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# Endosperm Balance Number and the polar-nuclei activation hypotheses for endosperm development in interspecific crosses of *Solanaceae* and *Gramineae*, respectively

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**Abstract** The Endosperm Balance Number (EBN) and the polar-nuclei activation (PNA) hypotheses have been developed to interpret, explain and predict interspecific and interploidy crossabilities in the Solanums and the Gramineae, respectively. Although these two hypotheses evolved independently, they share a number of common features. Assignment of EBNs and 'activation/response values' (AVs/RVs) depend on plumpness, size, and germinability of hybrid seeds. Also, both hypotheses emphasize the importance of a balanced parental genic contribution for the normal development of endosperm. However, in the EBN hypothesis a 2 maternal:1 paternal EBN ratio is a prerequisite for successful interspecific crossability, while the PNA hypothesis is based on the stimulative strength of the male nuclei to initiate mitotic divisions in the primary endosperm nucleus and is idependent on a 2:1 ratio between the RV of the polar nuclei and the AV of the male gamete. Differences and similarities betweeen the EBN and PNA hypotheses are summarized and contrasted. It is proposed that EBN and PNA be considered as the same concept.

**Key words** Endosperm development · Evolution · Imprinting · Incompatibility · Reciprocal crosses

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## Introduction

In many interspecific crosses, normal development and viability of hybrid seed can be inhibited by either pre- or post-fertilization barriers. The former include any incompatibility or incongruity mechanisms that prevent fertilization at any stage, from deposition of pollen grains on stigmas to the union of sperm nuclei with the egg cell and the polar nuclei cells. These phenomena and their genetic control systems have been described by de Nettancourt (1984). Post-fertilization barriers include any blocking or breakdown events following double fertilization.

Brink and Cooper (1947) established the concept that the primary cause of seed failure after double fertilization in interspecific crosses was due to endosperm breakdown. For normal endosperm development, Müntzing (1930) proposed that a 2 maternal tissue:3 endosperm:2 embryo ploidy ratio was necessary, while Watkins (1932) suggested that a 3 endosperm:2 embryo ploidy ratio was critical, and Valentine (1954) presented evidence that a 2 maternal tissue:3 endosperm ratio was most important. However, von Wagenheim (1957) indicated that endosperm function relied on its own constitution, independent of maternal tissue and/or embryo ploidy ratios. Nishiyama and Inomata (1966), based on cross-incompatibility studies in Brassicas and on earlier observations by Kihara and Nishiyama (1932), suggested that the sperm nuclei possess a "stimulative strength" to activate and initiate mitosis of the fertilized egg and polar nuclei. For normal development of endosperm, a 2 maternal:1 paternal genomic ratio was found to be necessary, otherwise the activation balance was disturbed, leading to embryo abortion.

Lin (1975, 1984), by utilizing inbred diploid maize lines carrying the indeterminate gametophyte (*ig*/*ig*) gene (Kermicle 1971), was able to demonstrate the importance of a 2 maternal:1 paternal genomic ratio. The presence of the indeterminate gametophyte gene can result in multiple eggs or the production of extra polar nuclei in the central cell. Lin (1975, 1984) reported that in (2*x*) *ig*/*ig* × (4*x*) *Ig*/*Ig*/*Ig*/*Ig* crosses the frequency of triploid hybrids was 12%, in comparison to crosses using *Ig*/*Ig* females, where the frequency of triploid hybrid development was near zero. The triploid hybrid frequency increase was found to be due to normal endosperm development, which consisted of four maternal and two paternal genomes (2:1 ratio preserved). The extra two polar nuclei in the female gametophyte were formed due to the presence of the indeterminate gametophyte gene. In the same study, Lin was able to identify that three or possibly four genes, termed "endosperm factors", in the long arm of chromosome 10 controlled endosperm development. Furthermore, Lin, by utilizing a 10-B translocation, obtained, in a single cross, two endosperms with the same chromosome make-up but differing in parentage for the long arm of chromosome 10 (10L). When all four 10Ls of the endosperm were contributed by the female parent, the endosperm was subnormal. However, normal endosperm was obtained when two out of the four 10Ls were contributed by the male parent. Lin concluded that a maternal effect was unlikely to explain the differences in endosperm development within a single ear and that the paternal source of 10L was responsible for the difference in endosperm growth.

