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A revival of effective ploidy: the asymmetry of parental roles in endosperm-based hybridization barriers

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Interest in understanding hybrid seed failure (HSF) has mushroomed, both in terms of identifying underlying molecular processes and their evolutionary drivers. We review phenotypic and molecular advances with a focus on the ‘effective ploidy’ concept, witnessing a recent revival after long obscurity. Endosperm misdevelopment has now been shown to underlie HSF in many inter-specific, homoploid crosses. The consistent asymmetries in seed size and developmental trajectories likely reflect parental divergence in key, dosage-sensitive processes. Transcriptomic and epigenomic studies reveal genome-wide, polarized expression perturbations and shifts in parental expression proportions, consistent with small-RNA imbalances between parental roles. Among-species differences in levels of parental conflict over resource allocation enjoy strong support in explaining why differences in effective ploidy may evolve.

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Introduction

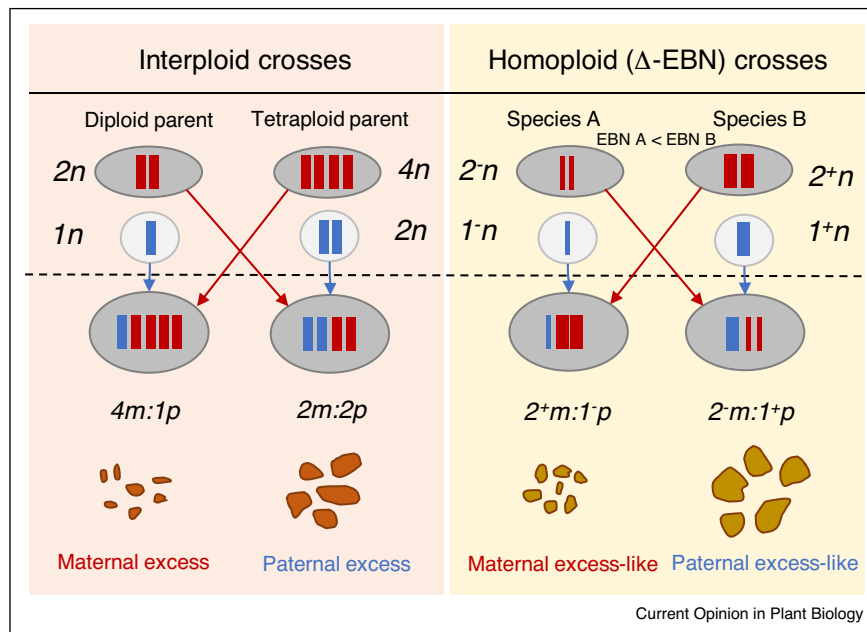
The build-up of reproductive isolation is a fundamental component of the speciation process, ultimately underlying the genesis of biodiversity. Here, we focus on hybrid seed failure (HSF), a postzygotic reproductive barrier widespread among flowering plants but traditionally underrepresented in the plant speciation literature [1,2]. While we draw on functional insights gained from experimental work using parents of different ploidies (i.e. interploidy crosses), our conceptual focus is on incidences of HSF between species of the same ploidy, that is, seed lethality revealed by inter-specific, homoploid crosses. Seminal crossing studies generated convincing evidence

that endosperm failure, rather than intrinsic F1 hybrid incompatibilities, is the major cause of embryo and seed abortion in numerous examples [3–6]. In flowering plants, the normally triploid endosperm arises by fusion of one of the two haploid sperm cells with the hypomethylated diploid central cell of the female gametophyte; it is this 2:1 ratio of maternal:paternal genomes—and the right balance of their epigenomic landscapes—that is required for normal endosperm development, at least for intra-specific crosses [7–9]. Importantly, this 2:1 genomic ratio contributes to asymmetries in relatedness between the maternal sporophyte and the filial seed compartments (fertilization products) endosperm and embryo, with the source and diversity of pollen donors playing an important role. These asymmetries are widely thought to underlie conflicts of interest over maternal seed provisioning [5,10–12]. Our review attempts to unite studies on plant model systems with a mechanistic focus and conceptually oriented work pursued mostly by plant evolutionary geneticists.

