



HAL
open science

Organ-wide and ploidy-dependent regulations both contribute to cell size determination: evidence from a computational model of tomato fruit

Valentina Baldazzi, Pierre Valsesia, Michel Génard, Nadia Bertin

► To cite this version:

Valentina Baldazzi, Pierre Valsesia, Michel Génard, Nadia Bertin. Organ-wide and ploidy-dependent regulations both contribute to cell size determination: evidence from a computational model of tomato fruit. 2018. hal-01953178

HAL Id: hal-01953178

<https://inria.hal.science/hal-01953178>

Preprint submitted on 12 Dec 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Organ-wide and ploidy-dependent regulations both contribute to cell size**
2 **determination: evidence from a computational model of tomato fruit**

3 Valentina Baldazzi^{1,2,3}, Pierre Valsesia¹, Michel Génard¹, Nadia Bertin¹

4

5¹INRA, PSH, France

6²Université Côte d'Azur, INRA, CNRS, ISA, France

7³ Université Côte d'Azur, Inria, INRA, CNRS, UPMC Univ Paris 06, BIOCORE,
8 France

9

10

11 **Abstract**

12

13 **The development of a new organ is the result of coordinated events of cell**
14 **division and expansion, in strong interaction with each other. This paper**
15 **presents a dynamic model of tomato fruit development that includes cells**
16 **division, endoreduplication and expansion processes. The model is used to**
17 **investigate the interaction among these developmental processes, in the**
18 **perspective of a neo-cellular theory. In particular, different control schemes**
19 **(either cell-autonomous or organ-controlled) are tested and results**
20 **compared to observed data from two contrasted genotypes. The model**
21 **shows that a pure cell-autonomous control fails to reproduce the observed**
22 **cell size distribution, and an organ-wide control is required in order to get**
23 **realistic cell sizes. The model also supports the role of endoreduplication**
24 **as an important determinant of the final cell size and suggests a possible**
25 **interaction through carbon allocation and metabolism.**

26

27 **Keywords:** cell size, division, development, expansion, endoreduplication,
28 growth, model, ploidy, tomato

29

30 **INTRODUCTION**

31 Understanding the mechanisms underpinning fruit development from its
32 early stages is of primary importance for biology and agronomy. Indeed, early
33 stages are highly sensitive to biotic and abiotic stresses, with important

34consequences on fruit set and yield. The development of a new organ is the
35result of coordinated events of cell division and expansion. Fruit growth starts
36immediately after pollination with intensive cell division. As development
37proceeds, the proliferative activity of cells progressively slows down giving way to
38a phase of pure cell enlargement during fruit growth and ripening. In many
39species, including tomato, the transition from cell division to expansion phases is
40accompanied by repeated DNA duplications without mitosis, a process called
41endoreduplication. The exact role of endoreduplication is still unclear. A strong
42correlation between cell ploidy (i.e number of DNA copies) and final cell size has
43been observed in different species (Bertin, 2005; Lee et al., 2007; Melaragno et
44al., 1993; Rewers et al., 2009), suggesting a role of endoreduplication into the
45control of organ growth (Breuer et al., 2010; Chevalier et al., 2011).

46 Understanding the way cell division, endoreduplication and expansion
47processes interact is crucial to predict the emergence of important morphological
48traits (fruit size, mass, shape and texture) and their dependence on
49environmental and genetic factors. Historically, a big debate has opposed two
50contrasting views, the cellular vs the organismal theory that set the control of
51organ growth at the level of the individual cell or of the whole tissue, respectively
52(reviewed in (Beemster et al., 2003; Fleming, 2006; John and Qi, 2008). In the
53recent years, a consensus view, the neo-cellular theory, has eventually emerged.
54Accordingly, although individual cells are the units of plant morphology, their
55behavior (division, expansion) is not autonomous, but coordinated at the organ
56level by cell-to-cell communication mechanisms, thus creating an effective
57interaction between cellular and whole-organ behavior (Beemster et al., 2003;
58Sablowski and Carnier Dornelas, 2014; Tsukaya, 2003). The existence of non-cell
59autonomous control of organ development has been demonstrated in *Arabidopsis*
60leaf (Kawade et al., 2010) but the underlying modes of action remain unclear
61and often system-specific (Ferjani et al., 2007; Han et al., 2014; Horiguchi and
62Tsukaya, 2011; Norman et al., 2011; Okello et al., 2015).

63

64Computational models offer a unique tool to express and test biological
65hypotheses, in a well-defined and controlled manner. Not surprisingly, indeed,
66computational modeling has been largely used to investigate the relationships
67between organ development and the underlying cellular processes. Many works
68have addressed the question of organogenesis, relating local morphogenetic
69rules and cell mechanical properties with the emerging patterns near the
70meristem (Boudon et al., 2015; Dupuy et al., 2010; Kuchen et al., 2012; Löffke
71et al., 2015; Lucas et al., 2013; Robinson et al., 2011) (von Wangenheim et al.,
722016). At the tissue scale, a few models have addressed the issue of cell's size
73variance based on observed kinematic patterns of cell division or growth rates,
74with a particular attention to the intrinsic stochasticity of cell-cycle related
75processes (Asl et al., 2011; Kawade and Tsukaya, 2017; Roeder et al., 2010). In
76most of these models, cell expansion is simply described via an average growth
77rate, possibly modulated by the ploidy level of the cell, without any reference to
78the underlying molecular mechanisms or to the environmental conditions.

79

80 To our knowledge, very few attempts have been made to explicitly model
81the interaction among cell division, expansion and endoreduplication from first
82principles and at the scale of organ development. In Fanwoua et al., 2013 a
83model of tomato fruit development have been developed that integrates cell
84division, expansion and endoreduplication processes based on a set of
85biologically-inspired rules. The fruit is described by a set of q classes of cells with
86the same age, ploidy and mass. Within each class, cell division and
87endoreduplication are described as discrete events that take place within a well-
88defined window of time, whenever a specific mass-to-ploidy threshold is reached.
89Cell growth in dry mass is modeled following a source-sink approach as a
90function of the thermal time, the cell ploidy-level and the external resources. The
91model is able to qualitatively capture the effect of environmental conditions
92(temperature, fruit load) on the final fruit dry mass, but hypotheses and
93parameters are hard to validate as comparison to experimental data is lacking.
94Moreover, the water content of the cell is not considered preventing the analysis

95of cell volumes.

96 Baldazzi and coworkers developed an integrated model of tomato fruit
97development which explicitly accounts for the dynamics of cell proliferation as
98well as for the mechanisms of cell expansion, in both dry and fresh masses,
99based on biophysical and thermodynamical principles (Baldazzi et al., 2012,
1002013). Here, a new version of this model, which includes cell endoreduplication,
101is proposed. The model was used to investigate different hypotheses concerning
102the regulation and the interaction among cellular processes, with special attention
103to 1) the importance of an organ-wide regulation on cell growth and 2) the
104mechanisms of interaction between endoreduplication and cell expansion
105processes.

106We focus on a natural, wild type organ development and we analyze the effect of
107organ-wide or cell ploidy-dependent regulation onto the dynamics of cell
108expansion. To this aim, different control schemes (either cell-autonomous or
109organ-controlled, with or without ploidy effect on cell expansion) were tested *in*
110*silico* by means of specific model variants. Simulation results were analyzed and
111compared to cell size distributions observed on two contrasted genotypes, a
112cherry and a large-fruited tomato variety.

113The model shows that a pure cell-based control cannot reproduce the observed
114cell size distribution, and an organ-wide control is required in order to get realistic
115cell and fruit sizes. The model also supports the role of endoreduplication as an
116important modulator of the cell expansion potential, although the strength of this
117interaction might be genotype-specific. In particular, results suggest a likely
118interaction through carbon allocation and metabolism.