Although interspecific hybrid embryos can be rescued from crosses showing postzygotic endosperm failure, the importance of understanding the genetic control of endosperm balance for normal development and support of embryo growth is apparent. Embryo rescue has been extensively used and described for immature embryos derived from such interspecific crosses that normally do not survive because of endosperm failure (Singsit and Hanneman 1991; Steward 1981; Watanabe et al. 1992; Williams et al. 1987). Two genetic systems to predict the outcome of interspecific crosses have been proposed. The Endosperm Balance Number (EBN) hypothesis was developed for *Solanum* (Johnson et al. 1980) and extended to *Lycopersicon* (Ehlenfeldt and Hanneman 1992) and *Impatiens* (Arisumi 1982), and is suggested to be present in alfalfa, beans, blueberries, rice, soybeans, forage legumes, grasses, ornamentals, and *Datura* (Ortiz and Ehlenfeldt 1992). The polar-nuclei activation (PNA) hypothesis was proposed for *Avena* species (Nishiyama and Yabuno 1978) and the Triticinae (Nishiyama 1983). The objective of this paper is to summarize, explain, compare, and contrast these two hypotheses.

## Endosperm Balance Number Hypothesis

Research directed at studying the triploid block (Johnston 1980) and the results from Lin's research work (1975) inspired potato researchers at the University of Wisconsin-Madison to develop the Endosperm Balance Factor (EBF) hypothesis for successful interspecific potato hybridization (den Nijs and Peloquin 1977; Johnston and Hanneman 1978). The EBF hypothesis was developed by Johnston and amplified by den Nijs and Peloquin (Johnston et al. 1980). According to crossing behaviour, each set of chromosomes had 1EBF; thus, diploids had 2EBFs and tetraploids had 4EBFs. For normal development a 2 maternal: 1paternal EBF ratio was a necessity in the endosperm. However, Mexican tetraploids and *Solanum acaule* (2n = 4*x* = 48) were assigned an EBF of 2 due to unusual crossing results.

The EBF evolved into the EBN hypothesis (Johnston et al. 1980). It soon became apparent that the EBN of a species did not correlate with its ploidy. Thus, initially *Solanum chacoense* (2*x*) was chosen as a crossing standard with an EBN of 2 (Johnston and Hanneman 1980). The rest of the *Solanum* species were assigned EBNs depending on their crossability results with 2*x*(2EBN)*S. chacoense*, colchicine-doubled 4*x*(4EBN) *S. chacoense* and with each other. An incomplete diallel crossing block was initiated by Johnston and Hanneman (1980) to identify EBNs for a total of 29 diploid, triploid, tetraploid, pentaploid and hexaploid potato lines. A cross was considered successful if "it averaged ten or more plump seeds per fruit and the chromosome count and/or morphology of the off-spring indicated hybridity". A cross was considered a failure if "the fruit had aborted seeds, or in crosses where no fruit was set, the styles showed many pollen tubes had reached the ovary". Hybrid ploidy was verified by chromosome counts. Every species that successfully crossed with diploid *S. chacoense* was assigned an EBN of 2. An EBN of 4 was assigned to species successfully crossed with colchicine-derived tetraploid *S. chacoense*. As a result, a number of diploid and some tetraploid clones were assigned an EBN of 2, while other tetraploid, all pentaploid, and all hexaploid clones were designated as 4EBN. A rare triploid clone derived from a tetraploid × diploid *S. chacoense* cross had an EBN of 3, the average of its parental EBNs. Assuming that the factors (genes) determining EBN were on one or only a few chromosomes, it was expected that the triploid would cross with both of its parents, due to random segregation of chromosomes during meiosis, which it did. In another study, several diploid 1EBN species were identified (Johnston and Hanneman 1982). The consistency of the EBN hypothesis was demonstrated by the assignment of a single EBN per species. The EBN hypothesis also serves as a selective screen for functional 2*n* gametes in potato (Johnston et al. 1980), since viable hybrids from 4*x* (4EBN) × 2*x* (2EBN) can be obtained only if the diploid produces 2*n* gametes; thus, preserving the 2:1 EBN ratio in the hybrid endosperm.

Combining knowledge on interspecific corssabilities gained through the EBN hypothesis and by utilizing 2*n* gametes, Ehlenfeldt and Hanneman (1984) were able to transfer and incorporate exotic germplasm into the cultivated potato. After the chromosome number of the non-tuber-bearing 2*x* (1EBN) species *Solanum brevidens* was doubled (Johnston and Hanneman 1982), the resulting 2EBN tetraploid was crossed with 2*x* (2EBN) *S. chacoense*. The 3*x* (2EBN) hybrid obtained was pollinated by the 4*x* (4EBN) breeding line 'Wis AG 231', and a single seed was set. The seed germinated giving rise to a 5*x* (4EBN) hybrid, derived from the fertilization of a 2*n* egg. Finally, the pentaploid was crossed with a number of 4*x* (4EBN) cultivars, and germinable hybrid seeds were obtained. The entire process was based on manipulating species EBNs and 2*n* gametes in order to preserve the 2 maternal: 1 paternal EBN ratio.

