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Varying importance of postzygotic isolation in polyploid speciation? A survey of the triploid block strength, its causal mechanisms, and evolutionary consequences

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Abstract:	<p>The triploid block, primarily caused by endosperm developmental issues, is known as a significant barrier to interploidy hybridization among flowering plants and thereby, polyploid speciation. However, its strength varies across taxa, with some instances of leakiness, questioning its universal role as a barrier. We conducted a literature survey to explore the causes of the variation in the strength of triploid block across angiosperms. We particularly assessed the impact of interploidy cross direction, types of endosperm development, endosperm persistence at seed maturity, and ploidy divergence. None of these factors had a significant impact on triploid seed viability, likely due to limited data and inconsistencies in estimation methods across the literature. In addition, triploid seed viability in experimental crosses was sometimes correlated to the occurrence of triploid hybrids in nature, sometimes not, suggesting a mixed role of the triploid block in shaping interspecies gene flow. Altogether, our study highlights the need for unified approaches in future studies on the triploid block to advance our understanding of its variation and evolutionary implications.</p>



CHARLES UNIVERSITY
Faculty of science

Department of Botany
Dr. Clément Lafon-Placette
Associate Professor

25th of June 2024

Dear editors,

We are pleased to submit our manuscript titled ‘Varying importance of postzygotic isolation in polyploid speciation? A survey of the triploid block strength, its causal mechanisms, and evolutionary consequences’ for consideration in *Plant Communications*.

Polyploidy is ubiquitous in plants, and is thought to be a major driver of plant diversity. Among the underlying processes, the triploid block (the inviability of triploid hybrid seeds) has been proposed as an “instant” reproductive barrier between polyploids and their diploid ancestor. However, the strength of the triploid block varies across clades, being close to absolute in some cases or largely “leaky” in others, raising the question of the underlying causes for this variation and its consequence on interploidy gene flow in nature. Our study focuses on these questions. In this review combined with a meta-analysis, we propose developmental and evolutionary causes influencing the strength of the triploid block across species and its consequences on gene flow. This study points at avenues for future research to combine developmental and evolutionary biology, as well as the need for standardized and rigorous methodologies, integrating crossing experiments with population genetics and cytogeography, to gain better understanding of the mechanisms and evolutionary implications of triploid block on gene flow in natural environments.

Given the importance of the triploid block and plant reproduction for biologists and breeders, and with our bridging between molecular and evolutionary biology, we believe this will be of broad interest to the readership of *Plant Communications* and hope you will agree.

Best regards,

On behalf of all authors, Clément Lafon Placette

A handwritten signature in blue ink, appearing to read 'Clément Lafon Placette'.

[Click here to view linked References](#)

1 **Varying importance of postzygotic isolation in polyploid speciation? A survey of the**
2 **triploid block strength, its causal mechanisms, and evolutionary consequences**

3

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13

14 **Abstract (250 words)**

15

16 The triploid block, primarily caused by endosperm developmental issues, is known as a
17 significant barrier to interploidy hybridization among flowering plants and thereby, polyploid
18 speciation. However, its strength varies across taxa, with some instances of leakiness,
19 questioning its universal role as a barrier. We conducted a literature survey to explore the
20 causes of the variation in the strength of triploid block across angiosperms. We particularly
21 assessed the impact of interploidy cross direction, types of endosperm development,
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23 significant impact on triploid seed viability, likely due to limited data and inconsistencies in
24 estimation methods across the literature. In addition, triploid seed viability in experimental
25 crosses was sometimes correlated to the occurrence of triploid hybrids in nature, sometimes
26 not, suggesting a mixed role of the triploid block in shaping interspecies gene flow.
27 Altogether, our study highlights the need for unified approaches in future studies on the
28 triploid block to advance our understanding of its variation and evolutionary implications.

29

30 **Keywords**

31

32 triploid block; hybrid seed lethality; polyploidy speciation; postzygotic barrier; seed
33 development

34

35 **1. Introduction**

36 Understanding the genetic basis of reproductive isolation is crucial to our understanding of
37 the speciation process. Reproductive isolation is the result of a combination of pre- and
38 postzygotic barriers, highlighting the importance of understanding both prezygotic and
39 postzygotic mechanisms (Rieseberg and Willis, 2007; Lowry et al., 2008). While prezygotic
40 barriers are typically emphasized due to their early emergence and possible prevention of
41 costly mating (Ramsey et al., 2003; Sobel and Chen, 2014; Arnegard et al., 2014), a deeper
42 understanding of postzygotic barriers is also crucial in speciation research (Coughlan and
43 Matute, 2020). Here we focus on the impact of whole genome duplication (WGD) on
44 postzygotic isolation. WGD is a dramatic mutation that leads to the production of an
45 additional copy of the entire genome of a lineage and is recognized as a major driver of
46 speciation (Lynch and Force, 2000; Servedio and Sætre, 2003; Rieseberg and Willis, 2007;
47 Coughlan and Matute, 2020; Fox et al., 2020). Genome duplication occurs across Eukaryotes,
48 though they are especially notable among plants (Cui et al., 2006). WGD is considered to
49 bring about an instant hybridization barrier between diploids and their polyploid derivatives
50 (Ramsey and Schemske, 1998), particularly crosses between diploid and tetraploid
51 individuals, which is the primary focus of our review. Often these crosses result in the failure
52 of viable seed formation, referred to as ‘triploid block’ (Marks, 1966). In the case of
53 comparatively weaker early-acting barriers, the triploid block may emerge as a significant
54 contributor to the genetic isolation between diploids and polyploids, and thereby polyploid
55 speciation. However there are many studies on the genetic basis of homoploid barriers
56 (Abbott et al., 2013; Yakimowski and Rieseberg, 2014; Nieto Feliner et al., 2017), and very
57 little is known about the drivers and basis of heteroploid barriers in nature, despite frequent
58 polyploid speciation (Kolář et al., 2017).

59 The triploid block is mostly caused by developmental problems in the hybrid
60 endosperm (Köhler et al., 2010). The endosperm is a tissue nourishing the embryo in the seed
61 and is the characteristic feature of most flowering plants (Baroux et al., 2002). In most
62 diploid species, the endosperm is predominantly triploid, with two copies of the maternal
63 genome and one copy of the paternal genome (2m:1p ratio). In interploidy hybrid seeds, this
64 2m:1p ratio is disturbed (Johnston et al., 1980; Haig and Westoby, 1991). Studies with
65 artificial *Arabidopsis thaliana* polyploid series have shown that the more pronounced the
66 deviation from the 2m:1p ratio, the stronger the defects in endosperm development and the
67 less viable the interploidy seeds (Scott et al., 1998). With this observation followed by a large
68 body of literature (Köhler et al., 2010; Schatlowski and Kohler, 2012; Birchler, 2014)), it is

69 now well-accepted that the primary cause for endosperm defects in interploidy hybrid seeds
70 is a gene dosage imbalance between maternal and paternal genomes related to the strict
71 requirement of the 2m:1p genome ratio (Johnston et al., 1980; Haig and Westoby, 1991). This
72 strict balance requirement suggests that paternal and maternal genomes are not equivalent,
73 i.e., they express different genes and impact endosperm development differently. This may
74 explain a particular feature of the triploid block, which is its parent-of-origin manifestation
75 (Feil and Berger, 2007). Depending on whether the polyploid is the paternal or maternal
76 parent, the phenotypic defects of the endosperm may vary as well as the rate of viability of
77 interploidy hybrid seeds (Stoute et al. 2012; Scott et al. 1998). In crosses where the maternal
78 parent possesses a higher ploidy level than the paternal one (referred to as 'mat-excess'
79 hereafter), such as in model species *A. thaliana*, the hybrids exhibit a high viability rate (Scott
80 et al., 1998). A similar pattern has been shown in other species (Von Bothmer and Jacobsen,
81 1986; Sekine et al., 2013), giving the impression that mat-excess seeds generally survive
82 better than paternal-excess ones (where the paternal parent possesses a higher ploidy level
83 than the maternal one, referred to as 'pat-excess' hereafter). Reports showing no parent-of-
84 origin impact on interploidy seeds (Burton and Husband, 2000; Sonnleitner et al., 2013)
85 question what may appear as a general trend.