119

120

121**MATERIALS AND METHODS**

122

123**Experimental data**

124

125Two datasets were collected from two glasshouse experiments performed at

126INRA Avignon (south of France) in 2004 and 2007 on large-fruited (cv Levovil)
127and cherry (cv. Cervil) tomato genotypes of *Solanum lycopersicum* L.
128
129The 2004 experiment fruits were collected from April to May (planting in February)
130whereas in the 2007 experiment fruits were sampled from October to December
131(planting in August). Plants were grown according to standard cultural practices.
132Trusses were pruned in order to homogenise truss size along the stem within
133each genotype. Maximum number of flowers left on each inflorescence was 12
134for Cervil and 6 for Levovil. Flowers were pollinated by bumblebees. Air
135temperature and humidity were recorded hourly in each experiment and input in
136the model as external signals.
137Flower buds and fruits were sampled at different time points relative to the time of
138flower anthesis (full-flower opening). Fruit fresh and dry mass and pericarp fresh
139mass were systematically measured at all times points, before further
140measurements. In 2004, half of the fruit pericarps were then analyzed by flow
141cytometry and the other half were used for the determination of cell number. The
142number of pericarp cells was measured after tissue dissociation according to a
143method adapted from that of Bünger-Kibler and Bangerth, 1982 and detailed in
144(Bertin et al., 2003). Cells were counted in aliquots of the cell suspension under
145an optical microscope, using Fuchs-Rosenthal chambers or Bürker chambers for
146the large and small fruits, respectively. Six to 8 aliquots per fruits were observed
147and the whole pericarp cell number was calculated according to dilution and
148observation volumes. The ploidy was measured in the pericarp tissue, as
149described in Bertin et al., 2007. The average value of three measurements per
150fruit (when allowed for by the fruit's size), was included in the analysis.
151
152In the 2007 experiment, the dynamics of cell number (but not endoreduplication)
153was measured following the same method as in the 2004 experiment. In addition,
154cell size distribution (smallest and largest radii and 2D-surface) distributions were
155measured with ImageJ software (imagej.nih.gov/ij/) in the cell suspension
156aliquots. About 20 to 25 cells per fruit were measured randomly on different
157fruits. Cell size distribution were derived for ripe fruits at about 43 days after

158anthesis (DAA) for Cervil and 60 DAA for Levovil in the considered growing
159conditions.

160

161

162**Model description**

163

164 The model is composed of two interacting modules, both issued from
165previously published models (Bertin et al., 2007; Fishman and Génard, 1998; Liu
166et al., 2007). The fruit is described as a collection of cell populations, each one
167having a specific age, ploidy and volume, which evolve and grow over time during
168fruit development. The number, age (initiation date) and physiological state
169(proliferating or endoreduplicating-expanding cells) of each population is
170predicted by the cell division-endoreduplication module (Bertin et al., 2007),
171based on genotype-specific parameters. It is assumed that the onset of
172endoreduplication coincides with the beginning of the expansion phase, i.e.
173expanding cells are endoreduplicating.

174

175At any time, mass (both fresh and dry component) of expanding cells is computed
176by a biophysical expansion module according to cell's characteristics (age,
177ploidy) and depending on available resources and environmental conditions
178(Fishman and Génard, 1998; Liu et al., 2007). Briefly, cell expansion is described
179by iteratively solving the Lockhart equation relating the rate of volume increase to
180the cell's internal pressure and cell's mechanical properties (Lockhart, 1965).
181Flows of water and solutes across the membrane are described by
182thermodynamic equations and depend on environmental conditions. The relative
183importance of each transport process may vary along fruit developmental stages,
184depending on specific developmental control. A full description of the model and
185its equations can be found in the section S2 of the Supplemental Material.
186In its standard version, the model assumes that all cells have equal access to
187external resources, independently from the number of cells (no competition). All
188the parameters of the division- endoreduplication module are considered to be
189independent from environmental conditions for the time being.

190

191

192 **Model initialisation and input**

193

194 The model starts at the end of the division-only phase, when the
195 proliferative activity of the cells declines and the expansion phase begins
196 (Baldazzi et al., 2013). For Cervil genotype this corresponds to approximately 8
197 days before anthesis and to 3 days before anthesis for Levovil genotype (Bertin
198 et al., 2007). The initial number of cells, n_0 , was estimated to $1e4$ for the cherry
199 tomato (Cervil) and $1.8e5$ for the large-fruited (Levovil) genotype based on a few
200 measurements.

201 At the beginning of the simulation, all cells are supposed to be proliferating
202 with a ploidy level of 2C (transient ploidy of 4C during cell cycle is not
203 considered). Proliferating cells are supposed to have a constant cell mass, m_0 ,
204 as often observed in meristematic cells (homogeneous/uniformity in cell size)
205 (Sablowski and Carnier Dornelas, 2014; Serrano-Mislata et al., 2015). The initial
206 mass of the fruit is therefore $M_f(0) = n_0 * m_0 = n_0 * (w_0 + s_0)$, where w_0 and s_0 are
207 initial cell water and dry mass, respectively. At any time, cells leaving the
208 proliferative phase start to grow, from an initial mass $2 * m_0$ and a ploidy level of
209 4C, according to the expansion model and current environmental conditions.

210

211 Cell expansion depends on environmental conditions and resources provided by
212 the mother plant. The phloem sugar concentration is assumed to vary daily
213 between 0.15 and 0.35 M whereas stem water potential oscillates between -0.05
214 and -0.6 MPa i.e. typical pre-dawn and minimal stem water potential measured
215 for the studied genotypes. Temperature and humidity are provided directly by
216 real-time recording of greenhouse climatic conditions.

217

218 **Choice of the model variants: control of cell expansion capabilities**

219

220 In the integrated model, a number of time-dependent functions account for
221 developmental regulation of cell's metabolism and physical properties during the

222expansion phase (Baldazzi et al. 2013, Liu et al. 2007). Two characteristic time-
223scales are recognizable in the model: the *cell age*, i.e. the time spent since an
224individual cell has left the proliferative phase, and *organ age* i.e. the time spent
225since the beginning of the simulation (Figure 1). Depending on the settings of the
226corresponding time-dependent functions, different cellular processes may be put
227under cell-autonomous or non-cell autonomous control (hereafter indicated as
228organ-wide control), allowing for an *in silico* exploration of alternative control
229hypotheses in the perspective of the cellular and organismal theories. Moreover,
230a direct effect of cell DNA content onto cell expansion capabilities may be tested
231according to biological evidences (Chevalier et al., 2011; Edgar et al., 2014;
232Sugimoto-Shirasu and Roberts, 2003).

233

234As a default all cellular processes are supposed to be dependent on cell age
235(cell-autonomous control) with the only exception of cell transpiration which is
236computed at the organ scale, on the basis of fruit external surface and skin
237conductance, and then distributed back to individual cells, proportionally to their
238relative water content (see section S2).

239

240 Based on literature information and on preliminary tests ((Baldazzi et al.,
2412013, 2017), the switch between symplastic and apoplastic transport, σ_p has
242been selected as the candidate process for an organ-wide control. Indeed,
243intercellular movement of macromolecules across plasmodesmata has been
244shown to be restricted by organ age in tobacco leaves (Crawford and Zambryski,
2452001; Zambryski, 2004) and it is known to be important for cell-to-cell
246communication (Han et al., 2013).

247

248The exact mechanisms by which cell DNA content may affect cell expansion
249remains currently unknown. Based on literature information and common sense,
250three distinct mechanisms of interaction between endoreduplication and cell
251expansion were hypothesized.