**Table 1** Endosperm Balance Numbers for potato (*Solanum*) species (from Hanneman 1994; Hawkes and Jackson 1992)

| Ploidy | EBN | Species |
|---|---|---|
| 2*x* | 1 | *S. brachistotrichum, S. brevidens, S. bulbocastanum, S. capsicibaccatum, S. cardiophyllum, S. chancayense, S. circacifolium, S. commersonii, S. etuberosum, S. fernandezianum, S. jamesii, S. lignicaule, S. mochiquense, S. pinnatisectum, S. stenophyllidium, S. trifidum* |
| 2*x* | 2 | *S. abancayense, S. acroglossum, S. amabile, S. ambosinum, S. berthaultii, S. brevicaule, S. boliviense, S. bukasovii, S. canasense, S. chacoense, S. chomatophilum, S.* × *doddsii, S. gandarillasii, S. gourlayi, S. huancabambense, S. infundibuliforme, S. kurtzianum, S. laxissimum, S. leptophyes, S. marinasense, S. medians, S. megistacrolobum, S. microdontum, S. multidissectum, S. multiinterruptum, S. pampasense, S. pascoense, S. paucissectum, S. raphanifolium, S. sanctae-rosae, S. sogarandinum, S. sparsipilum, S. spegazzinii, S. tarijense, S. tuberosum* (Groups Phureja and Stenotomum), *S. venturii, S. vernei, S. verrucosum, S. violaceimarmoratum, S. weberbaueri* |
| 4*x* | 2 | *S. acaule, S. agrimonifolium, S. colombianum, S. fendleri, S. hjertingii, S. oxycarpum, S. papita, S. paucijugum, S. polytrichon, S. stoloniferum, S. tuquerrense* |
| 4*x* | 4 | *S. curtilobum, S. gourlayi, S.* × *sucrense, S. tuberosum* (Groups Andigena and Tuberosum) |
| 6*x* | 4 | *S. albicans, S. brachycarpum, S. demissum, S. guerreroense, S. hougasii, S. iopetalum, S. moscopanum, S. oplocense* |

The genetic control of EBN was described by Ehlenfeldt and Hanneman (1988) by using exceptional 2*x* (1 1/2 EBN) hybrids from a 2*x* (1EBN) *Solanum commersonii* × 2*x* (2EBN) *S. chacoense* cross. A complete diallel, including the exceptional hybrids and their parents, was set up to observe viable to aborted seed ratios, which were then compared to predicted values. The data were best explained by a three unlinked loci model operating in a threshold-like system. It was also observed that an extra EBN maternal dosage produced viable seeds of reduced size, while any extra paternal dose resulted in seed abortion. Thus, although maternal and paternal EBN genes appear to be identical, their behaviour is different and a specific balance is required for normal seed production.

Endosperm Balance Number has also been used for evolutionary studies in potato (Hawkes and Jackson 1992). A good correlation between EBN and the postulated evolution and present taxonomy of potatoes was reported, although EBNs have been determined for only about 50% of the described tuber-bearing Solanums (Table 1) (Hanneman 1983; 1994; Hawkes and Jackson 1992). Mexican diploid species, which are considered the most primitive of the wild potato species, all have an EBN of 1, except 2*x* (2EBN) *Solanum verrucosum*. Their EBNs are considered to be under the same genetic control as those of the South American 2*x* (1EBN) species *S. commersonii* (Bamberg and Hanneman 1990). It is postulated that the 2EBN condition arose in South America as an isolating mechanism, during the evolution of the Series Yungasensa species from *S. commersonii* (Hawkes and Jackson 1992). The EBN hypothesis has also been extended to *Lycopersicon* species (Ehlenfeldt and Hanneman 1992), where the EBN values in the "peruvianum group" may be approximately double that of the "esculentum group".

## Polar-Nuclei Activation Hypothesis

A number of authors have tried to interpret and explain the results of interspecific and interploidy crosses in the Gramineae (Thompson 1930; Kihara and Nishiyama 1932; Wakakuwa 1934; Boyes and Thompson 1937). The general observation was that germinable seeds were recovered from interploidy crosses only when the species with the higher chromosome number was used as the female parent. In the reciprocal crosses, although more seeds were produced, the seeds were shriveled and failed to germinate. Kihara and Nishiyama (1932) intercrossed diploid, tetraploid, and hexaploid *Avena* species to study their crossabilities, the development of the embryos, and the chromosome pairing in the hybrids. From 143 diploid × tetraploid crossing attempts, 90 seeds were obtained, but none germinated. However, from 439 tetraploid × diploid pollinations, 136 seeds were obtained with 83% germinability. Similar results were recorded in diploid × hexaploid crosses and their reciprocals, and by Wakakuwa (1934) for *Triticum* reciprocal crosses.

Based on morphological and anatomical observations, Kihara and Nishiyama (1932) indicated that the differences in hybrid seed development were not associated with the chromosome number of the male nuclei and the egg or polar nuclei, but rather with an "activating stimulus" carried by the male nuclei that has the ability to induce nuclear divisions in the egg (to form the embryo) and in the polar nuclei (to develop the endosperm).