86 Also, there is a growing realization that the triploid block is sometimes leaky,
87 questioning how strong and universal barrier to interploidy gene flow it represents across
88 angiosperms. Experimental crosses using a variety of natural and artificially produced
89 diploids and tetraploids (Dinu et al., 2005; Stoute et al., 2012; Greiner and Oberprieler, 2012;
90 Sabara et al., 2013; Sekine et al., 2013; Behrend et al., 2015; Roccaforte et al., 2015; Vallejo-
91 Marín et al., 2016) indeed show variation in the manifestation of the triploid block across
92 taxa, both in terms of survival rate of interploidy hybrids and its parent-of-origin asymmetry.
93 Therefore, the triploid block may or may not be a strong barrier to interploidy hybridization,
94 and the reason behind it is unclear. Understanding the underlying causes of this variation may
95 help predict general patterns of interploidy gene flow in nature. However, the consequences
96 of the triploid block on gene flow in nature are also unclear. The triploid block is one of
97 many potential reproductive barriers preventing interploidy gene flow, and in fact,
98 postzygotic barriers such as hybrid seed lethality are thought to play a minor role in
99 regulating gene flow since they act late in the hybridization sequence (Coyne and Orr, 2004;
100 Rieseberg and Willis, 2007; Lowry et al., 2008). The triploid block may therefore have little
101 evolutionary consequences, and it is important to re-evaluate the assumption of the triploid
102 block as an important driver of polyploid speciation.

103 In this review, we investigated the variation of the triploid block across angiosperms
104 and assessed its role as a barrier to interploidy hybridization. We provide a synthesis of
105 current knowledge to 1) evaluate how often the triploid block is a leaky barrier across
106 angiosperms; 2) propose and test causes to explain why the strength of the triploid block
107 varies between taxa; and 3) critically assess whether the triploid block and its variation do
108 have an evolutionary significance in terms of realized interploidy gene flow in nature.

109

110 **2. Variation in the strength of triploid block across angiosperms**

111 The triploid block has been studied in several mixed-ploidy species taxa, using a variety of
112 natural and artificially produced diploids and polyploids (Dinu et al., 2005; Stoute et al.,
113 2012; Greiner and Oberprieler, 2012; Sabara et al., 2013; Sekine et al., 2013; Behrend et al.,
114 2015; Roccaforte et al., 2015; Vallejo-Marín et al., 2016). In this section, we assess to what
115 extent the strength of the triploid block shows natural variation across controlled crossing
116 experiments. The variation in the strength of the triploid block may occur at two different
117 levels. First, the intensity of the barrier itself might vary across taxa. Second, the cross-
118 direction effect observed in the triploid block may vary, i.e., the viability may not always be
119 the highest in a given cross direction (mat- or pat-excess), or even sometimes this effect may
120 be absent, leading to equivalent triploid seed viability independent of the cross direction.

121 Regarding the general intensity of the triploid block, a striking range of triploid seed
122 viability has been observed across studies. The variation spans from the virtual absence of
123 viable seeds (0%) in crosses between diploid *Mimulus guttatus* and their natural tetraploids
124 (Salony et al. in review) to a majority (about 80%) of triploid seeds being viable on average
125 in *Cyclamen persicum* Mill. (Takamura and Miyajima, 1996), and anything in between. This
126 data indicates the triploid block may be a permeable barrier to interploidy gene flow in some
127 species, while it may represent a strong barrier in others (Fig. 3). In addition, the cross-
128 direction effect on triploid seed viability, a commonly assumed feature of the triploid block,
129 is inconsistent across studies. Earlier surveys of interploidy crossing data suggested that mat-
130 excess crosses are consistently more successful than pat-excess crosses at producing viable
131 triploid seeds (Ramsey and Schemske, 1998). Indeed, germination results often showed a
132 higher survival upon interploidy crossing with higher maternal contribution in *Brassica*
133 *oleracea*: 90% in mat-excess to complete lethality in pat-excess (Stoute et al., 2012),
134 *Arabidopsis arenosa*: 69% in mat-excess to 7% in pat-excess (Morgan et al., 2021a), *Oryza*
135 *sativa*: 50% in mat-excess to complete lethality in pat-excess (Sekine et al., 2013) or

136 *Salpiglossis sinuata*: 8% in mat-excess to complete lethality in pat-excess (Needham and
137 Erickson, 1992), to only cite a few (Fig. 3).

138 However, the commonly accepted idea that mat-excess seeds are more viable than
139 paternal excess does not always hold. Only recently pat-excess crosses have been found to be
140 more successful in *Mimulus guttatus* (Phrymaceae), showing an increase from 0.5% in mat-
141 excess to 4% in pat-excess (Meeus et al., 2020). However, in Salony et al. (in review), similar
142 experimental crosses in *M. guttatus* revealed a strong barrier to triploid formation in both
143 directions, resulting in non-germinable seeds. Further evidence for better survival of pat-
144 excess hybrids comes from the experimental crossings in *Pilosella echioides* (Asteraceae):
145 44% viability in mat-excess to 66% in pat-excess (Chrtek et al., 2017). While emasculation is
146 not possible in Asteraceae, the high rate of viability is unlikely due to self-pollination as the
147 species was shown to be self-incompatible in this study. While mature seeds from reciprocal
148 crosses in *Galeopsis pubescens* (Lamiaceae) were found to be non-germinable (Håkansson,
149 1952), the seed viability in reciprocal crosses of other taxa, such as Solanaceae, remains
150 unclear due to incomplete crossing designs (missing pat-excess viability) and the lack of data
151 on seed viability (Sansome et al., 1942; Cooper and Brink, 1945; Wangenheim, 1957).
152 Additional evidence against the parent-of-origin effect on the viability of triploid seeds
153 emerges from *Oenothera hookeri* (Onagraceae), where a robust triploid block is observed in
154 both cross directions, with a viability of 5% in mat-excess and 4% in pat-excess crosses
155 (Wangenheim, 1962). Similarly, in *Chamerion angustifolium* (Onagraceae; 69% in mat-
156 excess/51% in pat-excess; (Burton and Husband, 2000), the triploid block is notably
157 consistent in both cross directions, however being rather relaxed. It is interesting to note that
158 despite similar triploid seed viability in both cross directions, the developmental defects in
159 mat-excess and pat-excess, as studied in *Oenothera*, differ (Wangenheim, 1962). This
160 suggests the existence of an asymmetric developmental basis for the triploid block in
161 *Oenothera*, leading to a symmetric outcome in terms of seed viability.

162 It thus appears that the strength of the triploid block is quite variable across studies,
163 ranging from acting as an absolute barrier to interploidy hybridization in some cases to being
164 considerably permeable in other cases. This variability seems to extend to related species as
165 well, with certain families exhibiting consistent patterns, such as Brassicaceae species
166 showing relatively high survival rates of mat-excess seeds (Scott et al., 1998; Stoute et al.,
167 2012; Morgan et al., 2021a), while Lamiaceae species display a robust triploid block in both
168 cross directions (Håkansson, 1952). In essence, the strength of the triploid block may show a
169 phylogenetic signal, with certain taxa showing a stronger triploid block than others. This

170 leads us to propose and evaluate the potential causes for the strength of the triploid block in
171 the subsequent section. First, the failure in endosperm development remains a consistent
172 characteristic during interploidy hybrid seed arrest irrespective of the taxa, suggesting the
173 developmental traits of the endosperm may influence the triploid block variations we
174 observed above. Furthermore, differences in interploidy genetic divergence may influence the
175 viability of hybrid seeds, as evidenced by variations in the extent of triploid block observed
176 across primary and secondary contact zones in *A. arenosa* (Morgan et al., 2021a).
177 Subsequently, we aim to bring some novel insight into these hypotheses through a meta-
178 analysis of inter-ploidy crosses conducted in the reviewed studies and uncover the underlying
179 causes and potential consequences on gene flow and speciation.

180

181 **3. Causes for the variation in the triploid block strength**

182

183 ***3.1 Developmental differences***

184 As introduced earlier, the triploid block is primarily manifested in the endosperm, a
185 nourishing tissue that serves to support embryo growth (Lopes and Larkins, 1993). Failure of
186 endosperm development ultimately causes embryo arrest and hybrid seed lethality in
187 interploidy crosses (Scott et al., 1998; Köhler et al., 2010). Endosperm shows a wide
188 diversity across angiosperms, ranging from its development, its persistence after seed
189 maturation, or its ploidy (Baroux et al., 2002). The endosperm development characteristics of
190 a given species could likely be a factor determining the extent of seed lethality upon
191 interploidy hybridization. However, little is known about how diversity in endosperm
192 characteristics across taxa can drive interspecific variation in the strength of the triploid
193 block. Identifying the link between endosperm characteristics and the strength of the triploid
194 block can serve as a basis for understanding the molecular basis of such postzygotic barriers.
195 Therefore, we discuss here the endosperm features that could potentially influence or
196 contribute to the variations in the strength of the triploid block. In this section, we will focus
197 exclusively on intraspecific interploidy crosses for the sake of eliminating potential
198 confounding factors.