Compromised endosperm development as the major cause of HSF

The last five years have witnessed a revival of interest in HSF from the dual perspectives of underlying molecular mechanisms and potentially rapid mode of speciation among flowering plants [13]. Prominent examples include work in the genera *Arabidopsis* [14], *Capsella* [15,16^{*}], *Solanum* [17,18] and *Mimulus* [19–23]. Histological and/or seed size data from these inter-specific, mostly homoploid crosses revealed both maternal and paternal effects, similar to those known from many interploidy crosses [5,6,24] (Figure 1). Specifically, reciprocal hybrid seeds differ markedly in size, with both classes of hybrid seeds often smaller than normally developing pure-species seeds [14,18], or one cross direction yielding larger-than-normal seeds [15,22]. These phenotypic patterns unite examples of nuclear-type (early syncytial phase before cellularization; *Arabidopsis*, *Capsella*) and cellular endosperm development (cell walls are formed from the first mitotic division; *Solanum*, *Mimulus*). Histologically, the patterns of endosperm misdevelopment differ between the two types of endosperm, but the relative timing and/or speed of developmental trajectories (precocious or delayed; Figure 2) is consistent in direction. Based on both phenotypic and molecular evidence, an accelerated versus slowed-down cell cycle may underlie these patterns [18,25,26,27^{**}]. In the interest of facilitating the narrative, we use the established terms ‘maternal-excess’ and ‘paternal-excess’ [5,6] to characterize the

Figure 1



Schematic comparison of reciprocal interploidy crosses (left) and inter-specific, homoploid crosses between species with diverged (Δ) EBN (right). The horizontal dotted line indicates fertilization, with the central cell (grey ellipses) and sperm cell (white circles) above and the endosperm (grey ellipses) below this line. Ploidies are represented by colored rods: paternal genomes in blue and maternal genomes in red. The unequal width of rods and '+' and '-' signs in the right half indicate diverged effective ploidies. Arrows symbolize the union of central cell (red) and sperm cell (blue), generating the endosperm. Mature seeds (bottom) are drawn to qualitatively convey the observed size differences in reciprocal crosses. EBN, endosperm balance number; n , ploidy level; m , genome of maternal origin; p , genome of paternal origin.

consistent asymmetries in hybrid seed size and development, even though most of the discussed examples refer to crosses among diploids (Figure 1).

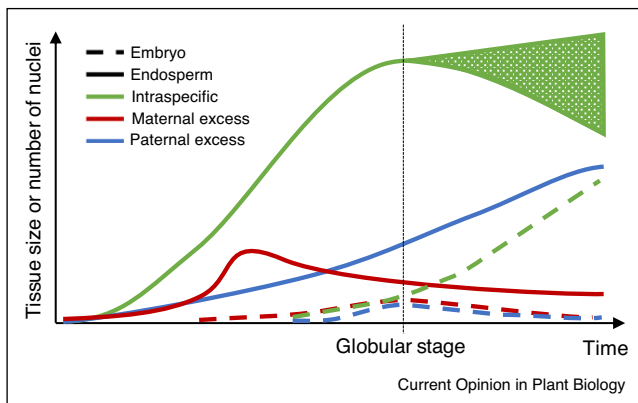
Changing perceptions of misregulated genomic imprinting in HSF