In most angiosperms, three major events lead to the development of seeds: (a) double fertilization, (b) initiation of mitotic divisions in the zygote and the primary endosperm nucleus, and (c) differentiation of the embryo and endosperm. Initiation of mitotic activity is presumably triggered by the interactions of the male nuclei with the egg and the polar nuclei (Nishiyama and Yabuno 1978, 1979). In the absence of the male nuclei, the ovule remains in a suspended developmental state. In intraspecific pollinations, the egg and the polar nuclei harmoniously interact with the male nuclei, leading to the normal development of seed. In interspecific crosses, this balanced interaction can be distorted due to the hyper- or hypo-activating action of the male nuclei, leading to disturbances in the physiology of embryogenesis. Thus, depending on the interaction strength between the polar nuclei and the male nucleus, interspecific crosses can result in: (a) overgrowth of the endosperm, when the stimulative strength of the male nucleus is too strong, (b) normal endosperm development (balanced interaction), of (c) slow and poor endosperm development due to the weak stimulative action of the male nucleus (Kihara and Nishiyama 1932). Since mitotic activity first starts in the primary endosperm nucleus, followed by the zygote, an early dependency of the embryo on endosperm development can be assumed (Lopes and Larkins 1993). Rapid endosperm development is believed to be the result of "epihybridity", since the interaction between the sperm nucleus and the polar nuclei involve epistatic combinations of epigenetic variation (Kermicle and Alleman 1990).

For 34 years the idea of the activating stimulus presented by Kihara and Nishiyama (1932) remained unexplored, until another study on cross-incompatibility between $2x$ and $4x$ Brassicas was conducted by Nishiyama and Inomata (1966). Hybrid seeds from crosses between autotetraploid *Brassica pekinensis* and diploid *Brassica chinensis*, sharing the same genome, were obtained only when the autotetraploid was used as female. Embryological events were similar to those described for *Avena*. Finally, the hypothesis of polar-nuclei activation (PNA) was proposed by Nishiyama and Yabuno (1978) to explain the diverse results of *Avena* interspecific crosses. The degree of the egg or polar nuclei activation is expressed by the 'activation index' (AI), which is the ratio of the 'activating value' (AV) of the male nucleus to the 'response value' (RV) of the egg or the two polar nuclei. In a self-pollinated plant AV = RV, and the

$$\text{AI of the egg} = (\text{AV}/\text{RV}) \times 100 = 100\%, \text{ and the}$$

$$\text{AI of the polar nuclei} = (\text{AV}/2\text{RV}) \times 100 = 50\%$$

The AVs are probably controlled by functional gene(s) called "activator(s)" (Nishiyama and Yabuno 1978, 1979).

Nishiyama and Yabuno (1978), using a diallel cross involving nine *Avena* species, classified the hybrid seeds according to germinability and kernel size into four classes: (a) small inviable kernels (Rd-), (b) small viable kernels (Rd+), (c) normal viable kernels (D+), and (d) shrivelled-empty inviable kernels (E-). They also arbitrarily assigned *Avena strigosa* as a standard, with an AV = 1.0. Based on hybrid seed development and viability, AVs were assigned to eight other species (Table 2). The relationship between the kernel type and the AI of the polar nuclei was defined as follows: (a) $\text{AI} < 20\%$ – kernel type Rd-, (b) $20\% < \text{AI} < 30\%$ – kernel type Rd+, (c) $30\% < \text{AI} < 80\%$ – kernel type D+, and (d) $80\% < \text{AI}$ – kernel type E- (Table 3). Thus, germinable kernels are obtained when the AI is between 20% and 80% although kernels are small when the AI is between 20% and 30%.

In an effort to alter crossabilities between *Avena* species, Nishiyama and Yabuno (1983) doubled the