199

200 ***3.1.1 Cellular vs nuclear type of endosperm***

201 There are mainly two types of endosperm development: nuclear and cellular. The nuclear
202 mode of development is the most common, found in ~ 160 angiosperm families, including
203 83% dicots (e.g., *Arabidopsis*, soybean, cotton) and monocots such as maize, rice, and wheat

204 (Kordyum and Mosyakin, 2020). In nuclear endosperm formation, an initial phase of repeated
205 free-nuclear divisions (called syncytial phase) is followed by a cellularization (cytokinesis)
206 phase (Lopes and Larkins, 1993; Brown et al., 1999). During the syncytial phase, the
207 endosperm central vacuole acts as the major resource sink, thereby allowing the early embryo
208 to uptake nutrients from the surrounding endosperm mostly through supporting structures
209 (such as the suspensor). With endosperm cellularization, there is a shrinkage of the central
210 vacuole, thereby marking the transition to the phase when the embryo acts as the primary
211 resource sink (additionally, the suspensor degenerates), being fed directly from the
212 endosperm (Lafon-Placette and Köhler, 2014). Hence, the shift from the syncytial phase to
213 the cellular phase is a crucial point (Hehenberger et al., 2012) for embryo nourishment as has
214 been demonstrated in *Arabidopsis thaliana* mutants with defective endosperm cellularization
215 (Kradolfer et al., 2013). Therefore, embryo arrest in response to endosperm cellularization
216 failure may be caused by a disrupted supply of nutrients to the embryo.

217 Interploidy crosses in species possessing nuclear-type endosperm demonstrated
218 deviations in the timing of cellularization in the endosperm (*Arabidopsis* - (Scott et al., 1998;
219 Morgan et al., 2021a); *Oenothera* - (Wangenheim, 1962). In mat-excess crosses, cell wall
220 formation is initiated earlier than the normal endosperm, leading to a shorter cell proliferation
221 phase. This leads to smaller but plump seeds. In the reciprocal cross (pat-excess),
222 cellularization is delayed, and as a result, cell proliferation continues longer than in normal
223 endosperm. In addition, the central vacuole still occupies major parts of the endosperm at
224 later stages of seed development, and hence, may remain the major nutrient sink in the seed
225 while the embryo suffers from reduced/blocked nutrient supply (Morley-Smith et al., 2008;
226 Hehenberger et al., 2012; Lafon-Placette and Köhler, 2014). This in turn may lead to reduced
227 embryo growth and finally embryo arrest. In maize (*Zea mays*), (Pennington et al., 2008)
228 reached comparable findings concerning the cellularization of endosperm and the size of
229 hybrid seeds. It was observed that seeds resulting from mat-excess crosses were smaller as
230 compared to those from pat-excess crosses. Moreover, the endosperm in mat-excess crosses
231 exhibited earlier cellularization, while in pat-excess it displayed an extended period of cell
232 proliferation. Furthermore, while the endosperm in mat-excess crosses accumulated
233 substantial starch, in pat-excess it accumulated minimal starch due to a delayed onset of
234 starch formation. Consequently, only a few (1.7% pat-excess, 0.83% mat-excess) seeds
235 germinated from the reciprocal interploidy crosses in maize (Pennington et al., 2008), unlike
236 in *Arabidopsis* where mat-excess interploidy hybrid seeds are highly viable (Scott et al.,
237 1998; Morgan et al., 2021a). Alterations in the accumulation patterns of storage compounds

238 were evident in rice (*Oryza sativa* - (Sekine et al., 2013) interploidy crosses, mirroring the
239 observed pattern in maize. The endosperm from mat-excess crosses accumulated notable
240 amounts of starch, whereas no starch granules were detected in crosses with pat-excess.
241 Despite a similar pattern of starch accumulation in maize and rice, they show a stark contrast
242 in germination outcomes. Pat-excess seeds showed no germination, while mat-excess seeds
243 exhibited high seed germination (50%) in rice. However, all resulting seedlings succumbed at
244 an early stage. This stands in contrast to maize (Pennington et al., 2008), where the triploid
245 block seems robust in both directions. Nevertheless, the failure of seeds involving parents
246 with different ploidy levels can be attributed to the altered progression of endosperm
247 cellularization- either delayed or accelerated and such aberrant endosperm development
248 patterns may be conserved across diverse angiosperm families. It appears that the occurrence
249 of precocious endosperm cellularization in mat-excess crosses is often less critical for seed
250 survival compared to its absence in pat-excess crosses, as mat-excess seeds tend to exhibit
251 better survival (i.e., seed viability) rates than pat-excess ones. However, this does not
252 consistently follow a trend, as some taxa with nuclear endosperm deviate from this pattern.
253 For instance, in Rye (*Secale cereale*), the viability rates are 47.2% in pat-excess and 30% in
254 mat-excess (Håkansson and Ellerström, 1950), and in *P. echinoides*, the rates are 66% in pat-
255 excess crosses and 44% in mat-excess (Chrtek et al., 2017).

256 While the cellular mode of endosperm formation is not very common, it is found in ~
257 80 angiosperm families, mostly dicots (e.g., balsam, petunia), and only a few monocot
258 families, such as Araceae, and Lemnaceae (Kordyum and Mosyakin, 2020). In the cellular
259 mode of development, nuclear divisions are followed by cell-wall depositions, thus, each
260 nuclear division (karyokinesis) is followed by a subsequent cytokinetic division, right from
261 the beginning (Lopes and Larkins, 1993). Similar to the nuclear mode of development, the
262 nutrient transfer in cellular endosperm takes place through adjacent maternal supporting
263 tissues, such as the integumentary cell layers present in the seed coat (Håkansson, 1952).
264 During the early stages of endosperm development in *Lycopersicon pimpinellifolium*
265 (Solanaceae), the endothelium (the inner epidermis of the integument) plays a crucial role in
266 nutrient transfer and support for the growing endosperm and embryo (Cooper and Brink,
267 1945). Following fertilization, the endothelium forms a single well-defined layer of densely
268 cytoplasmic cells surrounding the expanding endosperm. As the seed grows, the endothelium
269 divides along the radial axis. The nutrient absorption process involves the chalazal aperture
270 opposite the end of the conducting tissue and the endothelium. Nutrients enter the seed
271 through the vascular bundle, moving partially through the chalazal aperture and the active

272 cytoplasmic endothelium. Endosperm cells, especially those opposite the chalazal pocket,
273 exhibit dense cytoplasm, suggesting an active role in nutrient absorption and hence, serving
274 as the primary resource sink. Additionally, nutrients may diffuse into the depleted
275 integument, being absorbed by the endosperm through the endothelium. Overall, endothelium
276 serves as a conduit for nutrient transport, facilitating the growth and development of the seed,
277 and any alterations in endothelium growth may be a crucial point for embryo development
278 (Cooper and Brink, 1945; Håkansson, 1952). This has been supported by experimental
279 crosses in *Lycopersicon pimpinellifolium* (Cooper and Brink, 1945), where the viable
280 homoploid crosses exhibit high endosperm to endothelium ratios when compared to inviable
281 reciprocal interploidy crosses, with smaller endosperm and thicker endothelium.