252

2531) Endoreduplication has been often associated to an elevated protein synthesis

254and transcriptional activity (Chevalier et al., 2014) suggesting a general activation
 255of the nuclear and metabolic machinery of the cell to sustain cell growth
 256(Sugimoto-Shirasu and Roberts, 2003). Following these insights, a first
 257hypothesis assumes an effect of endoreduplication on cell expansion as a ploidy-
 258dependent maximal import rate for carbon uptake. For sake of simplicity, the
 259relation was supposed to be linear in the number of endocycles. The
 260corresponding equation, as a function of the cell DNA content (DNA_c, being 2 for
 261dividing cells, 4 to 512 for endoreduplicating cells), was

262

$$263 \quad v_m = \langle v^0 \rangle * \log_2(DNA_c) \quad ,$$

264

265where $\langle v^0 \rangle$ is the average C uptake activity per unit mass.

266

2672) In addition to a high transcriptional activity, endoreduplicating cells are
 268characterized by a reduced surface-to-volume ratio with respect to 2C cells. As a
 269consequence, it is tempting to speculate that one possible advantage of a high
 270ploidy level may reside in a reduction of carbon demand for cell wall and
 271structural units (Barow, 2006; Pirrello et al., 2018). Such an economy may impact
 272cell expansion capabilities in two ways. First, the metabolic machinery could be
 273redirected towards the synthesis of soluble components, thus contributing to the
 274increase of cell's internal pressure and consequent volume expansion.
 275In the model, the *ssrat* fraction of soluble compound within the cell is

276developmentally regulated by the age *t* of the cell (Baldazzi et al. 2013) as

277

$$278 \quad ssrat = b_{ssrat} (1 - e^{-a_{ssrat} * t}) + ssrat_0$$

279

280In the presence of a ploidy effect, the final *bssrat* value was further increased as

281

$$282 \quad b_{ssrat} = b_{ssrat}^0 * \log_2(DNA_c)$$

283

2843) Alternatively, "exceeding" carbon may be used to increase the rate of cell wall
 285synthesis or related proteins, resulting in an increase of cell wall plasticity

286(Proseus and Boyer, 2006).

287

288In the original expansion model on tomato (Liu et al., 2007) cell wall extensibility

289Phi declines during cell maturation (Proseus et al., 1999) as
290

$$291 \quad \Phi = \Phi_{min} + \frac{(\Phi_{max} - \Phi_{min})}{1 + e^{k(t - t_0)}}$$

292

293In the presence of a ploidy effect, the maximal cell wall extensibility was

294increased as

295

$$296 \quad \Phi_{max} = \Phi_{max}^0 * \log_2(DNAc)$$

297

298

299The individual and combined effects of organ-wide and ploidy-dependent control

300(one process at time) on cell expansion were investigated and compared to a full

301cell-autonomous model. A total of 8 model variants have been tested for each

302genotype, following the complete experimental design shown in Table 1.

303

304**Model calibration**

305Calibration has been performed using genetic algorithm under R software (library

306'genalg'). A two-steps procedure has been used for each tomato genotype.

307First, the division-expansion module (7 parameters) was calibrated on data from

308the 2004 experiment by comparing measured and simulated values of the total

309cells number and the proportion of cells in different ploidy classes, all along fruit

310development. The best fitting parameters were selected and kept fixed for the

311second phase of the calibration, assuming they are independent from

312environmental conditions.

313The expansion module was calibrated on the evolution of pericarp fresh and dry

314mass from the 2007 experiment, for which cell size distribution were measured.

315Six parameters have been selected for calibration based on a previous sensitivity

316analysis (Constantinescu et al., 2016), whereas the others have been fixed to the

317original models' values (Baldazzi et al., 2013; Fishman and Génard, 1998; Liu et

318al., 2007). An additional parameter was estimated for model variants M3 to M7 in

319order to correctly evaluate the strength of the ploidy-dependent control (see

320section S3 for more information).

321Due to their different structures, the expansion module was calibrated
322independently for each model variant. The quality of model adjustment was
323evaluated using a Normalized Root Mean Square Error (NRMSE):

$$324 \quad NRMSE(x) = 100 \frac{\sqrt{\frac{1}{n} \sum (O_i - S_i(x))^2}}{\frac{1}{n} \sum O_i}$$

325

326where O_i and S_i are respectively, the observed and simulated values of pericarp
327fresh and dry masses, and n is the number of observations. $x = \{x_1, x_2 \dots x_p\}$ is
328parameter set of the evaluated solution. The smaller the NRMSE the better the
329goodness-of-fit is. A NRMSE < 20% is generally considered good, fair if 20% <
330NRMSE < 30% and poor otherwise.

331To avoid high mean fitting errors in each condition, the choice of the best
332calibration solution was based on a min-max decision criterion (Constantinescu et
333al., 2016):

$$334 \quad \min_{x \in X} \left\{ \max \left(NRMSE_{FM}(x), NRMSE_{DM}(x) \right) \right\}$$

335where $NRMSE_{FM}$ and $NRMSE_{DM}$ are the normalized root mean square errors for
336fresh and dry masses respectively. Selected parameters are reported in tables S3
337and S4.

338Model comparison and selection

339On the basis of the best calibration solution, model selection has been performed
340by comparing measured and simulated cell size distribution, for each model
341variant. A *semi-quantitative* comparison approach has been used due to the limited
342experimental information available: the general distribution characteristics (shape,
343positioning and dispersion) have been characterized rather than a perfect fit. To
344this aim, 8 descriptive statistical indicators have been computed for each model

345variant and compared to those derived from real- data distribution, namely:

- 346 • skewness and kurtosis (distribution's shape)
- 347 • mean and median cell size (positioning)
- 348 • standard deviation (SD) and median absolute deviation (MAD) (data
- 349 dispersion)
- 350 • maximal and minimal cell size (data dispersion)

351In order to compare different calibration solutions, a *principal component analysis*
352(PCA) was performed on the descriptors of cell distribution arising from each
353model estimation. The ade4 library of R software was used for this purpose (R
354development Core Team 2006).

355

356**RESULTS**

357

358**A characteristic right-tailed distribution of cell areas**

359

360The distribution of cell sizes at a given stage of fruit development directly
361depends on the particular cell division and expansion patterns followed by the
362organ up to the considered time. Any change in the cell division or expansion rate
363will have a consequence on the shape and position of the observed distribution.
364For both tomato genotypes considered in this study, the cell area distribution at
365mature stage shows a typical right-tailed shape (Figure 2), compatible with a
366Weibull or a Gamma distribution (see section S1). The observed cell sizes span
367up to two orders of magnitude, with cell areas (cross section) ranging from 0.004
368to 0.08 mm² for Cervil genotype, and from 0.005 to 0.28 mm² for Levovil (Table
3693 and 4). The average cell area is calculated to be 0.026 mm² for the cherry
370tomato and 0.074 mm² for the large-fruited genotype, values in agreement with
371data from other tomato varieties (Bertin, 2005; Renaudin et al., 2017). Data
372dispersion is higher for the large-fruited genotype, but the shape of the
373distribution, as measured by its skewness and kurtosis values, is pretty similar for
374both tomato varieties.

375

376 Assuming that cell area distribution is a good descriptor of the underlying
377 interaction patterns among cellular processes, a principal component analysis
378 was performed on 8 statistical descriptors of cell size distribution in order to
379 compare predictions of M0-M7 models (see M&M and Tables 2 and 3). For both
380 genotypes, the two first principal components explained approximately 90% of
381 observed variance and were able to correctly separate models with cell-
382 autonomous control (M0, M5, M6) from models including an organ-wide control
383 of cell expansion (Figure 3 and 4). For Cervil genotype, the intra-model
384 variability of cell size distribution was lower than then inter-model differences,
385 whereas for Levovil models including a ploidy-dependent effect resulted in a
386 similar cell size distribution.

