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# *Constraints to Germplasm Evaluation*

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This paper discusses the procedural and genetic constraints to evaluating and using t wild relatives of wheat. The constraints include biosystematic and nomenclatu constraints, physical constraints arising from the difficulty of handling wild germpla in field experiments, genetic characters that hinder field or greenhouse evaluation, a characters that affect use in breeding programmes.

## BIOSYSTEMATIC CONSTRAINTS

One of the major problems confronting workers involved in the evaluation of w wheat germplasm is that authors of the classification systems used for wild mate do not agree on the biosystematic validity of the various taxa. We are referring h to: the validity of *Triticum urartu* Tum. as a species biosystematically separate fr the other wild diploid wheat, *T. monococcum* L. ssp. *boeoticum* Boiss.; the validity *Aegilops sharonensis* Eig as a species biosystematically separate from *Ae. longissi* Schweinf. and Muschli.; and the validity of *Ae. peregrina* (Hackel) Maire and Weil (syn. *Ae. variabilis* Eig) as a species biosystematically separate from *Ae. kotsc* Boiss.

These questions are important because *T. urartu* was recently proposed as the do of the A genome of durum and bread wheat on the grounds of similarity of restricti fragment length polymorphisms (RFLPs) of repeated nucleotide sequences (Dvo et al., 1988). *Ae. sharonensis* was proposed as the donor of the B genome of durum a bread wheat (Kushnir and Halloran, 1981), and its seed storage proteins are disti (Waines and Johnson, 1972). It is important, for the purposes of germplasm collecti preservation and evaluation, for us to be clear on the biosystematic speciation in t wheat group.

The confusion arises because a group of cytogeneticists presently working in Missouri and Palestine base species boundaries mainly on genome analysis — that is, on chromosome pairing at meiosis in $F_1$ hybrids (Kimber and Feldman, 1987; Kimber and Sears, 1987). If chromosomes pair in these intertaxa $F_1$ hybrids, then the two taxa have the same genome, and are considered the same species. This has the effect of lumping together species that are reproductively isolated. Genome analysis is not a very finely tuned experimental technique. There are even differences in the treatment of species between Kimber and Feldman (1987) and Kimber and Sears (1987), which are not explained and hence are confusing to the average breeder. On the other hand, a group of geneticists and biosystematists working mostly in California and France base species boundaries on either genetic or ecological reproductive isolation. If there are substantial barriers to gene exchange between two taxa in the field, irrespective of whether they share the same genome, then they are considered separate species (Johnson and Dhaliwal, 1976; Yaghoobi-Saray, 1979; Sharma and Waines, 1981; Waines et al., 1982; Lucas and Jahier, 1987; Waines and Payne, 1987; Dvorak et al., 1988; Smith-Huerta et al., 1989). If there is reproductive isolation between wild wheat taxa in natural populations, there is the possibility of genetic differentiation.

We believe this has happened sufficiently between *T. urartu* and *T. monococcum* ssp. *boeoticum*, and between *Ae. sharonensis* and *Ae. longissima*, for them all to be considered separate biosystematic species with different genes useful to the plant breeder. We are not sure about *Ae. peregrina* and *Ae. kotschyi*. There is a need for further field studies involving natural populations of these three pairs of species to clarify the situation. To use wild germplasm effectively, we must have a useful system of species classification.

## PROBLEMS WITH GENOME FORMULAE

Another problem in the wheat group arises from the fact that Kimber and Sears (1987) are still using an incorrect method of writing the genome formula. That the B genome donor contributed the cytoplasm of durum wheat, and was presumably the female parent, was demonstrated by Suemoto (1968, 1973). As plant breeders, we have to write the female parent first in cross notation. Hence we should write the genome formula of durum wheat as BBAA, and that of bread wheat as BBAADD. This is important when amphiploids are made of *Ae. speltoides* or *Ae. searsii* with *T. urartu*. If the *Aegilops* species is the female parent, then the genome formula is SSAA, and we shall attempt to equate SSAA with BBAA, not with AABB.