282 Seeds obtained from pat-excess crosses are characterized by highly vacuolated cells
283 of endosperm and enlarged endothelial cells (Cooper and Brink, 1945). As the depletion of
284 cells in the inner part of the integument occurs at a slower pace, there is a notable
285 accumulation of nutrients just outside the chalazal aperture during the developmental process,
286 thereby, blocking the nutrient supply towards the growth of the embryo. The endosperm cells
287 in mat-excess crosses exhibit a lag in development compared to the pat-excess crosses and
288 the seeds exhibit a smaller endosperm cavity. The endothelium cells exhibit a hyperplastic
289 pattern, with a notable scarcity of cytoplasm in the endosperm cells adjacent to the
290 overgrown endothelial tissue. Although accumulation of nutrients in the chalazal pocket was
291 observed in mat-excess hybrid seeds, the embryo collapsed in the end. This suggests there
292 could be other factors preventing the proper utilization of these reserves, leading to potential
293 starvation of the endosperm. Although the development in reciprocal crosses proceeds quite
294 differently and the mat-excess crosses yield significantly more mature seeds compared to that
295 of pat-excess crosses, the seeds are non-germinable in the end (Cooper and Brink, 1945).
296 Similarly, no germinable seeds were obtained in *Galeopsis pubescens* (Lamiaceae)
297 (Håkansson, 1952), although there is a pronounced difference between endosperm
298 development in pat-excess and mat-excess crosses. In the former, the development stops very
299 early, and degeneration sets in rapidly. In the latter, the endosperm development continues for
300 a longer period, although at a very slow rate; but eventually degenerates. Thus, the embryo in
301 the mat-excess cross is large compared with the small endosperm tissue, but further growth is
302 neglectable. No clear defects of the endothelium could be observed besides a longer
303 persistence in both pat-exc and mat-exc crosses (Håkansson, 1952). In *Datura stramonium*
304 (Sansome et al., 1942), the rate of development of the proembryo and endosperm in the
305 reciprocal interploidy hybrids slows down when compared to the control homoploid hybrids

306 as the development progresses. Subsequently, the contents of the seeds disintegrate about 7 –
307 13 days after pollination. The disintegration may occur first in either the proembryo or in the
308 endosperm. Endothelium cells were found to be enlarged in mat-excess crosses which
309 eventually degenerate along with the embryo-sac contents. On the other hand, in pat-excess
310 crosses, while the endothelium appeared as a single layer like in viable homoploid crosses,
311 with a few large endosperm cells and some small compact endosperm cells, subsequently
312 disintegration takes place in both proembryo and endosperm. It is interesting to note that the
313 opening in the endothelium layer, which forms a communication between the contents of the
314 embryo sac and the outer maternal tissue at the chalazal end, was still evident in the inviable
315 interploidy crosses. However, the seed contents degenerate, thereby, suggesting a poor
316 utilization of the nutrient reserves, leading to potential embryo arrest. Only 0.5 % of seeds
317 were germinable in mat-excess crosses, while in pat-excess crosses, germinable seeds were
318 rarely obtained.

319 To conclude, for the cellular type of endosperm, changes in the somatic tissue of the
320 seed, especially the maternal endothelium tissues surrounding the embryo sac, and the altered
321 development of endosperm, may cause an interrupted nutrient supply to both the endosperm
322 and the embryo. The mechanism through which such altered development leads to the
323 observed disintegrations, or the involvement of other factors, remains unknown at present,
324 despite the common incidence of the cellular mode of development. In our study, we did not
325 observe a clear trend of mat-/pat-excess asymmetry concerning triploid seed viability, unlike
326 in nuclear endosperm. Both mat- and pat-excess crosses exhibited low survival rates, despite
327 showing significantly different developmental courses.

328

329 ***3.1.2 Transient vs permanent endosperm***

330 In many species, the endosperm is consumed before seed maturation (transient endosperm)
331 (Vijayaraghavan and Prabhakar, 1984; Becraft et al., 2001; Simpson, 2010). However, it is
332 persistent (permanent endosperm) in others, such as cereal grains where the endosperm stores
333 the seed reserves and represents a major source of food and industrial value for human
334 nutrition (Vijayaraghavan and Prabhakar, 1984; Becraft et al., 2001; Simpson, 2010; Awika,
335 2011). In permanent endosperm, the embryo continues to use nutrients from the endosperm
336 during seed germination, while in the transient endosperm, the endosperm ceases to exist and
337 instead cotyledons represent the major reservoir for nutrients required during seed
338 development and germination (Yan et al., 2014). Transient endosperms are often a
339 characteristic feature of dicot crops such as soybeans and peas, and model plants such as

340 *Arabidopsis* (Li, 2017).

341 In rice and maize (Pennington et al., 2008; Sekine et al., 2013), virtually no
342 interploidy seedling could be retrieved. This was due to non-germinable seeds to a large
343 extent, but interestingly, in rice, while mat-excess seeds could germinate at relatively high
344 rates (50%), seedlings died at an early stage. This may reflect the role of the endosperm in
345 supporting the embryo after germination, and its impaired function in interploidy seeds.
346 Similarly, reciprocal hybrids in *Galeopsis* and *Oenothera* are barely viable (Hákansson,
347 1952; Wangenheim, 1962). However, in taxa such as *Cyclamen* and *Pilosella* (Takamura and
348 Miyajima, 1996; Chrtek et al., 2017), reciprocal interploidy crosses exhibit relatively higher
349 hybrid viability, despite these species sharing the common characteristics of having a
350 permanent endosperm. On the other hand, in taxa with a transient endosperm, the variation
351 also ranges from mostly viable seeds in crosses with higher maternal dosage, as observed in
352 *Arabidopsis* and *Brassica* (Scott et al., 1998; Stoute et al., 2012) to the near absence of viable
353 seeds in reciprocal crosses in *Mimulus* (Meeus et al., 2020); Salony et al. in review).

354 To conclude, cereal crop species appear more sensitive to endosperm defects during
355 interploidy hybridization. This sensitivity cannot be solely attributed to the persistence of the
356 endosperm, but may be influenced by the characteristics of the family and other factors that
357 reinforce the triploid block. We also hypothesized that taxa such as Brassicaceae or Fabaceae,
358 which have transient endosperms, might better survive endosperm defects as this tissue
359 appears to play a role only during a restricted developmental window, which we tested
360 further in this study (Chapter 3.3).

361

362 ***3.2 Genetic divergence between diploids and their polyploid relatives***

363 Triploid block is a postzygotic barrier, primarily caused by a dosage imbalance between the
364 maternal and paternal genomes in the endosperm. However, endosperm-based hybrid seed
365 lethality also occurs between species of same ploidy (Städler et al., 2021) as a result of
366 negative epistasis between mutations that arose independently in each species, following the
367 Bateson-Dobzhansky-Meyer incompatibility model (Orr, 1996). In this model, the probability
368 of incompatible mutations increase exponentially with divergence time between lineages. It is
369 therefore likely that the divergence time between diploid and polyploid lineages will affect
370 the survival of interploidy hybrid seeds. In other words, we can expect that the more genetic
371 divergence between diploids and polyploids, the more time to accumulate incompatible
372 mutations and thus the stronger the manifestation of the triploid block.

373 Alternatively, after WGD, polyploid genomes gradually decrease, through genome
374 downsizing and the loss of duplicated genes and non coding regions (Buggs et al., 2009;
375 Wendel et al., 2018). These changes lead to a gradual reduction in the genome size in
376 polyploid individuals, which could weaken the triploid block by reducing gene dosage
377 imbalances and skewing gene expression patterns towards the diploid-like state. This is likely
378 to be more prominent in "older" polyploid lineages than younger ones.

379 Altogether, genetic divergence between diploids and polyploids is likely to have
380 conflicting effects on the strength of the triploid block depending on the specific polyploid
381 system and the age of the polyploid lineage. However, these hypotheses remain to be tested
382 empirically, which is the focus of the subsequent section.

383

384 ***3.3 Testing of the hypotheses with a meta-analysis***

385 To go beyond mere literature review and speculation, we tested formally with a meta-analysis
386 whether the type of endosperm (cellular/nuclear; transient/permanent) or the divergence
387 between diploids and polyploids could be associated with the strength of the triploid block or
388 with the parent-of-origin pattern of hybrid seed viability. While we could directly assign a
389 type of endosperm to each species based on the literature, we had to rely on a proxy to assess
390 the divergence between diploids and polyploids. We compared synthetic and natural
391 polyploids, assuming that synthetic polyploids are (nearly) isogenic while natural polyploids
392 already evolved independently from diploids, therefore synthetics are genetically closer to
393 diploids than natural polyploids.

394

395 ***3.3.1 Compilation of the dataset***

396 We reviewed 29 published studies of mixed-ploidy species in the literature that comprised
397 diploids and natural auto-tetraploids and involved interploidy crossing data to address the
398 variation in the strength of the triploid block and identify a common pattern, if any. We
399 performed the literature survey using Google Scholar, using the keywords ["triploid block" or
400 "triploid hybrids" or "interploidy hybrids" or "interploidy crosses" or "hybrid seed failure"]
401 and [tetraploids] and [plants] from the 1900s to the present. A given study was included in
402 our dataset only if (1) the focus was comparing diploid plants with their tetraploid
403 counterparts from the same species (intraspecific crosses) (2) either or both of the diploid or
404 tetraploid control estimates for hybrid seed viability was measured (3) either or both of the
405 pat- or mat-excess estimates for hybrid seed viability was measured (4) strength of triploid
406 block was estimated by phenotyping of mature seeds (and not ovules) (5) germination or seed

407 abortion rates were used as a proxy for seed viability. The proportion of seeds that appeared
408 viable was used as a proxy when germination rate data was unavailable, provided there was a
409 proper description of seed morphology. In most papers, it was possible to extract numerical
410 values from tables, but with a single exception, where we extracted the data directly from the
411 figures using ImageJ (Schneider et al. 2012). The selected studies generally reported hybrid
412 seed viability as either the germination proportion or morphologically described and
413 categorized the viable and inviable seeds. In the later cases, we computed the viability proxy
414 by taking a ratio of viable seeds to the sum of viable and inviable seeds. Rarely, some studies
415 reported hybrid seed viability data using multiple replicates of control and reciprocal
416 interploidy hybridizations. In such cases, we considered the average value for each
417 hybridization treatment.