387

388 Separation was mainly performed by the first principal component on the basis of
389 the width of the distribution (sd, mad and maximal cell size) and its skewness,
390 both generally increased in models including an interaction between
391 endoreduplication-expansion processes (Figure 3 and 4). Models without organ-
392 wide control are characterized by a reduced dispersion and a larger median
393 value. With respect to experimental data, models combining an organ-wide and a
394 ploidy-dependent control gave the best results.

395

396 In the following, the effect of specific control mechanisms on the resulting cell
397 area distribution is analysed in details, based on the results obtained for the best-
398 fitting solution. The corresponding statistical descriptors are reported in Table 2
399 and 3, for Cervil and Levovil genotype respectively. Note that predicted minimal
400 cell sizes for Levovil genotype are systematically lower than experimental
401 measurements and correspond to the size of proliferating cells. This is partly due
402 to the dissociation method employed for cell counting that underestimates small
403 sub-epidermal cells. These cells although quite numerous contribute little to total
404 pericarp volume and mass (Renaudin et al., 2017).

405

406 **A simple cell-autonomous control scheme leads to unrealistic cell size**
407 **distribution**

408

409 As a benchmark model, the case of a simple cell-autonomous control,
410 without ploidy-dependent effect, was first considered (version M0 of the model).
411 Accordingly, two cells with the same age, even if initiated at different fruit
412 developmental stages, behaved identically in what concerns carbon metabolism,
413 transport and wall mechanical properties. In this scheme, therefore, cell size
414 variations derived exclusively from the dynamics of cell division, which caused a
415 shift in the initiation date for different cohort of cells. When applied to our
416 genotypes, the cell-autonomous model was able to reproduce the observed fruit
417 mass dynamics but the corresponding cell size distribution was extremely narrow
418 (Figure 5 A and D), with standard deviation less than $3e-3$, and strongly left-tailed
419 (see Table 2 and 3).

420

421 Including an organ-wide mechanism that controls cell size (model M1)
422 introduces a source of variance among cells. In this case, two cells of the same
423 age which were initiated at different fruit stages did *not* behave identically,
424 resulting in different expansion capabilities and growth patterns (Baldazzi et al.
425 2013). Following literature information and preliminary studies (Baldazzi et al.,
426 2017) carbon symplastic transport had been supposed under organ-wide control,
427 via the progressive closure of plasmodesmata with fruit age (Zambryski,
428 2004) (see M&M section). As a result, the cell size distribution got larger, and
429 skewness increased towards zero values, indicating a symmetric cell size
430 distribution, both for cherry and large-fruited tomatoes. Indeed, the typical right-
431 tail observed in experimental data was absent and the maximum cell size
432 predicted by the model was much smaller than expected. This suggested that a
433 mechanism controlling cell expansion was lacking in the model.

434

435 **Endoreduplication and cell growth: possible interactions and genotypic**

436 **effect**

437

438 A significant correlation between cell size and endoreduplication level has been
439 often reported. However, the molecular mechanism by which ploidy could

440modulate cell expansion capabilities remain elusive. In this work, three time-
441dependent cell properties have been selected as possible targets of ploidy-
442dependent modulation (see M&M section): *a*) the maximum carbon uptake rate
443(model version M2), *b*) carbon allocation between soluble and non-soluble
444compounds (model version M3) *c*) cell wall plasticity (model version M4).
445The three hypotheses were tested independently on both genotypes, in
446combination with an organ-wide control. Results are shown in Table 2 and 3. In
447most cases, the addition of a ploidy effect on cell expansion resulted in a positive
448skewed distribution, with comparable or increased cell size dispersion and
449maximum cell size with respect to the M0 and M1 models. The strength of the
450effect however strongly depended on the genotype, with different ranking among
451model versions.

452

453For Cervil genotype, a potential control of endoreduplication on both the V_{max}
454(model M2) and cell's allocation strategy (model M3) provided distribution
455agreement with data, i.e. right-tailed distributions with good dispersion and
456correct positioning in both mean and median cell area (Figure 5 B and C). The
457shape of the distribution however was better reproduced by the M2 model, which
458showed a skewness and kurtosis values close to the observed ones, but the
459adjustment to fruit growth dynamics, especially for dry mass, was only partially
460satisfactory (Table 2).

461In both models, a high ploidy level resulted in a larger cell size, although the
462maximum predicted cell size was slightly lower than the observed one.
463Correlation between ploidy level and cell area was significant with a p-value
464 <0.0001 (Table S5). The heterogeneity of cell sizes usually observed at each
465ploidy level was correctly captured by the models as a consequence of the
466asynchrony in cell division and endoreduplication patterns (Bourdon et al., 2011;
467Roeder et al., 2010). In comparison to the M2 and M3 models, the potential effect
468of endoreduplication onto cell's mechanical properties (M4) failed to increase cell
469size variance beyond the values already obtained without any ploidy effect (M1).

470

471For Levovil genotype, the interaction between endoreduplication and expansion

472 had less impact on the resulting cell distribution. For both model M2 and M4, the
473 addition of a ploidy-dependent effect on cell expansion, although significant, was
474 not able to produce a right-tailed distribution, as observed in experimental data
475 (Figure 5E). The shape of the distribution was pretty symmetric, with skewness
476 values close to zero, reduced dispersion and mean cell size. Only the inclusion of
477 a ploidy-dependent effect on cell's strategy for carbon allocation (model M3)
478 allowed increasing the skewness value up to a reasonable value, but the width of
479 the distribution remained lower than expected with maximal cell sizes not
480 exceeding 0.13 mm² (against the 0.28mm² observed) (Figure 5F).

481

482

483 **The mechanisms that contribute to cell size variance are likely genotype-** 484 **dependent**

485

486 The above results showed that cell distribution can be significantly affected by
487 both an organ-wide control of fruit development and a direct interaction between
488 endoploidy and cell expansion capabilities. In order to better discriminate their
489 respective role, the effect of a ploidy dependent expansion was tested alone,
490 without the contribution of an organ-wide control of cell growth (models M5-M7).
491 Results confirmed that the best results are obtained by a combined action of both
492 an organ-wide control and a ploidy-dependent modulation of cell expansion but
493 the relative importance of the two mechanisms is likely genotype-dependent.

494

495 In the case of Levovil, organ-wide control turned out to be a major regulatory
496 mode. Independently of the interactions between endoreduplication and
497 expansion, indeed, models without organ-control (models M5-M7) completely
498 failed to reproduce the observations, resulting in a very narrow and left-tailed cell
499 size distribution (Table 3). Organ-wide coordination of cell expansion appeared to
500 be the main responsible for positive skewness of cell size distribution whereas
501 the addition of an endoreduplication-mediated modulation of cell expansion
502 capabilities, alone, resulted only in a marginal improvement of model's
503 performances.

504

505The relative roles of ploidy-dependent and organ-wide control of cell growth
506appear more balanced in cherry tomatoes. Indeed, with the exception of model
507M6, both the organ-wide and the ploidy-mediated control of cell expansion were
508able to reproduce the expected right-tailed distribution shape (Table 2). However,
509their concomitant action was needed in order to get a realistic cell size variance.
510The two mechanisms thus seem to act in synergy to increase cell expansion and
511final cell size.

512

513

514**A combination of ploidy-dependent control of both carbon uptake and**
515**allocation better explains data**

516

517In spite of a good agreement with pericarp data, all tested models failed to fully
518reproduce the observed cell area distribution for Levovil genotype. In particular,
519the maximum reachable cell size predicted by the models was far lower than the
520observed data. Up to now, for seek of simplicity, interaction between
521endoreduplication and expansion has been supposed to affect a single process at
522time but in reality a combination of effects cannot be excluded. We therefore tried
523to combine a ploidy-dependent effect on both the maximum carbon uptake and
524the cell's allocation strategy between soluble and non-soluble compounds (model
525version M23).