Parental effects on seed size have long attracted interest from theoreticians and were mostly interpreted as reflecting the action of imprinted genes (IGs) [5,28,29^{*}], although credible alternatives based on more inclusive dosage-dependent interactions have been proposed [30–32]. Genomic imprinting refers to parent-of-origin-dependent expression, with expression at Maternally Expressed Genes (MEGs) mostly of maternal origin, and *vice versa* at Paternally Expressed Genes (PEGs). Epigenetic asymmetries such as differences in methylation patterns and/or histone modification are established before fertilization and thus generate epigenetically unequal parental landscapes [8,9]. The correct balance of these parental landscapes underlies imprinting and modulates gene expression, with consequences for seed development and mature seed size [33]. Hence, the plausible idea of misexpressed IGs underlying HSF has been pursued vigorously. Studies in *Arabidopsis* [34,35], *Mimulus* [21], *Solanum* [36] and *Oryza* [37] indeed

revealed expression perturbations of IGs in crosses subject to HSF, but also provide evidence for global misexpression of genes [35,36], transposable elements (TEs) [34], and small RNAs [37,38] in failing hybrid endosperm. Rather than being able to pinpoint causal genes, these parent-specific patterns of genome-wide perturbations may provide insights into the epigenetic machinery underlying dosage imbalances.

The recent discovery of allelic dosage being mediated by a small-RNA pathway engaged in RNA-directed DNA methylation (RdDM) in *Arabidopsis* endosperm may have far-reaching implications [38,39,40^{**}]. A mutant version of the gene coding for the largest subunit of RNA Polymerase IV (Pol IV; *npr1*) was shown to shift genome-wide expression in the endosperm to higher maternal proportions, and a normally inviable $2n \times 4n$ interploidy cross produced mostly viable seeds when the tetraploid father (or both parents) carried the *npr1* mutation [38,39]. Follow-up work using a larger suite of mutants for RdDM pathway and downstream genes implies that expression differences distinguishing viable from lethal seed development are rather subtle, and appear to rule out models of HSF based on derepressed TEs and misexpressed IGs [40^{**}]. These discoveries may precipitate a modified view recognizing deregulated imprinting—and

Figure 2



Schematic view of altered growth trajectories in abortive, parental-excess seeds compared to viable seeds from intra-specific crosses. Continuous lines refer to the endosperm and dashed lines to the embryo. In abortive seeds, embryo arrest is typically observed at (or shortly after) the globular stage. This qualitative drawing illustrates commonalities among results from hybrid crosses in taxa with cellular endosperm (*Solanum* [18,26], *Mimulus* [21]) and those with nuclear-type endosperm such as rice and maize ([24,26], and references therein). The dotted green area indicates the potential for the endosperm to persist (endospermic seeds such as in rice) or degenerate after the globular stage (non-endospermic seeds such as in tomato).

altered maternal:paternal expression ratios genome-wide [36,39]—as a downstream consequence of epigenomic imbalances, possibly mediated by small RNAs that define the endosperm methylation landscape [38,40^{**}]. Unbalanced epigenomic states may lead to cascading effects if key regulatory genes are impacted, with repercussions for seed viability. Naturally, it remains to be established to what extent these experimental findings in *Arabidopsis* can be extrapolated to homoploid crosses among nonmodel species resulting in partial or complete HSF [17–23].

A revival of the Endosperm Balance Number (EBN) hypothesis

Classical work on diploid and polyploid wild potato species (*Solanum*) spurred the important insight that what was conceptualized as EBN or ‘effective ploidy’—and not necessarily the karyotypic ploidy [7]—must conform to a 2:1 maternal:paternal endosperm ratio to allow viable seed formation in interspecific crosses [41–43]. Three decades ago, it was recognized that only few genes might underlie interspecific differences in EBN, and thus the possibility of rapid speciation due to HSF; experimental crosses using rare F1 hybrids suggested a system of three unlinked loci [42,43]. While this body of work facilitated predictions of breeding success between untested combinations of germplasm, it was underutilized regarding its conceptual contribution to models of speciation and

dosage phenomena [13]. The ‘weak inbreeder/strong outbreeder’ (WISO) hypothesis [44] connects the effective-ploidy concept with differences in levels of parental conflict as the proposed evolutionary driving force for diverged EBNs. At the mechanistic level, parental expression differences at dosage-sensitive genes might occur asymmetrically between reciprocal hybrid crosses [16^{*},27^{**}], driving phenotypic polarization between maternal-excess and paternal-excess types in endosperm and seed development upon hybridization [30,31].