418 In total, we reviewed 19 angiosperm species representing 19 genera from 11 families
419 (see Supplementary Table 1). Three species belong to Poaceae and Solanaceae; four families
420 are represented by two species, and five families are represented by a single species ($X^2 =$
421 190 , $df = 180$, $p = 0.2903$) (see Supplementary Table 1). Of the 19 species examined, 11
422 exhibited a nuclear type of endosperm and 8 exhibited a cellular type. In total, we have 31
423 estimates for hybrid viability from diploid ($N = 15$) and tetraploid ($N = 16$) control crosses
424 (the remaining values were not available, indicated as NA), while 16 and 19 estimates
425 respectively for seed viability of pat- and mat-excess hybridizations. It's crucial to emphasize
426 that we obtained estimates inconsistently distributed across species when considering all the
427 factors examined to account for variations in the triploid block strength. While having all four
428 estimates (seed viability in 2x-control, 4x-control, pat-excess, and mat-excess crosses) from a
429 single species would enhance the accuracy of gauging the triploid block's strength for that
430 species, unfortunately, this is not the case (see Supplementary Table 1).

431

432 **3.3.2 Statistical analysis**

433 We chose to perform a Bayesian meta-analysis by using the MCMCglmm package (Hadfield,
434 2010). We wrote the following model:

$$435 W_{ijk} = \mu + \text{parental excess } i : X_j + \text{genus } k + \varepsilon_{ijk} \quad (1)$$

436 where W_{ijk} is the fitness of triploids, μ is the mean value, and parental excess i is the effect
437 of the parental excess i (pat-excess or mat-excess), in interaction with another fixed factor X_j
438 being potentially

$X_j =$ *type of*
endosperm
endosperm
persistence
type of
polyploids

439

440 . In these factors, the type of endosperm can be cellular or nuclear, the persistence of
441 endosperm in mature seeds can be transient or permanent, and the polyploid can be either
442 natural or synthetic. We only included a single random effect: genus k is the effect of the k th
443 genus, and ϵ_{ijk} is the residual error. We chose to control the variability among genera rather
444 than among species (which is generally the case in meta-analyses). We assumed that the
445 residual error followed a Gaussian distribution. We performed these models with two
446 different datasets, one using the relative performance of triploid individuals compared to
447 parental lines, and the other using the absolute viability of triploids. The relative performance
448 of mat-excess or pat-excess triploids was determined by taking the ratio of absolute values of
449 the respective interploidy hybrid to the average value of the homoploid hybrids.

450 For all analyses, we used the weakly informative, default priors proposed in
451 MCMCglmm (Hadfield, 2010). For fixed effects, the prior is a normal distribution with the
452 mean being equal to zero and a variance of 10^{10} . For random effects, inverse-Wishart priors
453 are implemented, with the degree of belief parameter being equal to zero and the expected
454 variance being equal to 1. For all models, we used a burn period of 1.000.000 iterations, with
455 a thinning interval of 50, and the MCMC chains were run for 6.000.000 iterations in total.
456 The parameter models and associated 95% credible intervals were thus inferred from the
457 sampling of the posterior distribution 100.000 times. We did a visual examination of the

458 convergence, posterior traces, and autocorrelation values of our models, as suggested in
459 (Hadfield, 2010).

460

461 **3.3.3. Results**

462 As the analyses based on the relative and absolute hybrid seed viability gave similar results,
463 we chose to only present the results for the relative performance. None of the factors we
464 tested were significant (Fig. S1). We found a consistent trend of mat-excess hybrids being
465 more viable than the pat-excess ones, but no significant differences have been found (Fig. 4).
466 We found no differences between species having cellular or nuclear endosperm (Fig. 4, *left*),
467 no differences between species having transient or permanent endosperm (Fig. 4, *middle*),
468 and finally no differences between triploids involving synthetic or natural polyploids (Fig. 4,
469 *right*).

470

471 **3.3.4. Discussion**

472 In this section, we investigated the impact of various aspects of endosperm development and
473 the degree of genetic divergence between ploidies on the variation of the triploid block
474 strength. However, none of the factors we tested demonstrated a significant effect on the
475 strength of the triploid block. Notably, Brassicaceae plants exhibited a pattern of strong mat-
476 excess and weak pat-excess triploid block, but this trend was not statistically significant.
477 Nevertheless, it is crucial to acknowledge that the limited number of studies included in our
478 analysis makes it difficult to draw definitive conclusions about the factors influencing triploid
479 block strength. The mechanistic basis of triploid block is primarily studied in species
480 possessing nuclear endosperm including cereal crops and the endosperm-embryo interaction
481 in heteroploid crosses is limited to model species, such as *A. thaliana*. Consequently, the
482 intricate interplay of the triploid block in cellular endosperm remains poorly understood, with
483 a lack of available information, which makes it hard to link triploid block strength and
484 development causes for this type of endosperm. Hence, there is an urgent need to explore the
485 strength of the triploid block in non-model species to gain deeper insights into the general
486 causes of large variations in triploid block strength across angiosperm diversity.

487 Furthermore, there is a significant lack of consistency and coherence among studies
488 concerning the estimation of triploid hybrid seed viability. Relying solely on heteroploid
489 crosses without including homoploid crosses as control can result in an incomplete and
490 inaccurate assessment of the triploid block strength. However, many studies did not include
491 control homoploid crosses in their investigation, limiting the power of their inference of the

492 triploid block strength for multi-species comparisons. In addition, the lack of a unified
493 approach in scoring triploid viability, particularly scattered over-assessments of ovules and
494 mature seeds or utilizing seed sets or even fruit sets as a proxy, further hampers an accurate
495 estimation. Therefore, there is a pressing need for a comprehensive and standardized method
496 to estimate the strength of the triploid block, one of them being simply seed germination with
497 sufficient sample size (>100 seeds per cross), which was, unfortunately, lacking in many
498 studies we found. Following such a standardized approach and conducting rigorous
499 comparative analyses can provide a better understanding of the cause for the variation of
500 triploid block strength across angiosperms.

501

502 **4. Evolutionary consequences of the triploid block**

503 The range of variation in the strength and parent-of-origin pattern of the triploid block may
504 bear consequences for interploidy gene flow in nature. For example, taxa with leakier triploid
505 block may show more pronounced gene flow compared to taxa with a strong triploid block.
506 The real manifestation of the triploid block, however, depends on the strength of the other
507 barriers acting prior (prezygotic) or after (later postzygotic) triploid block. While prezygotic
508 barriers may have a key role in restricting gene flow (Husband and Sabara, 2004),
509 postzygotic barriers such as seed inviability are presumed to have a minor role in building
510 reproductive isolation since they act late in the hybridization sequence (Coyne and Orr,
511 2004). In this section, we evaluate how well we can predict genetic isolation between ploidies
512 by comparing data on the triploid block from experimental crosses with the measure of
513 interploidy gene flow in nature.

514 One way to assess the strength of interploidy isolation is to measure the presence of
515 triploid hybrids in natural populations. The occurrence of triploid hybrids largely varies
516 depending on the species, ranging from a large number of triploids in *Pilosella echioides* (up
517 to 73%; (Trávníček et al., 2011) or *P. rhodopea* (up to 58%; (Šingliarová et al., 2011), or
518 *Galax urceolata* (up to 23%; (Burton and Husband, 1999), to the virtual absence of such
519 hybrids in other systems such as *Arabidopsis arenosa* (0.14%; (Morgan et al., 2020) or
520 *Mimulus guttatus* (0, Salony et al. in review). The variation in the occurrence of triploid
521 hybrids in nature could be underlain by variations in the strength of the triploid block
522 between taxa. However, it could also be the result of other factors, such as clonal propagation
523 promoting the propagation of the odd-ploidy cytotypes (Trávníček et al., 2011; Chrtek et al.,
524 2017), or a large set of prezygotic barriers preventing any interploidy hybridization in the
525 first place. It is therefore important to evaluate whether the triploid block or other factor(s)

526 are most likely to explain the range of triploid hybrid occurrence observed in different
527 systems.