526

527Results showed that, for both genotype, the two mechanisms successfully
528combined together to improve cell's expansion capabilities. As a consequence,
529the tail of the distribution straightened and the maximal cell size increased
530towards realistic values (Figure 6). For Cervil genotype, indeed, the predicted cell
531size distribution approached the experimental one, as showed by the projection of
532the M23 model onto the first principal plane (Figure 3). For Levovil genotype, in
533spite of a significant increase of the maximal cell size, the width of the distribution
534remained much lower than in experimental data, resulting into a very long-tailed
535distribution shape.

536

537**DISCUSSION**

538

539 The present paper describes an improved version of an integrated cell
540 division-expansion model that explicitly accounts for DNA endoreduplication, an
541 important mechanism in tomato fruit development. The model is used to
542 investigate the interaction among cell division, endoreduplication and expansion
543 processes, in the framework of the neo-cellular theory (Beemster et al., 2003). To
544 this aim, 8 model variants including or not an organ-wide control of cell
545 development, have been tested and compared to data from two contrasting
546 tomato genotypes. Model simulations showed that a pure cellular control was
547 unable to reproduce the observed cell size distribution in terms of both average
548 cell size and cell variance. In agreement with the neo-cellular theory, the model
549 supported the need for an organ-wide control of cell growth, mediated by cell-to-
550 cell communication via plasmodesmata (Han et al., 2014; Norman et al., 2011)
551 and confirmed the role of endoreduplication as an important modulator of the cell
552 expansion potential.

553

554 According to the model, organ-wide control was the main responsible of
555 cell-to-cell variance but a ploidy-mediated effect on cell expansion was needed in
556 order to obtain large cell surface as the ones observed in experimental data. A
557 positive significant correlation between cell size and ploidy level was clearly
558 reproduced, independently of model version, in agreement with recent analysis
559 of cell size in both leaf epidermis (Kawade and Tsukaya, 2017; Roeder et al.,
560 2010) and fruit pericarp (Bourdon et al., 2011).

561

562 However, the strength of the correlation may be genotype-dependent. For the
563 cherry tomato variety, our modelling approach showed that a ploidy-dependent
564 control of carbon metabolism (uptake, allocation or a combination of the two), in
565 combination to an organ-wide modulation of cell expansion, was able to generate
566 a cell size distribution close to the observed one. For large-fruited tomato
567 genotype, interaction between endoreduplication and cell expansion via a single
568 process did not suffice to get large cells. Independently of the specific interaction
569 mechanism, the model proved unable to correctly reproduce the observed cell

570 sizes, resulting in a too narrow and symmetric distribution.
571 The addition of a double effect of cell's endoploidy on both carbon uptake and
572 allocation was able to increase the maximal cell size close to the correct values,
573 but dispersion remained lower than expected.

574

575 **Cell stochasticity may be important to explain cell size distribution in fruit**

576

577 A few reasons may account for the discrepancy observed for the large-
578 fruited variety. A first issue may reside in the quality of experimental data for
579 Levovil genotype. A rapid computation of the average cell size as the pericarp
580 volume over the number of cells at maturity, yields a value significantly lower than
581 the mean of the experimental distribution. This means that the proportion of small
582 cells is underestimated in our dataset, resulting in a distribution that is probably a
583 little flatter and less skewed than the real one.

584

585 A second, more fundamental reason of discrepancy is rooted in our modelling
586 approach. Our model is an example of population model: the fruit is described a
587 collection of cell groups, each having specific characteristics in terms of number,
588 mass, age and ploidy level, that dynamically evolve during time. Although
589 asynchrony in the emergence of cell groups allowed to capture a considerable
590 part of cell-to-cell heterogeneity, intrinsic stochasticity of cellular processes
591 (Meyer and Roeder, 2014; Robinson et al., 2011b; Smet and Beeckman, 2011)
592 are not accounted for. Variations in the threshold size for division (often
593 associated to a change in the cell cycle duration) as well as asymmetric cell
594 divisions are considered as important determinant of the final cell size (Dupuy et
595 al., 2010; Osella et al., 2014; Roeder et al., 2010; Stukalin et al., 2013) they may
596 contribute to significantly spread the size distribution of both dividing and
597 expanding cell groups, from the early stages. Moreover, the degree of additional
598 dispersion introduced by cell expansion is likely to depend on the specificity of the
599 underlying mechanisms, with possible interactions with ploidy-dependent and
600 organ-wide controls. Of course, these mechanisms are intrinsic of the
601 development of any new organ, independently of the genotype, but they can

602prove more sensitive for Levovil genotype than for Cervil in reason of its higher
603cell number and longer division windows.

604In perspective, the addition of stochastic effects could help to fill the missing
605variance for both Cervil and Levovil genotypes. To this aim, a novel modelling
606scheme is needed in which the average cell mass of a group is replaced by a
607distribution function of cell sizes, whose parameters can evolve with time under
608the effect of cell expansion processes.

609

610**Endoreduplication could regulate cell size through carbon allocation and** 611**metabolism**

612

613 From a biological point of view, the model suggested that
614endoreduplication may interact with cell's carbon metabolism, increasing the
615substrate potentially available for cell expansion. This is in line with literature
616data pointing to ploidy level setting the maximum potential cell size that can be
617attained or not, depending on internal (hormones) and external (environmental)
618factors (Breuer et al., 2010; Chevalier et al., 2011; De Veylder et al., 2011). In
619particular, the model revealed that high ploidy level may increase both the carbon
620uptake rate and its relative allocation to soluble compounds, thanks to an overall
621economy in cell wall synthesis (Barow, 2006; Pirrello et al., 2018).

622

623 It is important to stress that the molecular basis of the supposed interaction
624between endoreduplication and expansion are not described in the model and
625could involve many molecular players. Moreover, the existence of other targets of
626a ploidy-dependent control cannot be excluded nor the contribution of other
627mechanisms to the control of the final cell size. In many fruit species including
628tomato, a negative correlation between average cell size and cell number has
629been observed, suggesting the existence of a competition for resources
630(Lescourret and Génard, 2003; Prudent et al., 2013). This kind of mechanism
631may contribute to broaden the range of attainable cell sizes, increasing size
632variance among first and late-initiated cells (see section S5).The importance of
633such an effect may vary with genotype and environmental conditions (Bertin,

6342005; Quilot and Génard, 2008). This may be especially important for large-
635fruited tomatoes for which cell number is large.

636

637**Measurement of cell size distribution: promises and challenges to** 638**understand the control of fruit growth**

639

640 Further work is needed in order to identify the mechanisms behind organ
641growth and cell size determination. To this aim, the analysis of cell size
642distribution shows up as a promising approach. When looking at our results,
643indeed, the NRMSE with respect to pericarp fresh and dry mass was always
644between 20% and 30% indicating a satisfactory agreement with data,
645independently from the model version and the tomato genotype. This highlights
646the fact that the dynamics of fruit growth alone is not enough to discriminate
647between several biologically-plausible models. In this sense, cell size distribution
648represents a much more informative dataset as it uniquely results from the
649specific cell division and expansion patterns of the organ (Halter et al., 2009).

650 The assessment of cell sizes in an organ is not an easy task though. As
651illustrated by the case of Levovil, the employed measurement technique may
652have important consequences on the resulting cell size distribution (Legland et
653al., 2012). Indeed, mechanical constraints acting on real tissues as well as
654vascularisation can largely modify cell shape, resulting in elongated or multi-lobed
655cells (Ivakov and Persson, 2013). Thus, if the orientation of 2D slices can
656potentially affect the resulting cell area estimation, possible differences between
657*in-vivo* tissues and dissociated cells, both in number and size, should also be
658checked.