## NOMENCLATURAL PROBLEMS

The nomenclatural problem in wild wheats and their relatives derives from the group being placed historically in two genera, *Triticum* L. and *Aegilops* L., by morphological



taxonomists. This system was followed by Hammer (1980), in the Flora of Turkey (Davis, 1985), and in other descriptions of regional floras where *Triticum* and *Aegilops* grow wild. The impetus to merge the two genera derives mostly from Morris and Sears (1967), who followed Bowden (1959) and the International Code of Nomenclature for Cultivated Plants (Fletcher et al., 1958).

The basic premise of these authors is that the B genome (maternal genome) of tetraploid wheat is from an *Aegilops* species and, hence, following the code, *Aegilops* must be merged with *Triticum*. However, geneticists and cytogeneticists do not yet agree which *Aegilops* species is the donor of the B genome of the BBAA tetraploid wheats (we have followed plant breeding practice and listed the maternal genome first). Perhaps we should wait until the source of the B genome is agreed upon before we change the names of genera. If the International Code of Botanical Nomenclature (Lanjouw et al., 1956) is followed, we may have to merge the genus *Dasypyrum* (*Haynaldia*) with *Triticum*, because *D. villosum* may form spontaneous amphiploids with durum and bread wheat in Sardinia and southern Italy, and a supposed amphiploid has been called *X Haynaldo-triticum sardoum* (Meletti et al., 1977). This claim of spontaneous amphiploid formation needs to be verified. Will we also have to merge *Secale* with *Triticum*?

These biosystematic and nomenclatural problems are a great constraint to germplasm evaluation. A workshop is needed to consider these problems in our major cereal group. This workshop should be held in southern Europe or West Asia, so that the majority of the people who work with *Triticum* and *Aegilops* can attend.

## PHYSICAL CONSTRAINTS

Physical constraints include the problems encountered in sowing, growing and harvesting wild wheats in field or glasshouse trials, and then in threshing the kernels.

### Sowing

The dissemination unit in wild *Triticum* species is a spikelet containing one or two kernels surrounded by pales and glumes, one or more of which are often awned. In *Aegilops* species, the inflorescence spike sometimes breaks up into individual spikelets, or more often it stays intact and separates from the culm at an abscission zone at the base of the spike. In *Ae. speltoides* Tauch, the population is dimorphic for the inflorescence spike: var. *speltoides* (syn. *aucheri*) has an inflorescence axis which persists through grain maturity and the lateral spikelets lack awns; var. *ligustica* has an inflorescence axis which breaks up when the grains are mature into individual spikelets, and the lateral spikelets have lemmas with awns. In *Aegilops*, the dissemination unit is either the entire spike, made up of several spikelets, or individual spikelets, as in wild *Triticum*.

The awns can be removed from a spikelet either by hand, or in a threshing box, or with a machine that will beat them off without damaging the grains. When the awns are removed, it is not difficult to sow spikelets by hand in rows in a field. It is difficult, however, to sow complete spikes by hand. In species with intact inflorescences, the awns are often more difficult to remove, and the inflorescence has to be broken by hand into individual spikelets, or the kernels threshed out by hand. To avoid threshing, we prefer to sow spikelets. Moreover, threshing may damage thin kernels.

## Emergence

Many of the diploid *Aegilops* and *Triticum* species have slender coleoptiles which, at Riverside, California (the site of our study), have difficulty breaking though the surface soils. Our soils are formed from decomposed granite and have very little humus. The surface sets like concrete after wetting. To overcome seedling emergence and obtain an even stand of wild diploid wheats, we usually sow spikelets in a University of California soil mix in flats and then transplant 1- to 2-month-old seedlings to plots in the field. Sowing spikelets in flats in the cold glasshouse also prevents bird damage to germinating seedlings. Migrating birds in autumn and winter can decimate small plots of wild wheats. Domesticated diploid and polyploid wheats have more robust coleoptiles and seedlings have less difficulty emerging in our soils. However, they are just as susceptible to bird damage as wild wheats at this stage.

## Population biology

Since most wild wheat and *Aegilops* spikelets contain two kernels, one somewhat larger than the other, sowing 100 spikelets in a plot will result in up to 200 seedlings. We sow 100 spikelets to avoid genetic drift. If the parental species tends to outcross, the two seedlings that develop from a spikelet can be half-sibs, or they can be full-sibs if the parent tends to self. In a species with many spikelets in a spike, the resulting seedlings can all have the same female parent, but they can have different male parents and show multiple paternity.