528 Although numerous natural cytotype surveys have been done in mixed-ploidy plants,
529 and numerous studies estimated the triploid block strength with experimental crossings, very
530 few studies have actually combined both in an attempt to relate the triploid block strength
531 with the presence of triploid hybrids in nature. In *Chamerion angustifolium*, a species with
532 relaxed triploid block (Burton and Husband, 2000), a significant proportion of triploids (9%)
533 were found in natural populations (Husband and Schemske, 1998). Also, we see a strikingly
534 high triploid frequency in *Pilosella echiodies* (or *Hieracium echiodies*) in the field (around
535 73%; (Trávníček et al., 2011), which corroborates the higher triploid viability (averaging 77
536 % in both directions) found in a crossing experiment performed by (Chrtek et al., 2017). On
537 the contrary, *Plantago media* exhibits robust triploid block, as evidenced by the rare triploid
538 formation in controlled crossing experiments (0.8% triploid hybrids; (Van Dijk and Van
539 Delden, 1990). Consistently, triploids were also rare in the mixed ploidy populations (0.44%,
540 (Dijk et al., 1992). Similarly, the triploid block was found to be strong in *Oenothera hookeri*
541 (approx. 5% survival; (Wangenheim, 1962), and no natural triploids were observed in this
542 species. From the above studies, one may propose that there is at least a correlation between
543 the triploid block and the occurrence of triploid hybrids in nature in the extreme cases: when
544 the triploid block is strong, logically, (nearly) no triploids are reported from the field. On the
545 other hand, frequent triploids are found in some species with known relaxed triploid block.

546 There are, however, cases where experimental data do not correspond with field
547 observations. For example, in *Arabidopsis arenosa*, mat-excess crosses result in largely
548 viable (~75%) triploid progeny (Morgan et al., 2021a), while triploid plants are virtually
549 absent from natural mixed-ploidy populations (Morgan et al., 2020), likely due to strong
550 prezygotic post-pollination barriers (Morgan et al., 2021b).

551 Overall, while reciprocal crosses in interploidy hybridization experiments provide a
552 valuable understanding of reproductive barriers, they may not necessarily translate to similar
553 patterns in nature due to the complex interplay of reproductive isolation pathways in natural
554 populations. However, studies comparing the manifestation of the triploid block after
555 experimental crosses with interploidy gene flow in nature are too rare to firmly conclude on
556 this point. Future interdisciplinary investigations of the mechanism (crossing experiments)
557 and realized impact in the field (population genetics and cytogeography) of triploid block
558 may provide valuable insights into the implications for reproductive isolation and
559 evolutionary processes.

560

561 **5. Conclusions & perspectives**

562 Understanding the strength of the triploid block is of significant importance as it has
563 implications for speciation and the dynamics of polyploid populations. Triploid block can act
564 as a reproductive barrier between different ploidy levels, contributing to speciation. If the
565 triploid block is strong and effectively prevents the formation of triploid hybrids, it can
566 promote the formation of distinct diploid and polyploid lineages, thereby leading to the
567 establishment of new species over evolutionary time. In contrast, an incomplete triploid block
568 may allow the formation of triploid hybrids, leading to increased levels of hybridization and
569 gene flow between different ploidy levels. This can result in higher levels of genetic diversity
570 within polyploid populations and potentially contribute to the generation of evolutionary
571 novelties with adaptive advantages and may promote neo polyploid establishment.

572 Our present study shows that the triploid block is not always a strong barrier to
573 interploidy hybridization. Instead, the strength of the triploid block largely varies depending
574 on the species, suggesting different outcomes for interploidy hybridization in nature
575 potentially due to mere phylogenetic signal. We tested the impact of types of endosperm
576 development and genetic divergence between diploids and polyploids, but could not identify
577 any clear cause for this variation. We also could not provide statistical support for the
578 commonly accepted idea that mat-excess seeds survive to a better extent than pat-excess
579 ones. While this could suggest that previous hypotheses related to the triploid block
580 mechanisms are wrong, a likely explanation is the lack of suitable and comparable data
581 available in the literature. Despite a large number of studies with interploidy experimental
582 crosses, too few have actually robust sample sizes, parental controls, or clearly defined
583 criteria to assess basic proxies of triploid block such as seed viability. This calls for future
584 works on the triploid block in a unified and rigorous setup, which should include: 1) a clear
585 definition of what "viable seed" is assessed, and seed germination should be the ultimate
586 measure for seed viability; 2) control crosses for both the diploid and the polyploid parent, as
587 many studies we encountered either had no control crosses or only one of the two parents; 3)
588 a clear definition of "seed set" as the total number of seeds (including inviable ones); when
589 defined, "seed set" sometimes referred either to the total number of seeds or to the number of
590 viable-looking seeds. The first definition measures prezygotic isolation while the second
591 confounds both pre and postzygotic isolation. Finally, the impact of the triploid block on gene
592 flow in nature remains questionable, even though, again, too few studies are available to draw
593 firm conclusions on this matter.

594

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600

601 **Author contributions**

602 C.L.P., S.S. and F.K. conceived the study. S.S. conducted the literature search. S.S. and J.C.
603 carried out data analysis. S.S., J.C., and C.L.P. wrote the manuscript. S.S., J.C., F.K and
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606

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611

612 **Declaration of interests**

613 The authors declare no competing interests.

614

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843

844 **Figure legends**

845 **Fig. 1** Factors influencing the variations in the strength of the triploid block, as proposed in
846 this study. These factors include the mode of endosperm development, the persistence of
847 endosperm in mature seeds, and the divergence time between diploids and polyploids, all of
848 which may potentially impact the strength of the triploid block.

849

850 **Fig. 2** Developmental progress of endosperm, embryo and surrounding maternal tissues in the
851 seeds obtained from control and interploidy hybridisations; a. nuclear endosperm b. cellular
852 endosperm; em: embryo, en: endosperm, ent: endothelium, int: integumentary cells, sc: seed
853 coat.