Molecular correlates and evolutionary drivers of effective ploidy

A few recent studies have leveraged endosperm transcriptomes of normal and failing hybrid seeds to probe for molecular correlates of HSF and differences in effective ploidy. Comparisons among three diploid *Capsella* species with different mating systems and/or antiquity of self-fertilization uncovered near-absolute HSF between the obligate outcrosser *Capsella grandiflora* (*Cg*) and the ancient selfer *Capsella orientalis* (*Co*), but also between the latter and the more recent selfer *Capsella rubella* (*Cr*), with inferred effective ploidies ranking $Cg > Cr > Co$ [16^{*}]. These patterns are entirely consistent with the WISO hypothesis because the species’ mating systems (or their duration since transition from ancestral outcrossing) should maintain the highest level of parental conflict in *Cg* and the lowest in *Co*. Transcriptomic data revealed that relative PEG abundance also scales $Cg > Cr > Co$, as do PEG expression levels $Cg > Cr$. Moreover, there are associations of TEs with many of the species-specific PEGs and differential TE spread dynamics among lineages [16^{*}], consistent with differences in TE abundance among the three *Capsella* species [45] and the proposed role for TE insertions in establishing imprinted expression at nearby genes [46].

Using laser-assisted microdissection of developing endosperm and transcriptomic analyses, studies in green-fruited wild tomato species (*Solanum* section *Lycopersicon*) have contributed to our understanding of genomic imprinting [47] and altered gene expression patterns in failing hybrid endosperm [27^{**},36]. In addition to perturbed imprinting, we uncovered genome-wide maternal expression biases in both (failing) hybrid endosperms between *S. peruvianum* (*Sp*) and *Solanum chilense* (*Sc*), with stronger maternal bias in the maternal-excess cross $Sp \times Sc$ [36]. Intriguingly, this transcriptomic signal mimics the patterns seen in both homoploid and $2n \times 4n$ *Arabidopsis thaliana* crosses with mutated *nprpd1* gene in the pollen donor (both cross types) or both parents (homoploid cross) [39,40^{**}]. Additional allele-specific expression data in *Sp*, *Sc* and a third lineage of wild tomatoes (*Solanum arcanum* var *marañón*, *Sa*) [18,47] confirm the maternal expression bias of hybrid endosperms, with larger bias in maternal-excess crosses