659The use of mutant or modified strains (Musseau et al., 2017) in combination with
660recent advancements in microscopy and tomography (Mebatsion et al., 2009;
661Wuyts et al., 2010) could now permit the acquisition of more reliable datasets,
662opening the way to an in-depth investigation of cell size variance in relation to the
663their position within the fruit (Renaudin et al., 2017) and to the underlying
664molecular processes. At term, improvements in the ability of computation models

665to integrate the multiple facets of organ development in a mechanistic way can
666help to evaluate and quantify the contribution of the different processes to the
667control of cell growth.

668

669

670**Acknowledgments**

671

672The authors warmly thank B. Brunel for help with cell measurements. The
673authors are grateful to Inria Sophia Antipolis – Méditerranée "NEF" computation
674cluster for providing resources and support. This work was partially funded by the
675Agence Nationale de la Recherche, Project "Frimouss" (grant no. ANR-15-
676CE20-0009) and by the Agropolis Foundation under the reference ID 1403-032
677through the « Investissements d'avenir » programme (Labex Agro:ANR-10-LABX-
6780001-01).

679

680

681**Literature cited**

682

683Asl, L.K., Dhondt, S., Boudolf, V., Beemster, G.T.S., Beeckman, T., Inzé, D.,
684Govaerts, W., and De Veylder, L. (2011). Model-based analysis of Arabidopsis
685leaf epidermal cells reveals distinct division and expansion patterns for pavement
686and guard cells. *Plant Physiol.* *156*, 2172–2183.

687Baldazzi, V., Bertin, N., Genard, M., and Génard, M. (2012). A model of fruit
688growth integrating cell division and expansion processes. In *Acta Horticulturae*,
689(International Society for Horticultural Science (ISHS); Leuven; Belgium), pp.
690191–196.

691Baldazzi, V., Pinet, A., Vercambre, G., Bénard, C., Biais, B., and Génard, M.
692(2013). In-silico analysis of water and carbon relations under stress conditions. A
693multi-scale perspective centered on fruit. *Front. Plant Sci.* *4*, 495.

694Baldazzi, V., Génard, M., and Bertin, N. (2017). Cell division, endoreduplication
695and expansion processes: setting the cell and organ control into an integrated
696model of tomato fruit development. *Acta Hortic.* *1182*.

697Barow, M. (2006). Endopolyploidy in seed plants. *BioEssays* *28*, 271–281.

698Beemster, G.T.S., Fiorani, F., and Inzé, D. (2003). Cell cycle: the key to plant
699growth control? *Trends Plant Sci.* *8*, 154–158.

700Bertin, N. (2005). Analysis of the tomato fruit growth response to temperature and
701plant fruit load in relation to cell division, cell expansion and DNA
702endoreduplication. *Ann. Bot.* *95*, 439–447.

703Bertin, N., Génard, M., and Fishman, S. (2003). A model for an early stage of
704tomato fruit development: cell multiplication and cessation of the cell proliferative
705activity. *Ann. Bot.* *92*, 65–72.

706Bertin, N., Lecomte, A., Brunel, B., Fishman, S., and Génard, M. (2007). A model
707describing cell polyploidization in tissues of growing fruit as related to cessation of
708cell proliferation. *J. Exp. Bot.* *58*, 1903–1913.

709Boudon, F., Chopard, J., Ali, O., Gilles, B., Hamant, O., Boudaoud, A., Traas, J.,
710and Godin, C. (2015). A Computational Framework for 3D Mechanical Modeling
711of Plant Morphogenesis with Cellular Resolution. *PLoS Comput. Biol.* *11*,
712e1003950.

713Bourdon, M., Coriton, O., Pirrello, J., Cheniclet, C., Brown, S.C., Poujol, C.,
714Chevalier, C., Renaudin, J.-P.P., and Frangne, N. (2011). In planta quantification
715of endoreduplication using fluorescent in situ hybridization (FISH). *Plant J.* *66*,
7161089–1099.

717Breuer, C., Ishida, T., and Sugimoto, K. (2010). Developmental control of
718endocycles and cell growth in plants. *Curr. Opin. Plant Biol.* *13*, 654–660.

719Bünger-Kibler, S., and Bangerth, F. (1982). Relationship between cell number,
720cell size and fruit size of seeded fruits of tomato (*Lycopersicon esculentum* Mill.),
721and those induced parthenocarpically by the application of plant growth
722regulators. *Plant Growth Regul.* *1*, 143–154.

723Chevalier, C., Nafati, M., Mathieu-Rivet, E., Bourdon, M., Frangne, N., Cheniclet,
724C., Renaudin, J.-P., Gévaudant, F., and Hernould, M. (2011). Elucidating the
725functional role of endoreduplication in tomato fruit development. *Ann. Bot.* *107*,
7261159–1169.

727Chevalier, C., Bourdon, M., Pirrello, J., Cheniclet, C., Gévaudant, F., Frangne, N.,
728Gévaudant, F., and Frangne, N. (2014). Endoreduplication and fruit growth in
729tomato: evidence in favour of the karyoplasmic ratio theory. *J. Exp. Bot.* *65*, 1–16.

730Constantinescu, D., Memmah, M.-M., Vercambre, G., Génard, M., Baldazzi, V.,

731Causse, M., Albert, E., Brunel, B., Valsesia, P., and Bertin, N. (2016). Model-
732Assisted Estimation of the Genetic Variability in Physiological Parameters Related
733to Tomato Fruit Growth under Contrasted Water Conditions. *Front. Plant Sci.* 7,
7341–17.

735Crawford, K.M., and Zambryski, P.C. (2001). Non-targeted and targeted protein
736movement through plasmodesmata in leaves in different developmental and
737physiological states. *Plant Physiol.* 125, 1802–1812.

738Dupuy, L., Mackenzie, J., and Haseloff, J. (2010). Coordination of plant cell
739division and expansion in a simple morphogenetic system. *Proc. Natl. Acad. Sci.*
740U. S. A. 107, 2711–2716.

741Edgar, B.A., Zielke, N., and Gutierrez, C. (2014). Endocycles: a recurrent
742evolutionary innovation for post-mitotic cell growth. *Nat. Rev. Mol. Cell Biol.* 15,
743197–210.

744Fanwoua, J., De Visser, P.H.B., Heuvelink, E., Yin, X., Struik, P.C., and Marcelis,
745L.F.M. (2013). A dynamic model of tomato fruit growth integrating cell division, cell
746growth and endoreduplication. *Funct. Plant Biol.* 40, 1098.

747Ferjani, A., Horiguchi, G., Yano, S., and Tsukaya, H. (2007). Analysis of leaf
748development in fugu mutants of *Arabidopsis* reveals three compensation modes
749that modulate cell expansion in determinate organs. *Plant Physiol.* 144, 988–999.

750Fishman, S., and Génard, M. (1998). A biophysical model of fruit growth:
751simulation of seasonal and diurnal dynamics of mass. *Plant Cell Env.* 21, 739–
752752.

753Fleming, A.J. (2006). The integration of cell proliferation and growth in leaf
754morphogenesis. *J. Plant Res.* 119, 31–36.

755Halter, M., Elliott, J.T., Hubbard, J.B., Tona, A., and Plant, A.L. (2009). Cell
756volume distributions reveal cell growth rates and division times. *J. Theor. Biol.*
757257, 124–130.

758Han, X., Kumar, D., Chen, H., Wu, S., and Kim, J.-Y. (2013). Transcription factor-
759mediated cell-to-cell signalling in plants. *J. Exp. Bot.*

760Han, X., Kumar, D., Chen, H., Wu, S., and Kim, J.Y. (2014). Transcription factor-
761mediated cell-to-cell signalling in plants. *J. Exp. Bot.* 65, 1737–1749.