**Table 2** Activating/Response Values (AV/RV) for *Avena* species

| Ploidy | Species | Genome | AV/RV | References |
|---|---|---|---|---|
| $2x$ | *A. strigosa* | $A_sA_s$ | 1.0 | Nishiyama and Yabuno 1978 |
| | *A. ventricosa* | $C_vC_v$ | 0.4 | Nishiyama and Yabuno 1978 |
| | *A. eriantha* | $C_pC_p$ | 0.5 | Nishiyama and Yabuno 1978 |
| | *A. clauda* | $C_pC_p$ | 0.45 | Nishiyama 1984 |
| | *A. hirtula* | $A_sA_s$ | 0.9 | Nishiyama and Yabuno 1978 |
| | *A. prostrata* | $A_pA_p$ | 0.8 | Nishiyama 1984 |
| | *A. damascena* | $A_dA_d$ | 0.95 | Nishiyama 1984 |
| | *A. canariensis* | $A_cA_c$ | 0.8 | Nishiyama 1984 |
| | *A. longiglumis* | $A_lA_l$ | 1.7 | Nishiyama and Yabuno 1978 |
| $4x$ | *A. barbata* | AABB | 1.9 | Nishiyama and Yabuno 1978 |
| | *A. murphyi* | AACC | 1.5 | Nishiyama 1984 |
| | *A. maroccana* | AACC | 1.4 | Nishiyama 1984 |
| | Autotetraploids | | | |
| | *A. hirtula* | $A_sA_sA_sA_s$ | 2.1 | Nishiyama and Yabuno 1983 |
| | *A. longiglumis* | $A_lA_lA_lA_l$ | 3.3 | Nishiyama and Yabuno 1983 |
| | *A. pilosa* | $C_pC_pC_pC_p$ | 0.85 | Nishiyama and Yabuno 1983 |
| | Interspecific hybrid | | | |
| | *A. hirtula* × *A. pilosa* | | 1.4 | Nishiyama and Yabuno 1983 |
| $6x$ | *A. byzantina* | AACCDD | 2.7 | Nishiyama 1984 |
| | *A sativa* | AACCDD | 2.8 | Nishiyama and Yabuno 1978 |
| | *A. fatua* | AACCDD | 2.9 | Nishiyama and Yabuno 1978 |
| | *A. sterilis* | AACCDD | 3.0 | Nishiyama and Yabuno 1978 |

**Table 3** Calculated ‘activation inidex’ (AI) percentages for *Avena* species, observed hybrid kernel development, and proposed EBN values (calculated AI percentages in bold indicate crosses between species within the same group)

| | Diploids | | | | | | | | | Tetraploids | | | Hexaploids | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A. ventricosa* (0.4) | *A. clauda* (0.45) | *A. eriantha* (0.5) | *A. prostrata* (0.8) | *A. canariensis* (0.8) | *A. hirtula* (0.9) | *A. damascena* (0.95) | *A. strigosa* (1.0) | *A. longiglumis* (1.7) | *A. maroccana* (1.4) | *A. murphyi* (1.5) | *A. barbata* (1.9) | *A. byzantina* (2.7) | *A. sativa* (2.8) | *A. fatua* (2.9) | *A. sterilis* (3.0) |
| *A. ventricosa* (0.4) | **50** | **D + 56** | **63**[a] | 100 | 100 | 113 | 119 | E − 125 | E − 213 | 175 | E − 188 | E − 237 | E − 338 | E − 350 | 363 | E − 375 |
| *A. clauda* (0.45) | **D + 44** | **50** | **D + 56** | 89[a] | 89 | E − 100 | E − 106 | 111 | 189 | 156 | E − 167 | 211 | 300 | 311 | 322 | 333 |
| *A. eriantha* (0.5) | **D + 40** | **D + 45** | **50** | 80[a] | E − 80 | E − 90 | 95 | 100 | E − 170 | E − 140 | E − 150 | E − 190 | 270 | E − 280 | 290 | E − 300 |
| *A. prostrata* (0.8) | 25 | 28[a] | 31[a] | **50** | **50** | **D + 56** | **D + 59** | **D + 63** | E − 106 | 88 | 94 | E − 119 | 169 | 175 | 181 | 188 |
| *A. canariensis* (0.8) | 25 | 28 | 31 | **50** | **50** | **56** | **D + 59** | **63** | 106 | 88 | 94 | 119 | 169 | 175 | 181 | 188 |
| *A. hirtula* (0.9) | 22[a] | 25 | Rd + 28 | **D + 44** | D + 44 | **50** | **D + 53** | **D + 56** | E − 94 | D + 78 | 83 | E − 106 | 150 | 156 | 161 | E − 167 |
| *A. damascena* (0.95) | 21 | 24 | D + 26 | **42** | **D + 42** | **D + 47** | **50** | **53** | 89 | 74 | 79 | 100 | 142 | 147 | 153 | 158 |
| *A. strigosa* (1.0) | 20 | 23 | Rd + 25 | **D + 40** | **40** | D + 45 | **48** | **D + 50** | E − 85 | D + 70 | D + 75 | E − 95 | 135 | E − 140 | E − 145 | E − 150 |
| *A. longiglumis* (1.7) | 12 | 13 | 15[a] | 24 | 24 | 26 | 28 | D + 29 | 50 | **41** | **D + 44** | **D + 56** | 79 | E − 82 | E − 85 | E − 88 |
| *A. maroccana* (1.4) | 14 | 16 | 18[a] | 29 | 29 | 32[a] | 34 | 36[a] | **61**[a] | **50** | **D + 54** | **68**[a] | E − 96 | 100 | 104 | 107 |
| *A. murphyi* (1.5) | 13 | 15 | Rd − 17 | 27 | 27 | 30 | 32 | D + 33 | **D + 57** | **D + 47** | **50** | **D + 63** | E − 90 | E − 93 | E − 97 | E − 100 |
| *A. barbata* (1.9) | 11 | 12[a] | Rd − 13 | D + 21 | D + 21 | Rd + 24 | 25 | Rd + 26 | **D + 45** | **D + 37** | **D + 39** | **50** | D + 71 | D + 74 | D + 75 | D + 79 |
| *A. byzantina* (2.7) | 7 | 8 | 9 | 15 | 15 | 17 | 18 | 19 | D + 31 | 26 | D + 28 | 35 | **50** | **52** | **54** | **56** |
| *A. sativa* (2.8) | 7 | 8 | Rd − 9 | 14 | 14 | 16 | 17 | Rd − 18 | D + 30 | 25 | D + 27 | D + 34 | **48** | **50** | **D + 52** | **D + 54** |
| *A. fatua* (2.9) | 7 | 8 | 9 | 14 | 14 | 16 | 16 | Rd − 17 | D + 29 | 24 | 26 | D + 33 | **47** | **D + 48** | **50** | **D + 52** |
| *A. sterilis* (3.0) | 7 | 8 | Rd − 8 | 13 | 13 | Rd − 15 | 16 | Rd − 17 | D + 28 | D + 23 | D + 25 | D + 32 | **45** | **D + 47** | **D + 48** | **50** |
| | **Group 1 = 1 EBN** | | | **Group 2 = 2 EBN** | | | | | **Group 3 = 3 EBN** | | | | **Group 4 = 4 EBN** | | | |