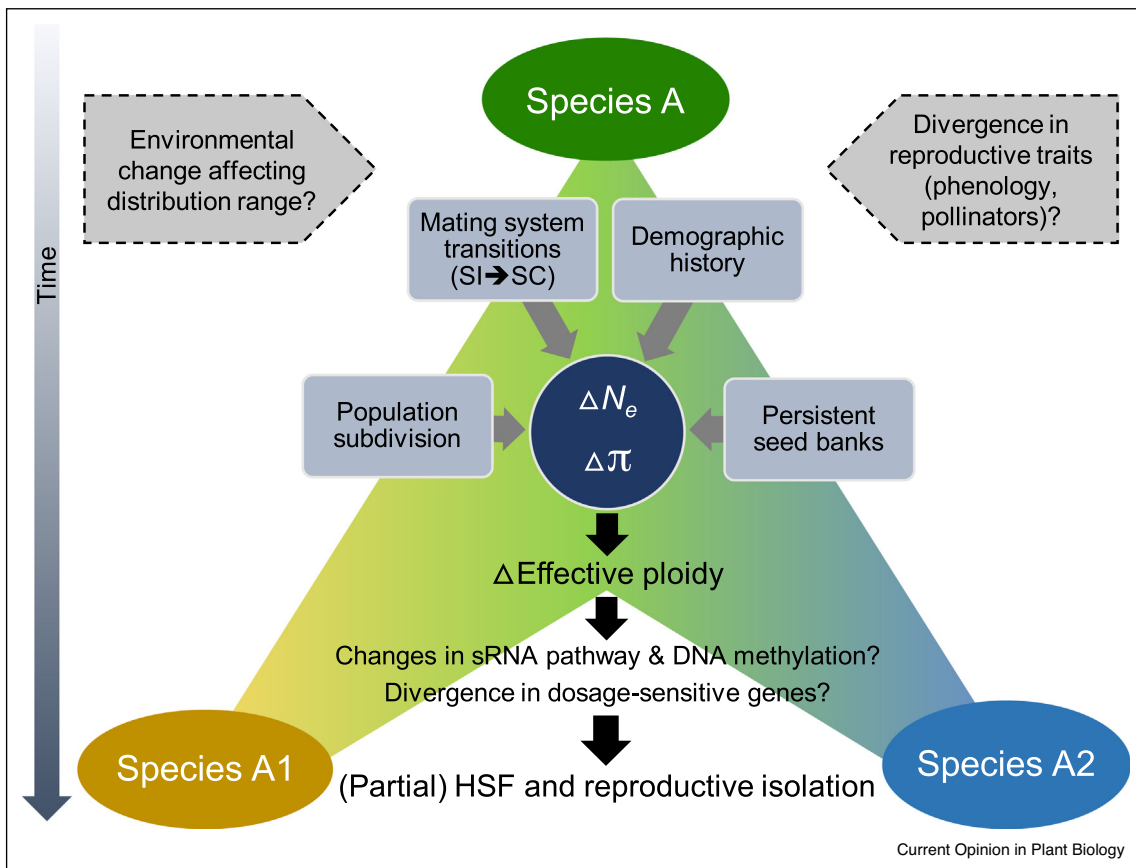
(MR, AMFR, TS, unpublished data). These results obtained in *Solanum* challenge the interpretation that maternal expression bias somehow buffers the effects of an additional paternal endosperm genome in *Arabidopsis* [39,40**].

Reciprocal hybrid crosses among the wild tomato species *Sp*, *Sc* and *Sa* show clear patterns of paternal and maternal excess, suggesting that effective ploidy ranks $Sp > Sc > Sa$ [18]. Recent transcriptome surveys revealed that for the two inter-specific crosses resulting in complete, bidirectional HSF, the largest expression divergence is between reciprocal hybrids—which have the exact same parents but in inverted parental roles—with high congruence between independent sets of maternal-excess and paternal-excess endosperms [27**]. This strong expression polarization is also evident for functionally important classes of genes such as MADS-Box transcription factors, E3 ubiquitin ligases, and genes related to the cell cycle. However, unlike in the

Capsella study [16*], the number and expression level of IGs is not statistically different between the three lineages [47].

Using the idea that differences in levels of parental conflict may lead to divergence in effective ploidy [44] allows us to broaden the scope of the WISO hypothesis beyond the strict dichotomy of inbred versus outbred mating systems [29*]. Indeed, kin conflict should decrease with increasing relatedness of mates [10,28], and evolutionary processes that modulate effective population size (N_e) might all play a role, such as demographic history, population subdivision and connectivity, and persistent soil seed banks [48] (Figure 3). Because the coalescent history of gene samples is affected by these demographic and life-history characteristics, levels of nucleotide diversity (π) of species or regional populations should reflect their long-term N_e [48,49]. Roth *et al.* [27**] used this rationale to unite

Figure 3



Simplified schematic of major ecological and evolutionary factors that impact the effective population size (N_e) of diverging populations or species. Other factors being equal, lower N_e should lead to higher relatedness among mates, thus lowering levels of parental conflict. Divergence in N_e (roughly estimated by nucleotide diversity π at selectively neutral genome regions) is hypothesized to facilitate divergence in effective ploidy (EBN), which may manifest as altered expression levels at dosage-sensitive genes or other molecular changes. Ecological and life-history features (that may or may not change during divergence) are in light-grey boxes, and major evolutionary factors/processes that impact N_e (for more details, see Refs. [48,49]) are in light-blue boxes. HSF, hybrid seed failure; SC, self-compatibility; SI, self-incompatibility.

phenotypic, transcriptomic, and π estimates for the three *Solanum* species, where levels of π perfectly mirror the inferred ranking of effective ploidies ($S_p > S_c > S_a$). These data suggest that HSF can evolve rapidly and without a fundamental change in mating system [18,27^{**},36], and imply that levels of π may be a reasonable proxy to gauge reproductive compatibility between recently diverged species. Our arguments received support from studies in the *Mimulus guttatus* complex, where π was likewise found to scale with inferred effective ploidy, which in turn predicts the severity and asymmetries of hybrid seed defects [22].