762Horiguchi, G., and Tsukaya, H. (2011). Organ Size Regulation in Plants: Insights
763from Compensation. *Front. Plant Sci.* 2, 24.

764Ivakov, A., and Persson, S. (2013). Plant cell shape: modulators and
765measurements. *Front. Plant Sci.* *4*, 439.

766John, P.C.L., and Qi, R. (2008). Cell division and endoreduplication: doubtful
767engines of vegetative growth. *Trends Plant Sci.* *13*, 121–127.

768Kawade, K., and Tsukaya, H. (2017). Probing the stochastic property of
769endoreduplication in cell size determination of *Arabidopsis thaliana* leaf epidermal
770tissue. *PLoS One* *12*, e0185050.

771Kawade, K., Horiguchi, G., and Tsukaya, H. (2010). Non-cell-autonomously
772coordinated organ size regulation in leaf development. *Development* *137*, 4221–
7734227.

774Kuchen, E.E., Fox, S., de Reuille, P.B., Kennaway, R., Bensmihen, S., Avondo, J.,
775Calder, G.M., Southam, P., Robinson, S., Bangham, A., et al. (2012). Generation
776of leaf shape through early patterns of growth and tissue polarity. *Science* *335*,
7771092–1096.

778Lee, H.-C., Chen, Y.-J., Markhart, A.H., and Lin, T.-Y. (2007). Temperature effects
779on systemic endoreduplication in orchid during floral development. *Plant Sci.* *172*,
780588–595.

781Legland, D., Devaux, M.-F., Bouchet, B., Guillon, F., and Lahaye, M. (2012).
782Cartography of cell morphology in tomato pericarp at the fruit scale. *J. Microsc.*
783247, 78–93.

784Lescourret, F., and Génard, M. (2003). A multi-level theory of competition for
785resources applied to fruit production. *Écoscience* *10*, 334–341.

786Liu, H.-F.F.H.-F., Génard, M., Guichard, S., and Bertin, N. (2007). Model-assisted
787analysis of tomato fruit growth in relation to carbon and water fluxes. *J. Exp. Bot.*
78858, 3567–3580.

789Löffke, C., Dünser, K., Scheuring, D., Kleine-Vehn, J., Barbez, E., Kubeš, M.,
790Rolčik, J., Béziat, C., Pěnčík, A., Wang, B., et al. (2015). Auxin regulates SNARE-
791dependent vacuolar morphology restricting cell size. *Elife* *4*, 119–122.

792Lucas, M., Kenobi, K., von Wangenheim, D., Voß, U., Swarup, K., De Smet, I.,
793Van Damme, D., Lawrence, T., Péret, B., Moscardi, E., et al. (2013). Lateral root
794morphogenesis is dependent on the mechanical properties of the overlaying
795tissues. *Proc. Natl. Acad. Sci. U. S. A.* *110*, 5229–5234.

796Mebatsion, H.K., Verboven, P., Melese Endalew, A., Billen, J., Ho, Q.T., and

797Nicolaï, B.M. (2009). A novel method for 3-D microstructure modeling of pome
798fruit tissue using synchrotron radiation tomography images. *J. Food Eng.* 93,
799141–148.

800Melaragno, J., Mehrotra, B., and Coleman, A. (1993). Relationship between
801endopolyploidy and cell size in epidermal tissue of Arabidopsis. *Plant Cell Online*
8025, 1661–1668.

803Musseau, C., Just, D., Jorly, J., G?vaudant, F., Moing, A., Chevalier, C., Lemaire-
804Chamley, M., Rothan, C., and Fernandez, L. (2017). Identification of Two New
805Mechanisms That Regulate Fruit Growth by Cell Expansion in Tomato. *Front.*
806*Plant Sci.* 8, 1–15.

807Norman, J.M. Van, Breakfield, N.W., Benfey, P.N., Carolina, N., Van Norman,
808J.M., Breakfield, N.W., and Benfey, P.N. (2011). Intercellular communication
809during plant development. *Plant Cell* 23, 855–864.

810Okello, R.C.O., Heuvelink, E., de Visser, P.H.B., Struik, P.C., and Marcelis, L.F.M.
811(2015). What drives fruit growth? *Funct. Plant Biol.* 42, 817.

812Osella, M., Nugent, E., and Cosentino Lagomarsino, M. (2014). Concerted control
813of *Escherichia coli* cell division. *Proc. Natl. Acad. Sci. U. S. A.* 111, 4–8.

814Pirrello, J., Deluche, C., Frangne, N., Gévaudant, F., Maza, E., Djari, A., Bourge,
815M., Renaudin, J.-P., Brown, S., Bowler, C., et al. (2018). Transcriptome profiling of
816sorted endoreduplicated nuclei from tomato fruits: how the global shift in
817expression ascribed to DNA ploidy influences RNA-Seq data normalization and
818interpretation. *Plant J.* 93, 387–398.

819Proseus, T.E., and Boyer, J.S. (2006). Identifying cytoplasmic input to the cell wall
820of growing *Chara corallina*. *J. Exp. Bot.* 57, 3231–3242.

821Proseus, T.E., Ortega, J.K.E., and Boyer, J.S. (1999). Separating Growth from
822Elastic Deformation during Cell Enlargement. *Plant Physiol.* 119, 775–784.

823Prudent, M., Dai, Z.W., Génard, M., Bertin, N., Causse, M., and Vivin, P. (2013).
824Resource competition modulates the seed number-fruit size relationship in a
825genotype-dependent manner: A modeling approach in grape and tomato. *Ecol.*
826*Modell.*

827Quilot, B., and Génard, M. (2008). Is competition between mesocarp cells of
828peach fruits affected by the percentage of wild species (*Prunus davidiana*)
829genome? *J. Plant Res.* 121, 55–63.

830Renaudin, J.-P., Deluche, C., Cheniclet, C., Chevalier, C., and Frangne, N.
831(2017). Cell layer-specific patterns of cell division and cell expansion during fruit
832set and fruit growth in tomato pericarp. *J. Exp. Bot.* *68*, 1613–1623.

833Rewers, M., Sadowski, J., and Sliwinska, E. (2009). Endoreduplication in
834cucumber (*Cucumis sativus*) seeds during development, after processing and
835storage, and during germination. *Ann. Appl. Biol.* *155*, 431–438.

836Robinson, S., Barbier de Reuille, P., Chan, J., Bergmann, D., Prusinkiewicz, P.,
837and Coen, E. (2011). Generation of spatial patterns through cell polarity
838switching. *Science* (80-.). *333*, 1436–1440.

839Roeder, A.H.K., Chickarmane, V., Cunha, A., Obara, B., Manjunath, B.S., and
840Meyerowitz, E.M. (2010). Variability in the control of cell division underlies sepal
841epidermal patterning in *Arabidopsis thaliana*. *PLoS Biol.* *8*, e1000367.

842Sablowski, R., and Carnier Dornelas, M. (2014). Interplay between cell growth
843and cell cycle in plants. *J. Exp. Bot.* *65*, 2703–2714.

844Serrano-Mislata, A., Schiessl, K., and Sablowski, R. (2015). Active Control of Cell
845Size Generates Spatial Detail during Plant Organogenesis. *Curr. Biol.* *25*, 2991–
8462996.

847Stukalin, E.B., Aifuwa, I., Kim, J.S., Wirtz, D., and Sun, S.X. (2013). Age-
848dependent stochastic models for understanding population fluctuations in
849continuously cultured cells. *J. R. Soc. Interface* *10*, 20130325.