[a] Crosses attempted but no seeds obtained

chromosome numbers of some diploid oat species with colchicine, giving autotetraploids. They found (Table 2) that when the chromosome number of a species is doubled, its corresponding AV is also nearly doubled, and its crossability is often altered. In the same study, an *Avena hirtula* (4*x*) × *A. eriantha* (4*x*) amphiploid was also obtained, and its AV was calculated to be the sum of its parental AVs. The AVs for seven additional *Avena* species (*A. clauda*, *A. prostrata*, *A. damascena*, *A. canariensis*, *A. murphyi*, *A. maroccana*, *A. byzantina*) were also estimated by Nishiyama (1984) (Table 2). The AVs for the AC-genome species *A. maroccana* and *A. murphyi* were estimated to be 1.4 and 1.5, respectively, which is very close to the AV sum of A-genome and C-genome diploid species.

To estimate the AV for a species, a set of diallel crosses with species of known AVs has to be established and the seed type obtained recorded. When the AV for one parent is known, a series of tentative AVs for the unknown parent can be assigned and the AIs for the endosperm calculated. Usually more than one AI calculated from different AVs will correspond to the kernel type. However, in a number of crosses, including reciprocals, only one or two AVs will give good correspondence between AIs and the kernel type observed (Nishiyama and Yabuno 1983; Nishiyama 1984). The assignment of an AV for diploid *A. clauda* (Nishiyama 1984) will serve as an example. This species was crossed with diploid species *A. hirtula*, *A. ventricosa*, *A. eriantha*, and tetraploid species *A. barbata*. In crosses between *A. clauda* and either *A. hirtula* (AV = 0.9) or *A. barbata* (AV = 1.9), the kernels were type E- (shrivelled-empty and inviable) (Table 3). Thus, the AI had to be more than 80%. When *A. clauda* was crossed either to *A. venticosa* (AV = 0.4) or *A. eriantha* (AV = 0.5), normal type (D+) kernels were obtained, indicating that the AI had to be close to 50% (Table 3). When AVs between 0.3 and 0.7 were given for *A. clauda*, the best estimate based on the kernel type is 0.45. The larger the number of testcrosses, the more accurate the assigned AV will be.

The same model hypothesis was extended for *Triticum* (Nishiyama 1979a), *Aegilops* (Nishiyama 1979b), and the Triticinae (Nishiyama 1983). Three diploid, five tetraploid, and two hexaploid *Triticum* species were used in an incomplete diallel to determine their AVs (Table 4). *Triticum boeoticum* was used as a standard and was assigned an AV = 1 (Nishiyama 1979a). Diploid and tetraploid *Aegilops* species were also assigned AVs (Table 4), again using *T. boeoticum* as a standard (Nishiyama 1979b). Intergeneric hybridizations were also made between *Aegilops* and *Triticum*, but the PNA hypothesis was only applicable to a few cases due to insufficient crossing data (Nishiyama 1981). More intergeneric hybridizations between *Secale cereale* and *Triticum* species, and between *Triticale* (6*x*) and rye (4*x*) or wheat (6*x*) were made to determine the AVs for rye and *Triticale*. According to crossability results, diploid rye was assigned an AV = 2.7 and 4*x* and 6*x* *Triticales* were assigned AVs of 3.6 and 4.4, respectively. The AVs for 4*x* and 6*x* *Triticales* correspond to the AV sums of *Triticum monococcum* (AV = 0.9) and *S. cereale* (AV = 2.7) for the former, and of 4*x* wheat (AV = 1.7) and *S. cereale* (AV = 2.7) for the latter (Nishiyama 1983). Thus, the AVs can be used to imply the evolutionary progression of a species.

