other parental species. For that reason, an additional marker, which would reflect recent hybridization events more sensitively, was desirable. For this purpose, we used ribosomal DNA (the ITS1-5.8S-ITS2 region) as a nuclear DNA marker (**paper #2**). The nuclear ribosomal internal transcribed spacer (ITS) has been widely used in many biosystematic studies, including hybridization (Álvarez & Wendel 2003). Since ITS is biparentally inherited, we expected to detect genetic evidence from both parents in hybrids. Indeed, all hexaploid hybrids determined by flow cytometry showed additivity of parental ITS copies (but see Discussion of the **paper #2**). The nuclear marker also revealed a hybrid origin of most nonaploid plants.

Chloroplast DNA (*trnT-F* region) markers helped us to determine the direction of hybridization in all cases (**paper #2**).

In conclusion, I can state that nuclear ribosomal and chloroplast DNA markers in addition to genome size measurements and chromosome numbers enabled us to determine hybrids with a high degree of certainty.

## Natural hybridization within the Triticeae as a source of genetic variation

One of the most important aspects that influence natural hybridization between related species is their genetic variation. Genetic variation of species has crucial influence on hybridization ability and vice versa. Genetic variation of species is determined by several key factors, such as species' age, distribution range, reproductive mode, etc. However, it is rather unknown for most of the wild Triticeae species. Chromosome numbers accompanied by genome constitution of particular species are often the only information concerning their genetic background. This variation is often underestimated, and such data should be always verified, at least within the territory where the respective studies are carried out. Global genetic variation of species is then resolved with an increasing number of focused studies. As described earlier, the tribe Triticeae displays extreme reticulation, which is, in part, a consequence of the ability of many Triticeae taxa to hybridize with other taxa. Many species thus display a surprisingly high genetic variation.



Mason-Gamer described in her excellent paper (Mason-Gamer 2004) reticulate evolution of allohexaploid *E. repens*. Her investigation revealed at least five distinct lineages in the genome of this species, suggesting that allopolyploidy and introgression took place during the evolution of *E. repens*. Chloroplast DNA data identified three potential candidates to be maternal genome donors—*Pseudoroegneria*, *Dasyphyrum* (Coss. et Durieu) T. Durand, and *Thinopyrum* Á. Löve—whilst nuclear DNA data confirmed the previously suggested *Pseudoroegneria* and *Hordeum* as genome contributors of hexaploid plants. Apart from the expected *Pseudoroegneria* and *Hordeum* nuclear genome donors, three additional genome donors were unexpectedly identified: *Taeniatherum* Nevski and two donors of unknown identity. One of them is placed within the Triticeae, although it remains unidentified. The second one falls outside the Triticeae and is close to the annual grass *Cutandia*. The author speculated that the genetic diversity was likely achieved through introgression rather than through multiple origins of the species. This study thus demonstrated the ability of *E. repens* to acquire genetic material through introgression from surprisingly divergent sources.

Another study of the same author (Mason-Gamer *et al.* 2005) showed that hexaploid *E. repens* is polyphyletic since it displayed different *Hordeum* nuclear gene variants within its genome. Whether this polyphyly originated from multiple origin of this species involving different *Hordeum* progenitors or from introgression is still unclear, although the authors inclined towards the latter.

More evidence of recurrent hybridizations within the Triticeae is provided by Liu *et al.* (2006), who studied phylogenetic relationships among Asiatic *Elymus* species based on ITS data. About 30 species with StY genome constitution are known from temperate Asia. While having the same genome constitution, the StY-genome species have been largely modified and display high genetic diversity. Authors suggested a multiple origin of some species, since different accessions of the same species appeared in different clades of a phylogenetic tree. In this case, multiple origins of some species caused by recurrent hybridization of parental species promoted rapid adaptation of *Elymus* species to different ecological conditions and resulted in the formation of many endemic genotypes and species (Liu *et al.* 2006).

During literature search I found that a high degree of crossability between wheat and *Elytrigia intermedia* exists and reaches (expressed as a quantity of viable seeds) up to 5.5 % of all pollinated florets (Sharma & Gill 1983a,b; Franke *et al.* 1992; Tyankova 2000). In addition, backcrosses were obtained. Chen *et al.* (2001) reported seed set after wheat-*Elytrigia intermedia* crosses, even up to 62.5 %. An important fact resulting from the crosses between wheat and *Elytrigia* is that crossability highly depends on the particular cultivars or strains of both wheat and *Elytrigia*. Sharma & Ohm (1990) found that the crossability of a wheat variety with *E. intermedia* differed even among individual *E. intermedia* plants used, probably due to the polymorphism of this species.

Therefore, I realize that the ability of particular species to hybridize with one another is strongly dependent on both large- and fine-scale genome constitution of particular taxa; this constitution largely differs among populations. It is apparent from the literature that many complex species from the tribe, such as *Elymus*, might have been affected by local hybridization events and that their accessions from distant parts of the world might have undergone quite different evolutionary histories.

A survey of published karyological data on *Elytrigia repens* and *E. intermedia* (**paper #1**) has revealed a considerable variation in ploidy levels of both species. Therefore, we at first

performed flow cytometric and karyological investigations on the samples from the Czech Republic. They showed that both species occur predominantly at the hexaploid level. Aside from hexaploids, several plants of a nonaploid cytotype ( $2n=9x=63$ ) were found, representing the first record from the field (**paper #1**).