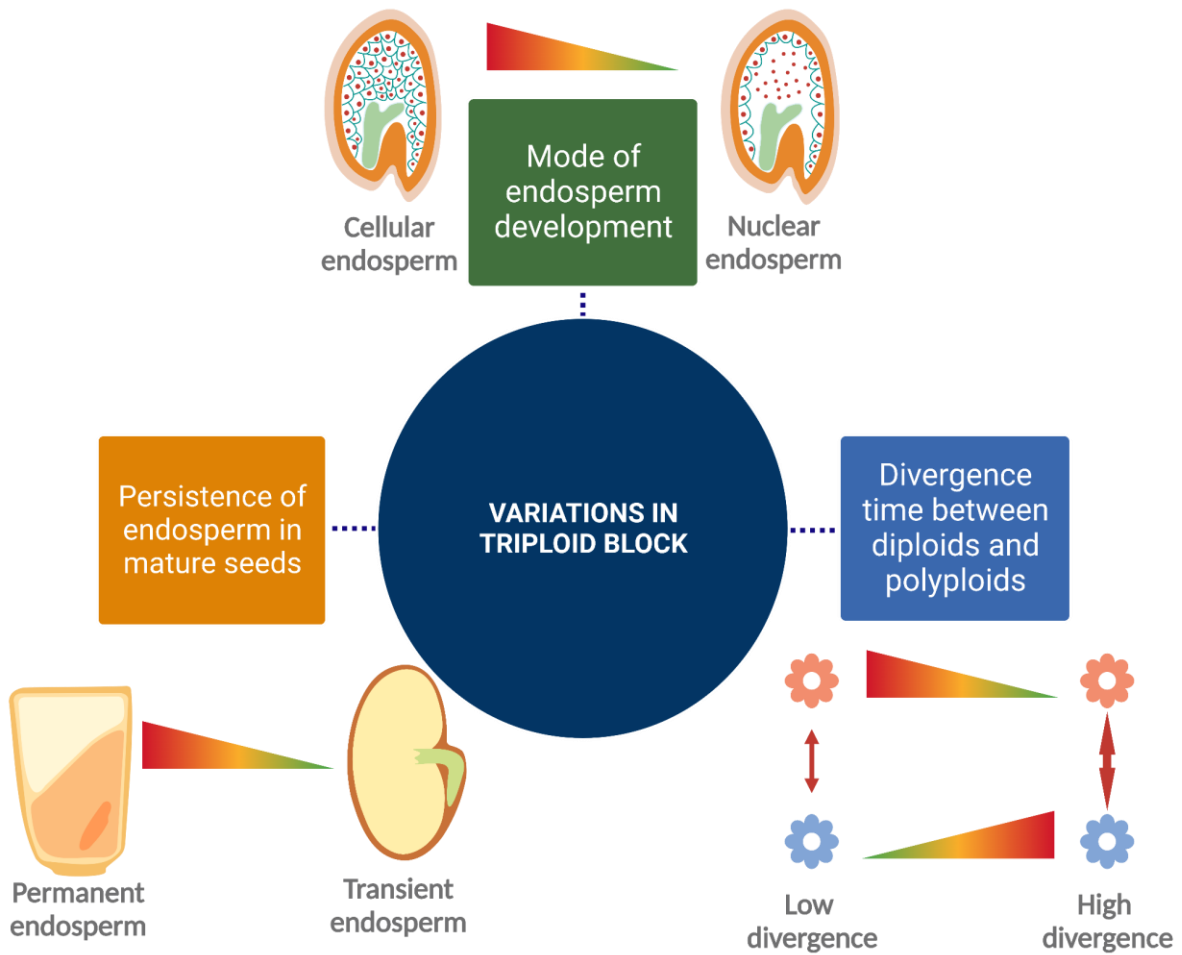
854

855 **Fig. 3** Proportion of absolute hybrid viability across species, grouped according to respective
856 taxa studied in the present review. Mat-excess and Pat-excess hybrids are represented by red
857 and blue respectively.

858

859 **Fig. 4** Asymmetry in the relative performance of triploid hybrids (seed germination) with
860 respect to control homoploid hybrids, tested against different factors that were hypothesized
861 to potentially affect the triploid embryo viability: type of endosperm development (*left*),
862 persistence of endosperm in mature seeds (*middle*), type of polyploids used in interploidy
863 crosses (natural or synthetic) (*right*). Mat-excess and Pat-excess hybrids are represented by
864 red and blue respectively.

865



867

868 **Fig. 1** Factors influencing the variations in the strength of the triploid block, as proposed in
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871 which may potentially impact the strength of the triploid block.

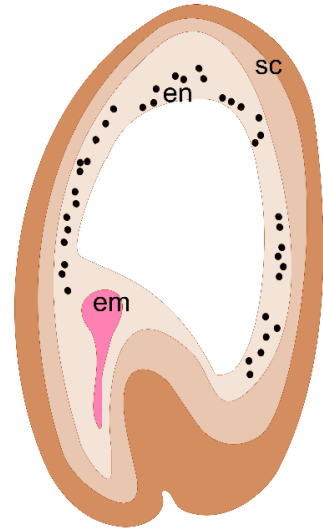
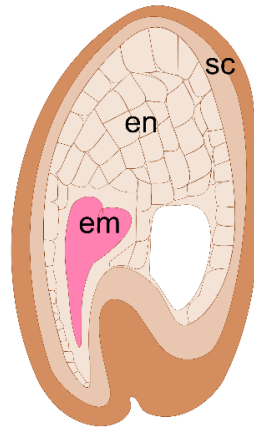
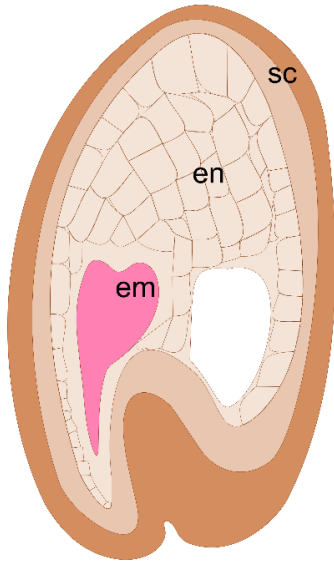
872

A. Nuclear endosperm

Balanced crosses:
 - normal endosperm development
 - normal embryo development

Maternal-excess:
 - early cellularisation
 - smaller seeds
 - embryo arrests

Paternal-excess:
 - delayed cellularisation
 - larger seeds
 - embryo arrests

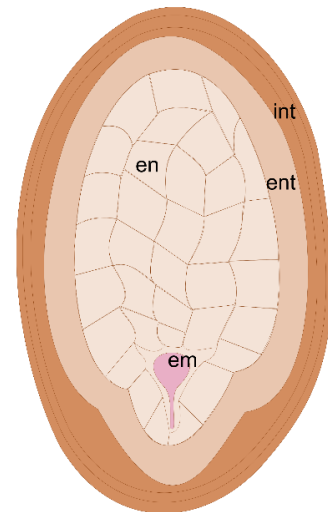
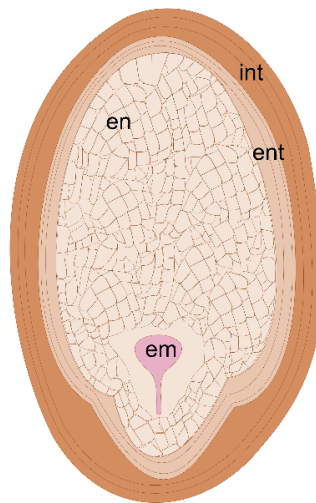
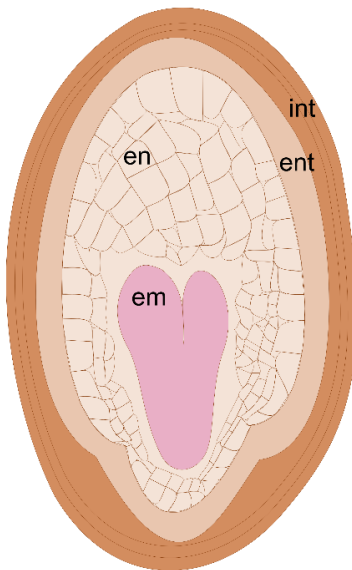


B. Cellular endosperm

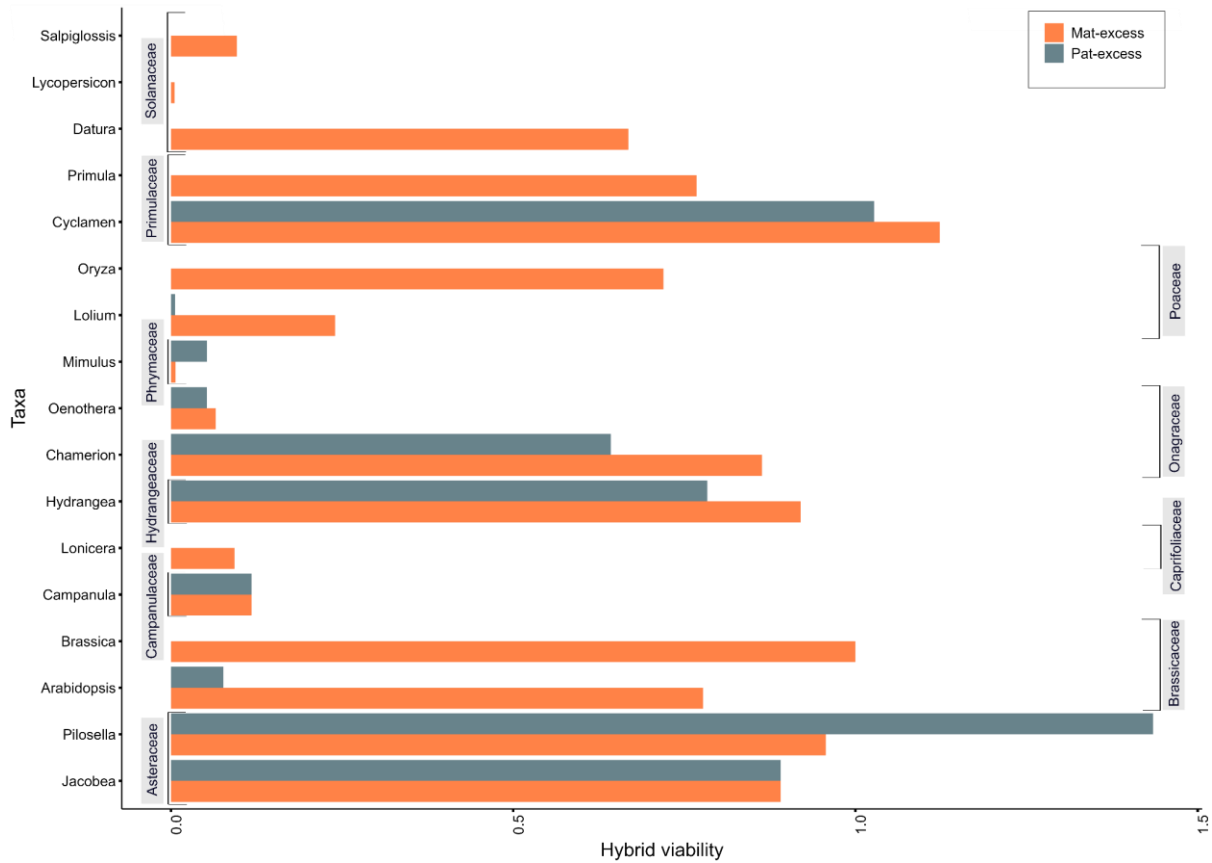
Balanced crosses:
 - thin endothelium layer, with exception in Galeopsis
 - normal endosperm development
 - normal embryo development