It is important to remember that many wild wheat and *Aegilops* accessions can and do outcross more than domesticated wheats. As pollen is wind blown, the extent of outcrossing will depend greatly on climatic factors. In cool, humid conditions, we would expect more outcrossing than in hot dry conditions. Estimates of outcrossing in wild diploid *Triticum* and *Aegilops* species can be found in Yaghoobi-Saray (1979) and Smith-Huerta et al. (1989). Only if grains are threshed out of spikelets and sown separately will individual plants be of a single genotype. If a two-grained spikelet is sown, the resultant plant-clump can contain two genotypes. Further study is needed of the reproductive and population biology of diploid wheat and *Aegilops* species in West Asia.



## Germplasm increase

For the past 3 years, with assistance from the International Board for Plant Genetic Resources (IBPGR), we have increased the wild wheat collection at the University of California (Riverside) and duplicated it at the United States Department of Agriculture (USDA) Small Grains Collection, which is now at Aberdeen, Idaho, and at the Kyoto Germplasm Collection in Japan.

We increased germplasm in the field in the following way. Four rows each on raised beds 76 cm apart were sown with spikelets by hand, or germinated seedlings were transplanted into the rows. The row length was 5 m. Each 15 $m^2$ plot contained 12 spikelets (thus, potentially, over 200 genotypes) and was surrounded by a 1 m-wide border (two rows) of domesticated oats. Irrigation was by sprinkler or furrow, in addition to natural winter rainfall. Weed control was mechanical between the beds and by hand hoe between plants within the row. Broad-leaved weeds were also sprayed with a selective herbicide. Such a patchwork of wheats and oats allows each accession to develop independently of others. Because we experience damage by jack rabbits and other herbivores, the whole germplasm increase area is surrounded by wire netting.

## Harvesting

If there are not too many plots, when wild wheats are mature, spikelets can be harvested by hand each morning when dew is still present. If there are many plots, spikelets can be allowed to drop to the soil surface, which has been kept weed free; they can be raked up, or picked up by hand or by a vacuum machine. Later, soil and leaf material will be separated from the spikelets. It is possible to obtain replicated plots and components of yield data of wild *Triticum* and *Aegilops* accessions in the field, with the help of patient graduate students and field workers. In this way, 70 accessions of *Aegilops* species with three replications were harvested in summer 1988. We are now analyzing the material for yield components, grain protein and lysine content.

Other techniques used in harvesting fragile, rachised wheats include taping the awns of the spike with scotch tape in two places, near the spike base and at the spike apex. Alternatively, it is possible to place all the effective spikes on a plant in a plastic bag which has holes cut into it with a paper punch. Cheesecloth bags or bags made of a fine net can also be made and used to enclose the spikes. However, all these methods are time-consuming and can be applied only to plants in a small number of plots. Furthermore, under our conditions, plastic bags tend to collect condensed moisture with the result that fungal saprophytes may grow on the glumes. For ease of handling we prefer to let the spikelets drop naturally and to rake them up from the soil surface. In this way, we have increased the seed of more than 500 accessions in a given year. Spikelets are put into large brown paper bags and taken to the field house for cleaning, packaging and shipping. If the *Aegilops* species under increase has a spike that falls entire, without breaking into individual spikelets, then it is easy to pick up spikes from

the soil surface. The border of oats that surrounds each plot prevents spike movement between plots, but it does not prevent cross-pollination.

### Location of increase

Wild wheat accessions grow better at a coastal field station, such as Irvine, California (elevation 123 m) or a mountain station such as Idyllwild (elevation 1615 m) than at intermediate elevations such as Riverside (297 m) or Moreno (463 m) (Guzy et al., 1989). This is because Riverside and Moreno have a shorter growing season and the temperature becomes too hot too quickly. High temperatures at flowering tend to kill the pollen of wild *Triticum* species, but not necessarily of *Ae. longissima* or *Ae. searsii* (Ehdaie and Waines, 1989). A small project that investigated phenotypic plasticity (Bradshaw, 1965) in diploid and polyploid wild wheats grown at these four sites in southern California indicated that diploid wheats have greater plasticities for yield components than other taxa (Guzy et al., 1989). *T. monococcum* exhibited higher spikelet numbers relative to wild tetraploids, although seed size was reduced.