Hybridization has also played an important role in natural populations studied by us (**paper #2**). The frequency of hexaploid hybrids between *E. repens* and *E. intermedia* differed among the localities, characterized by different degrees of anthropic disturbances. At sites of agricultural land-use, hybridization was common whereas no hybrid was found at the steppic locality, underlining the importance of different ecological conditions for hybrid formation or establishment. The direction of hybridization was highly asymmetric as cpDNA identified *E. intermedia* as the maternal parent in 61 out of 63 cases. Hybridization also played an important role in the formation of eight nonaploids, which likely arose by fusion of reduced and unreduced gametes of both species. One hybrid nonaploid was partially fertile and the variable chromosome numbers in its progeny suggested backcrossing of the nonaploid mother with hexaploid cytotypes and also fusion of two reduced gametes of nonaploids (either through self- or out-pollination).

Recently, we discovered a population of heptaploid ( $2n=7x=49$ ) plants intermixed with hexaploid *E. intermedia* at one locality (unpubl. observation). Besides heptaploids, several aneuploids ( $2n=47, 48, 50$ ) were present there, too. Apparently, the heptaploid cytotype was dominating at one part of the locality. Whether these plants originated through hybridization of different cytotypes or species is unclear yet and further investigation is needed. But it is clear that such cytotypes can be viable under natural conditions and some of them may persist and take part in further hybridizations.

In conclusion, we identified at least five (I do not take into account the progeny of one nonaploid since the plants were not found growing spontaneously in the field but were sown and cultivated in a greenhouse; for details see **paper #2**) to date unknown cytotypes among approximately 500 analysed plants from the *E. repens*–*E. intermedia* complex. This cytological variation is not assumed to be comprehensive; e.g., a tetraploid cytotype of *E. repens* has been described from Eastern Europe and Asia and it also occurs in neighbouring Poland (Mizianty *et al.* 2001). Hybridization between different species or cytotypes can apparently generate large variation of geno- and cytotypes that can serve as raw material for evolution. These data confirmed our presumption that natural populations comprise much more variation than is generally thought and that hybridization is an important force generating the variation. Also, they demonstrated the necessity to evaluate at least the cytological variability of investigated species within the respective area.

### **Ecological and evolutionary implications of hybridization between *E. repens* and *E. intermedia***

This issue is discussed in **paper #2**, hereafter I only briefly summarize it. Hybridization in plants is perceived as a creative force in their evolution (Arnold 1997). Together with introgression they may cause transfer of genetically encoded adaptations whereby genetic diversity and ecological amplitude of involved species may be increased (Stutz & Thomas 1964; Arnold & Bennett 1993; Kim & Rieseberg 1999). Namely *E. intermedia* is known to possess many valuable traits, such as biotic and abiotic resistances, wherefore it is often used in wheat improvement (Fedak 1999; Fedak & Han 2005). Although *E. repens* is rather

unexplored in this respect, its ecological amplitude is even wider than that of the former species. I assume that hybridization between both *Elytrigia* congeners may cause bidirectional transfer of adaptive traits between the species.

Nowadays, agricultural practice changes towards reduced tillage management (MacIlwain 2004). Such a practice reduces infiltration of water through the upper layer, especially of heavy soils. As a consequence, an increased frequency of local floods have already occurred. I measured the response of both species and their hybrid to flooding intensity (**paper #3**), estimated as biomass accumulation after the flooding period. *E. repens* was the most flooding-tolerant of the three taxa tested. *E. intermedia* was the least flooding-tolerant but still able to survive under flooded conditions for one month. This was a quite surprising finding for this xerophilous species. The response of *E. repens* × *E. intermedia* hybrids to flooding tended to be intermediate between that of the parents. This was likely caused by enhanced rhizome production inherited from the highly rhizomatous *E. repens*. Such an adaptation may gain high importance in environments changing towards more frequent local floods. Increased rhizome formation in hybrids may also enhance their weedy potential compared to *E. intermedia*; such hybrids could benefit from acquisition of *E. repens*-specific adaptations to the weedy, disturbed habitats. Vegetative propagation through rhizomes may also be important in cases of low fertility, such as in hybrids. Survival of plants at a locality for many years through vegetative propagation increases the chance of hybridization in the future because (1) multiplication of individuals increases the probabilities simply in a mathematical way; and (2) local ecological conditions change through time whereby the chance to meet a compatible sexual counterpart increases.

In my view, hybridization in general provides an effective way for species to enrich their gene pools. Mason-Gamer (2004) speculated that an obvious ability of *E. repens* to hybridize with other species and to acquire genetic material through introgression from divergent sources may be responsible for the success and rapid spread of this species throughout North America. In this thesis, I demonstrate that natural hybridization between *E. repens* and *E. intermedia* is common in the field, generates a wide array of cyto- and genotypes, and provides ample raw material for evolution and adaptation. In its consequences, such hybridization may broaden the ecological amplitude of both species.

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**Papers:**

**#1 Genome size discriminates between closely related taxa *Elytrigia repens* and *E. intermedia* (Poaceae: Triticeae) and their hybrid.**

Mahelka V., Suda J., Jarolímová V., Trávníček P., Krahulec F.  
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**#2 Recent natural hybridization between two allopolyploid wheatgrasses (*Elytrigia*, Poaceae): ecological and evolutionary implications.**

Mahelka V., Fehrer J., Krahulec F., Jarolímová V.

**#3 Response to flooding intensity in *Elytrigia repens*, *E. intermedia* (Poaceae: Triticeae) and their hybrid.**

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