**Table 4** Activating/Response Values (AV/RV) for *Triticum* and *Aegilops* species

| Ploidy | Species | Synonyms[a] | Genome[b] | AV/RV |
|---|---|---|---|---|
| 2*x* | *T. monococcum* | | AA | 0.9 |
| | | *T. boeoticum* | | 1.0 |
| | | *T. urartu* | | 0.5 |
| | *Ae. squarrosa* | *T. tauschii* | DD | 0.6 |
| | *Ae. uniaristata* | *T. uniaristata* | $U_nU_n$ | 0.7 |
| | *Ae. umbellulata* | *T. umbellulatum* | UU | 0.9 |
| | *Ae. comosa* | *T. comosum* | MM | 1.1 |
| | *Ae. caudata* | *T. dichasians* | CC | 1.4–(1.7) |
| | *Ae. speltoides* | *T. speltoides* | SS | 1.5 |
| 4*x* | *T. timophevii* | | AAGG | 1.5 |
| | *T. pyramidale* | | AABB | 1.6 |
| | *T. turgidum* | | AABB | |
| | | *T. dicoccoides* | | 1.7 |
| | | *T. dicoccum* | | 1.7 |
| | | *T. durum* | | 1.75 |
| | *Ae. ventricosa* | *T. ventricosum* | $DDU_nU_n$ | 1.6 |
| | *Ae. ovata* | *T. ovatum* | UUMM | 1.65 |
| | *Ae. triaristata* | *T. triaristatum* | UUMM | 2.1 |
| | *Ae. cylindrica* | *T. cylindricum* | CCDD | 2.2 |
| | *Ae. triuncialis* | *T. triunciale* | UUCC | 2.2 |
| 6*x* | *T. aestivum* | | AABBDD | 3.0 |
| | | *T. spelta* | | 2.9 |

[a] Synonyms based on nomenclature by Kimber and Sears (1987)
[b] Genome designations by Kimber and Sears (1987)

## Discussion

In an effort to predict the outcome of interspecific crosses, two independent but parallel hypotheses have evolved, the Endosperm Balance Number (EBN) hypothesis for the tuber-bearing *Solanums* and their close non-tuber-bearing relatives (Johnston et al. 1980), and the polar-nuclei activation (PNA) hypothesis for the Gramineae (Nishiyama and Yabno 1978; Nishiyama 1983, 1984). Both were introduced in the late 1970s and evolved during the following years.

The PNA hypothesis was proposed in order to explain the results of interspecific crosses and their reciprocals and was based on "... an advanced hypothesis of an opinion of Kihara and Nishiyama..." (Nishiyama and Yabuno 1978). Nishiyama tried to provide evidence for the existence of a stimulative action of the male nuclei for initiation of mitotic divisions in embryo and endosperm development through embryological studies. However, Lin (1975, 1984), by utilizing the indeterminate gametophyte (*ig*) gene (Kermickle 1971), was able to demonstrate the importance of an imprinting genomic balance (2 maternal:1 paternal) for normal endosperm development in maize. He further demonstrated that when chromosome arms of paternal origin were missing from the endosperm, a reduction in kernel size resulted, and replacement of the missing arms by those of maternal origin did not eliminate this defect. Based on activity differences between maternally and paternally derived genomes, chromosones, or even genes, Kermicle and Alleman (1990) reported that many aspects of endosperm development could be controlled by diffierentially imprinted genes. The EBN hypothesis is essentially an expansion of the 2 maternal:1 paternal genomic ratio value. However, the genome of each species is assigned an EBN, and it is the EBN in the endosperm that must be in a 2 maternal:1 paternal ratio.

A number of similarities exist between the two hypotheses. Assignment of species' EBNs or AVs are based on interspecific crossabilities using principally *S. chacoense* (2EBN) for potato and *A. strigosa* (AV = 1.0) for oats as standards. A single value is assigned to each species in each genus. Plumpness, size, and germinability of hybrid seed are all important characteristics for assigning EBNs and AVs. Ploidy of the progeny is also important in determining EBN. In hybrid potato seeds, the number of viable seeds per fruit is actually counted, since the endosperm is difficult to evaluate, while in oats the endosperm development is scored. On the basis of a species' EBNs and AVs, predictions can be made on the success or failure of an interspecific cross, even if there is no previous crossability knowledge (Table 3). Furthermore, species can be manipulated by altering their ploidy levels to overcome endosperm collapse and obtain interspecific hybrid seeds (Johnston and Hanneman 1980, 1982; Nishiyama and Yabuno 1983; Ehlenfeldt and Hanneman 1984). In this way, exotic germplasm can be transferred to the cultivated species. Both theories give consistent and reproducible predictions for species crossabilities.