### Threshing

The tough glumes of wild wheats protect grains from birds, but they can be a problem at threshing time. Many wild wheats and primitive domesticates, such as *T. dicoccum* and *T. monococcum*, can be threshed in a threshing box lined with ribbed rubber. A wooden brick is used to rub the glumes against the rubber, releasing the grains. However, this can be time-consuming. It may be better to weigh a sample of spikelets, take a subsample, weigh it, and then thresh the subsample by hand with a pair of forceps. The weight of threshed grains can be determined and hence the weight of grains in the original sample.

## GENETIC CONSTRAINTS

### Evaluation

Only small samples of grain are necessary for micro protein and lysine determination. Large quantities of threshed grain (up to 200 g) are necessary for micro loaf-baking tests and cookie diameter tests. Fortunately, many electrophoretic and seed protein techniques can be performed on single grains of wild wheats or on single leaves (Waines and Payne, 1987; Smith-Huerta et al., 1989), and the genetic control of protein bands studied. The flag leaves of some wild *Triticum* and *Aegilops* species are small and thin, and this may hinder water retention experiments. Austin et al. (1986) reported that a *T. urartu* accession with a high photosynthetic rate had wide flag leaves, which is encouraging.



### Using the evaluated germplasm

Most crosses of wild *Triticum* and *Aegilops* species as males with tetraploid and hexaploid wheat cultivars as females are successful if sufficient care is paid to setting up the cross. The male parent can affect the development of hybrid seed (Gill and Waines, 1978), and this is thought to be under simple genetic control. The genes that control intergeneric hybrid formation in the wheat group are known, and are thought to be similar to the rye-wheat crossability system (Thomas et al., 1981). We have found that some accessions of *T. urartu* give semi-lethal or lethal seedlings with other species of *Triticum* and *Aegilops*. There are accessions that hybridize more easily. We have not investigated the genetics of this characteristic.

Accessions of diploid species with desirable characteristics should be crossed with tetraploid and hexaploid wheats to incorporate the character into material that can be crossed with modern cultivars. Roberts et al. (1982) reported that a collection of *Ae. squarrosa* L. from Afghanistan was resistant to the root knot nematodes *Meloidogyne incognita* and *M. javanica*. The resistance is dominant and is inherited as a single Mendelian trait (Kaloshian, 1988). The resistance is expressed in an amphiploid, Prosquare, formed from *T. turgidum* var. *durum* Produra and *Ae. squarrosa* G3489 (Kaloshian et al., 1989a). This amphiploid is also resistant, in glasshouse tests, to the Columbia Basin root nematode, *M. chitwoodi* (Kaloshian et al., 1989b). Lacking evidence to the contrary, the simplest explanation is that a single locus controls resistance to these three *Meloidogyne* species. Further tests may indicate that more than one locus is involved. The 'gene' conferring resistance to *M. javanica* is located on chromosome 6D of *Ae. squarrosa* (Kaloshian, 1988). There is reason to suspect that Produra may confer hybrid necrosis genes to the amphiploid that will be expressed in crosses with other commercial wheats. To overcome this possible problem, we are attempting to cross the durum wheat Cocorit with *Ae. squarrosa* G3489. Field tests are being conducted this summer in southern and northern California to see if the amphiploid showing resistance to *M. javanica* and other root knot nematodes will express the resistance under field conditions. If so, the resistance in the Prosquare-based and Cocorit-based amphiploids will be transferred to commercial wheats.

A more direct way to transfer a desirable trait in *Ae. squarrosa* into bread wheat would be to cross it directly to the bread wheat cultivar and backcross the tetraploid hybrid to the bread wheat cultivar. This technique was successful when bread wheat was the female parent (Merkle and Starks, 1985) and when *Ae. squarrosa* was the female parent (Gill and Raupp, 1987). *T. monococcum* ssp. *boeoticum* was successfully crossed with bread wheat (The and Baker, 1975), as was *Ae. speltoides* (Dvorak, 1977), and characters were transferred. To what extent this is possible with other *Aegilops* and related species needs to be investigated.