Maternal-excess:
 - overgrown endothelium layer
 - small, irregular endosperm cells
 - embryo arrest

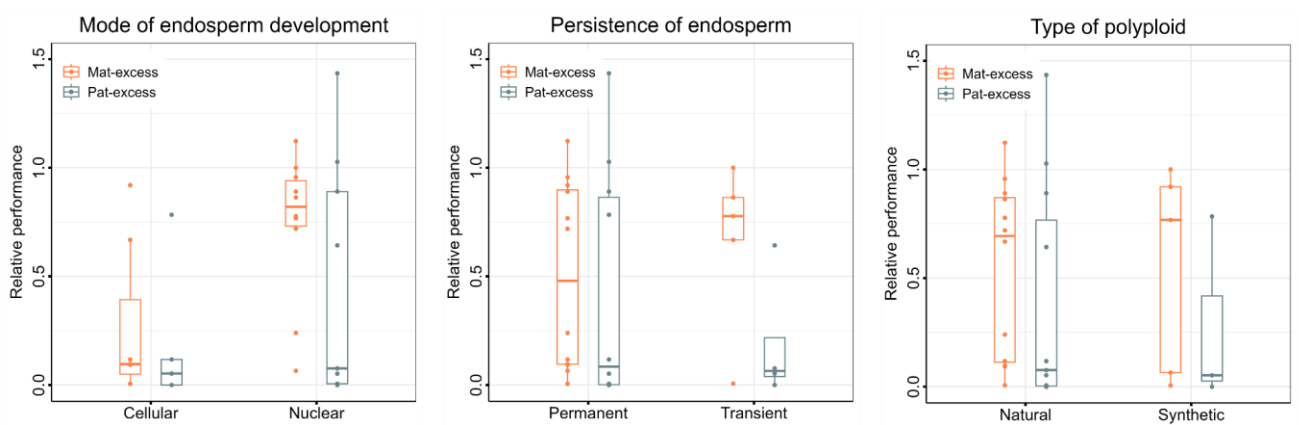
Paternal-excess:
 - enlarged endothelium layer
 - large, irregular endosperm cells
 - embryo arrest



875 **Fig. 2** Differences in the developmental profile of endosperm, embryo and surrounding
 876 maternal tissues in the seeds obtained from control and interploidy hybridisations; a. nuclear
 877 endosperm b. cellular endosperm; em: embryo, en: endosperm, ent: endothelium, int:
 878 integumentary cells, sc: seed coat.
 879



880
 881 **Fig. 3** Proportion of absolute hybrid viability across species, grouped according to respective
 882 taxa studied in the present review. Mat-excess and Pat-excess hybrids are represented by red
 883 and blue respectively.
 884

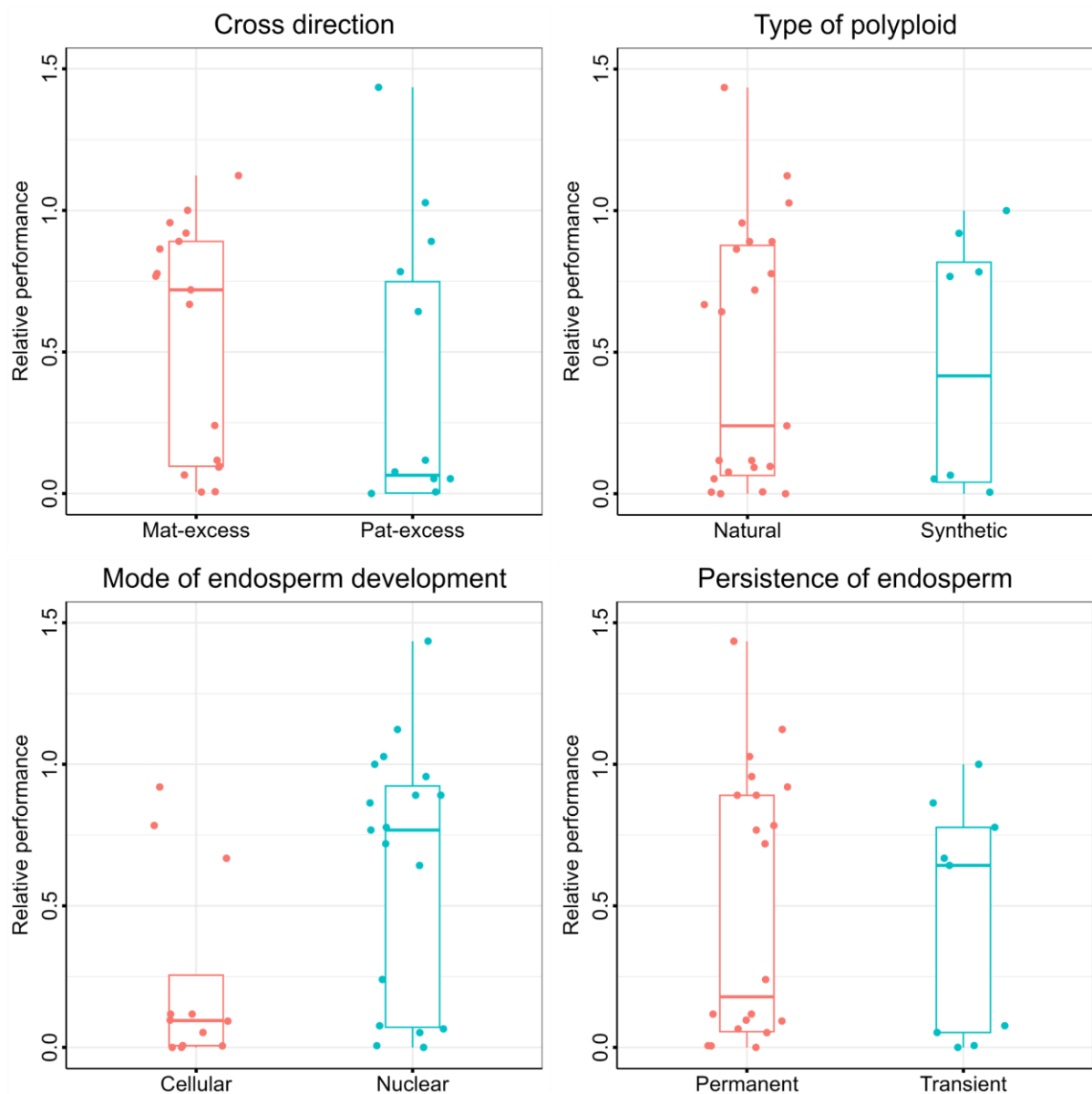


885

886 **Fig. 4** Asymmetry in the relative performance of triploid hybrids (seed germination) with
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 888 to potentially affect the triploid embryo viability: type of endosperm development (*left*),
 889 persistence of endosperm in mature seeds (*middle*), type of polyploids used in interploidy
 890 crosses (natural or synthetic) (*right*). Mat-excess and Pat-excess hybrids are represented by
 891 red and blue respectively.

892

893 **Supplemental information**

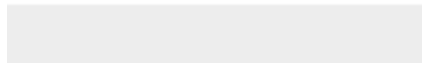


894

895 **Fig. S1** Relative performance of triploid hybrids wrt different factors that could potentially
 896 affect the triploid viability: asymmetry in parental genome contribution (during reciprocal
 897 interploidy crosses) (*top left*), type of polyploids (*top right*), type of endosperm development
 898 (*bottom left*), persistence of endosperm in mature seeds (*bottom left*).



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