Mechanistically, however, the link between N_e and effective ploidy is largely a black box; previous suggestions that quantitative or qualitative inadequacies between parental sRNA populations may lead to HSF [38,50], for example, due to sequence mismatches or copy-number variations, may point in the right direction. If divergence in dosage-sensitive genes and/or upstream changes in the DNA methylation pathway are involved (Figure 3), it will be difficult to disentangle their effects from comparative endosperm expression data alone. One plausible avenue is to study the effects of candidate-gene mutants in a comparative framework.

Potential links between EBN and parental conflict over resource allocation

Evolutionary models predict parental (or kin) conflicts to mediate seed development, in particular regarding levels of maternal resource allocation that, in turn, impact seed size and organismal fitness [5,10–12,28,51]. An important assumption regarding conflict over seed provisioning is the ability of sires to affect seed weight via the expression of paternal alleles in endosperm and/or the embryo, yet this has rarely been rigorously tested. Using a novel statistical approach, Cailleau *et al.* [52^{**}] showed in maize (*Zea mays*) that half-sib endosperms aggressively compete for maternal resources, and that different sires have different competitive abilities; maternal resources were ‘wasted’ due to male–male competition via unrelated male alleles in adjacent kernels. Similarly, interpopulation crosses in *Dalechampia scandens* (Euphorbiaceae) revealed modest but significant effects on seed size, depending on whether the more outcrossed population served as pollen or seed parent, yielding paternal-excess or maternal-excess phenotypes, respectively [29^{*}]; similar results were previously obtained in *Arabidopsis lyrata* [53]. Such subtle effects do not interfere with successful endosperm/seed development, but more strongly asymmetric resource allocation—whether driven by diverged levels of parental conflict or other factors [54^{*}—might cause strongly compromised endosperm development resulting in HSF. We thus envision a continuous scale of effective ploidy, with success or failure of particular parental

combinations reflecting the extent of unbalanced resource allocation to developing seeds.

Conclusions and future prospects

To achieve representative assessments of both molecular mechanisms and evolutionary drivers of HSF, the field needs more studies on nonmodel taxa, representing a range of life-history and mating systems among closely related species within such groups. Study systems of special interest from ecological and evolutionary vantage points due to their exceptional diversity, but also with increasing genomic resources such as *Solanum* and *Mimulus*, offer great potential to move beyond phenotypic studies of HSF [17–19,23] and expand to molecular surveys of normal and failing seed development [20,21,55]. Methodologically, technical challenges are shared between model and nonmodel species, such as potential contamination of endosperm and/or embryo samples with maternal seed coat tissue that confounds allele-specific expression estimates [56]. In addition, novel approaches to assess influential evolutionary hypotheses with molecular data [57^{**}], or by exploiting ancient plant lineages with nonstandard endosperm composition and seed development [58^{*}], may also offer crucial insights. The still rather fragmentary nature of our understanding of the ‘how and why’ of HSF in nonmodel systems may not warrant firm conclusions, but genome-wide assessments of (altered) endosperm gene expression [27^{**}], parent-specific expression patterns [36,40^{**}] and epigenomic landscapes [16^{*},40^{**}] are consistent with small-RNA-mediated dosage imbalances underlying HSF. Finally, we ought to remain open-minded regarding the evolutionary drivers of this now increasingly appreciated type of inter-specific post-zygotic barrier.

Conflict of interest statement

Nothing declared.

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