If *T. urartu* is the A genome donor of commercial tetraploid and hexaploid cultivars, as the work of Dvorak et al. (1988) suggests, this will allow further possibilities for the use of wild germplasm. *T. urartu* contains accessions with high concentrations of protein and lysine (Waines et al., 1987). The protein concentration of six accessions

ranged from 22.5 to 27.8 per cent; lysine content ranged from 4.49 to 6.50 µg/mg. The adjusted lysine/protein ratio of one accession, G1754, was 27.05 µg/mg. We would like to investigate the possibility of improving the nutritional quality of bread wheat by substituting *urartu* chromosomes for their homoeologous chromosomes in the A genome of a commercial bread wheat. We are attempting to cross the Glenson monosomic series to *urartu* G1754 to see if this line of research is feasible. The Glenson monosomic series was developed by the Institute for Plant Science Research, Cambridge, UK on behalf of the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT).

## CONCLUSION

Although working with wild *Triticum* and *Aegilops* species is not as easy as working with domesticated wheats, it can be just as rewarding. It is worth bearing in mind that, some 10 000 years ago, hunter gatherers and early farmers in West Asia harvested and evaluated wild wheats. If they could do it then, we should certainly be able to harvest, evaluate and use the same species today!

## *Discussion*

L. TANASCH: Genome analysis has shown that the donor of B genome in bread wheat is not yet certain. Some researchers think that *T. urartu* may be the donor. Is there some work done using the RFLP technique to solve this problem?

J. G. WAINES: B. L. Johnson said *T. urartu* was the B genome donor in bread wheat. But this was disproved by Miller and Chapman in Cambridge and by Dvorak who was in Canada at that time, with chromosome pairing studies using the telocentrics of Chinese Spring. Johnson assumed that *T. monococcum* was the A genome donor, as did everyone else. Work by Professor Konarev in Leningrad suggested that on immunochemical grounds *T. urartu* was a parent of *T. turgidum*. However, recent work conducted by Dvorak and others (1988) suggests that *urartu* donated the A genome on the basis of RFLP analysis. The RFLP pattern of *T. turgidum* is identical to that of *urartu*, but differs from that of *monococcum*.

S. JANA: What is the basis for selecting the figure '120' as a population size that would prevent genetic drift in wild accessions in rejuvenation nurseries?

J. G. WAINES: It is based on textbooks of population genetics and recommendations of the International Board for Plant Genetic Resources (IBPGR).

S. JANA: Why do wild wheats maintain seed viability longer in storage if preserved as spikes rather than as cleaned seeds?



J. G. WAINES: Threshing wild wheats and *Aegilops* species which have hard glumes requir a great deal of pressure. This is usually applied from a block of wood or brick. The blo can damage either the embryo or the endosperm of the kernels, resulting in loss of viabilit

M. VAN SLAGEREN: Do *Aegilops speltoides* variants grow in different habitats? In western Sy I have observed them growing together but they are easily distinguished, and there are ve few intermediate forms present.

J. G. WAINES: Yes, they do grow together, but the *ligustica* type variants are more frequent high elevation and less so at sea level, at least in Turkey. We really know very little abo wild wheats and their ecological distribution.

M. VAN SLAGEREN: Why is there no distinction between *boeoticum* and *urartu* in the Flora West Asia?

J. G. WAINES: In the Flora of Turkey, *Triticum* was not treated by a wheat specialist a omissions were caused by lack of herbarium specimens and poor literature surveys Ankara and Edinburgh where the Flora was prepared. Another reason may be that althou *urartu* and *boeoticum* tend to grow together in mixed populations, the former tends flower and mature a little earlier. So if one arrives at a collection site slightly later, then on *boeoticum* gets sampled, the *urartu* having shattered. But once you get to know bo materials, the differences are obvious. The seed colour in *boeoticum* is blue or yello whereas in *urartu* it is red; this could be the origin of the red grain colour in tetraplo wheats. The shape of the grain is also quite distinct. Quite often, there is a third awn in t spikelet, so there are a series of characters which, when you put them together, c distinguish the two species. Crosses can be made only one way, invariably with *boeotic* as a female parent. If crossed the other way around, then you do not get a hybrid embry If *urartu* is crossed with *monococcum*, with the former as female parent, then again t hybrid is sterile and this sterility continues in backcrosses 1 and 2. So they are reprodu tively isolated species, although they grow together. The populations of wild diplo wheats in Turkey are composed of 90 per cent *boeoticum* and 10 per cent *urartu*. There some mechanism that is keeping them apart.