Although the foundation (predictability of crosses) and principle (endosperm development) for both hypotheses are the same, the EBN focusses on the 2 maternal:1 paternal ratio in the endosperm, while the PNA is based on the stimulative strength of the male nuclei to initiate mitotic dividions in the primary endosperm nucleus. Thus, the PNA hypothesis adds another level of complexity by introducing the 'activation index' (AI), with which success and/or failure of reciprocal crosses can be explained. For example, in *A. sativa* (RV = 2.8) × *A. murphyi* (AV = 1.5), the AI = 27%, and thus the kernel will be small but viable (kernel type Rd+). The reciprocal cross *A. murphyi* (RV = 1.5) × *A. sativa* (AV = 2.8) will have an AI = 93% and the kernel will be empty and inviable (kernel type E-) (Table 3). Nishiyama and Yabuno (1978) reported that all 6 seeds obtained from the former cross germinated, while all 38 seeds obtained from the latter cross failed to germinate. In the EBN hypothesis, however, differences in hybrid seed development in reciprocal crosses were explained as a directional effect due to dosage tolerance effects in the endosperm (Johnston 1980; Ehlenfeldt and Hanneman 1988), where a slightly higher female EBN dosage might be tolerated, giving rise to smaller but viable seeds.

Assignment of EBNs and AVs is based on species crossabilities. Assigned EBNs of potato are whole numbers because an interspecific cross will either produce hybrid seed (2 maternal:1 paternal EBN ratio preserved) or not (2 maternal:1 paternal EBN ratio violated). Whenever the 2:1 ratio was violated and hybrid seed was produced, it was an indication of 2*n* gametes functioning (Johnston et al. 1980). The genetics of EBN has been studied by Ehlenfeldt and Hanneman (1988), who proposed that three unlinked loci operating in a threshold-like system control EBN. It has also been demonstrated that the EBN of North American species is under the same genetic control as that of the South American species (Bamberg and Hanneman 1990). Activating values in the PNA hypothesis are usually integers because the hybrid seeds produced from an interspecific cross are classified in four categories. They also have to fall in the corresponding AI range depending on their morphological description. No genetic system has been identified yet for *Avena*, although Nishiyama and Yabuno (1978, 1979) refer to the presence of "activator gene(s)".

The EBN hypothesis (Johnston et al. 1980) and the PNA hypothesis (Nishiyama and Yabuno 1978) seem to be the same biological concept described by two different groups independently investigating the crossing relationships of species using different crops and related species. Oat lends itself to the determination of seed characteristics because of its large seededness, but less so to crossing success because of the limitation of obtaining a maximum of one seed per pollination. In potato, one pollination yields a fruit with many seeds, so seed set is a relevant measure, but seed size is small and less amenable to analysis. Therefore, in potato, the EBN concept embraces a broad grouping of species based on their crossability (seed set), while in oats, the PNA concept deals with the crossability of individual species and is concerned with the condition of the seed produced. EBNs must be in a 2 maternal:1 paternal relationship in the endosperm for normal seed development, just as the PNA requires the AI of the polar nuclei to be in an acceptable range based on the ratio of 1 AV to 2 RVs for successful seed development. Although a 2 maternal:1 paternal EBN ratio for potato and an AI percentage between 30 and 80 for oats are necessary for normal hybrid seed development, they are not the only criteria for a successful interspecific cross. Other incompatibility barriers preventing fertilization might be present. However, these two hypotheses provide a solid basis for explaining and predicting interspecific crossabilities.

One can group oat species into four broad crossability groups on the basis of their AVs and crossing behaviour and assign a different EBN for each group (Table 3): Group 1 (1EBN) includes all oat species having AVs between 0.4 and 0.5; Group 2 (2EBN) represents species with AVs between 0.8 and 1.0; Group 3 (3EBN) includes all species with AVs between 1.4 and 1.9; and finally species having AVs between 2.7 and 3.0 are included in Group 4 (4EBN). Crossabilities between species within the same group, having the same EBN, are possible no matter which species is used as the pollen source. A 2 maternal:1 paternal EBN ratio is preserved in such crosses. However, production of hybrid seed between species belonging to adjacent groups is tolerated only when the species with the higher AV is used as the female. This agrees with the observation of Ehlenfeldt and Hanneman (1988) that an extra EBN maternal dosage produced viable seeds of reduced size (corresponding to Rd+ hybrid oat kernels), while an extra paternal dose resulted in seed abortion. Similarly, AVs could be assigned to potato species with the relevant determination of seed condition. These common points justify viewing the Endosperm Balance Number hypothesis and the polar-nuclei activation hypothesis as a single biological concept. Analysis of existing and additional crossing data will provide the corresponding necessary information to expand this common concept to other genera as well.

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