850Sugimoto-Shirasu, K., and Roberts, K. (2003). “Big it up”: endoreduplication and
851cell-size control in plants. *Curr. Opin. Plant Biol.* *6*, 544–553.

852Tsukaya, H. (2003). Organ shape and size: a lesson from studies of leaf
853morphogenesis. *Curr. Opin. Plant Biol.* *6*, 57–62.

854De Veylder, L., Larkin, J.C., and Schnittger, A. (2011). Molecular control and
855function of endoreplication in development and physiology. *Trends Plant Sci.* *16*,
856624–634.

857von Wangenheim, D., Fangerau, J., Schmitz, A., Smith, R.S., Leitte, H., Stelzer,
858E.H.K., and Maizel, A. (2016). Rules and Self-Organizing Properties of Post-
859embryonic Plant Organ Cell Division Patterns. *Curr. Biol.* *26*, 439–449.

860Wuyts, N., Palauqui, J.-C., Conejero, G., Verdeil, J.-L., Granier, C., and
861Massonnet, C. (2010). High-contrast three-dimensional imaging of the
862*Arabidopsis* leaf enables the analysis of cell dimensions in the epidermis and

863mesophyll. Plant Methods 6, 17.

864Zambryski, P.C. (2004). Cell-to-cell transport of proteins and fluorescent tracers
865via plasmodesmata during plant development. J. Cell Biol. 164, 165–168.

866

867

868

869

870

871

872

873

874

875

876

877

878

879

880

881

882

883

884**Figures**

885

886

887Figure 1: Scheme of the integrated model. The fruit is described as a collection of cell populations,
888each one having a specific age, ploidy and volume. Cells can be either proliferating or expanding-
889endoreduplicating. The number of cells in each class is predicted by the division-
890endoreduplication module, assuming a progressive decline of cells' proliferating activity.
891Expanding cells grow according to the expansion module which provides a biophysical description
892of the main processes involved in carbon and water accumulation. It is assumed that the onset of
893endoreduplication coincides with the beginning of the expansion phase. Two timescales are
894recognizable in the model: the organ age i.e. the time since the beginning of the simulation, and
895the cell age i.e. the time since the cell left the mitotic cycle and entered the expansion-
896endoreduplication phase. Depending on the model version, cell expansion may be modulated by
897organ age (organ-wide control) and/or by cell's ploidy.

898

899

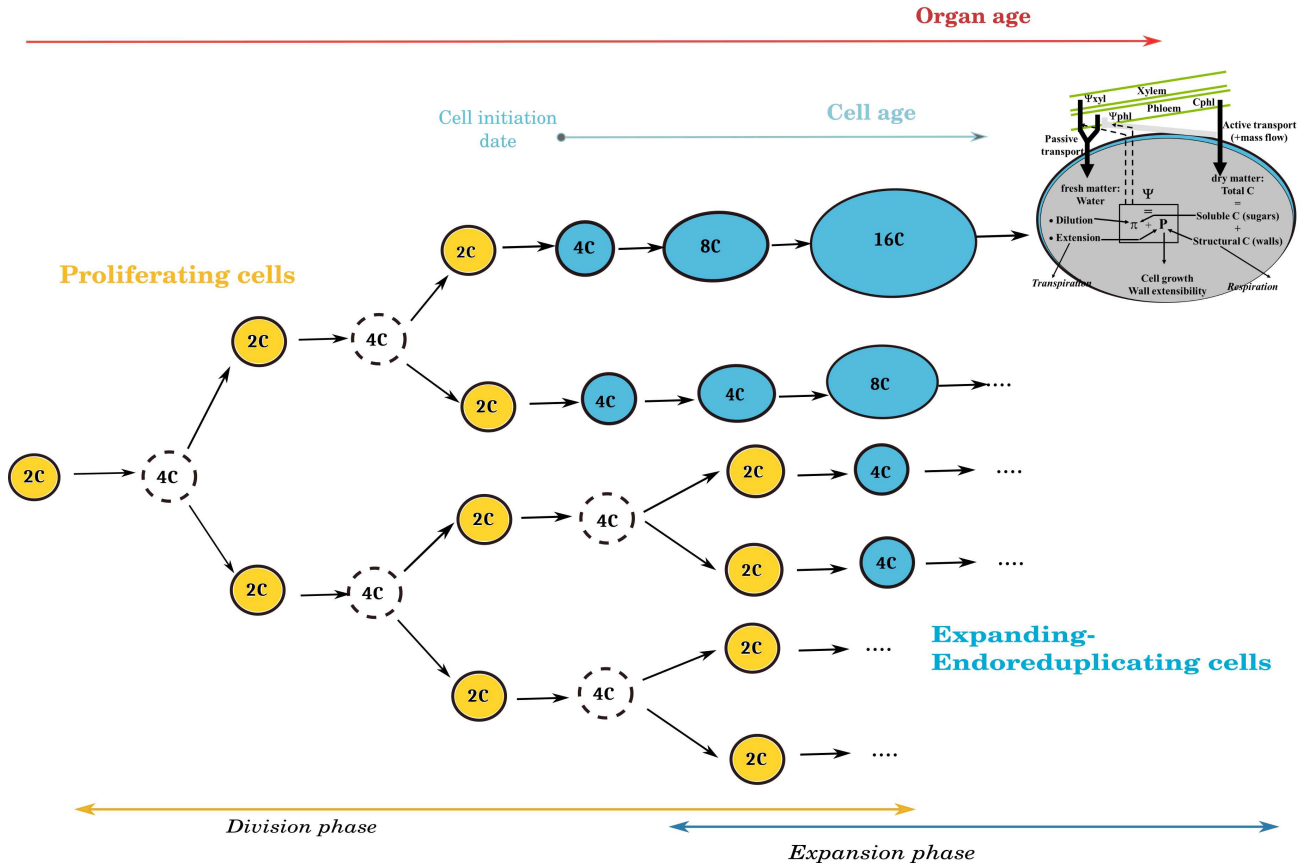
900

901

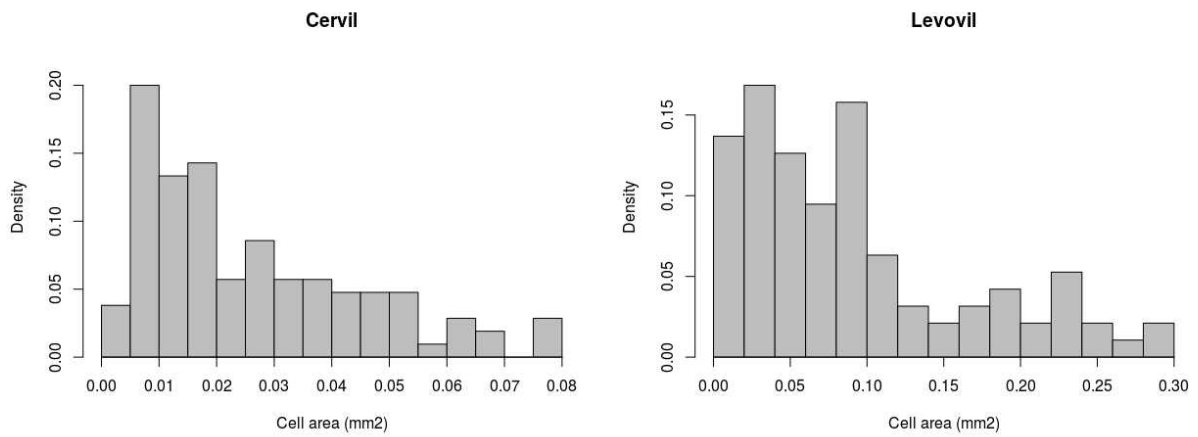
902

903

904



906



908Figure 2: Measured cell size distribution at fruit maturity Left: Cervil genotype. Right: Levovil

909genotype.

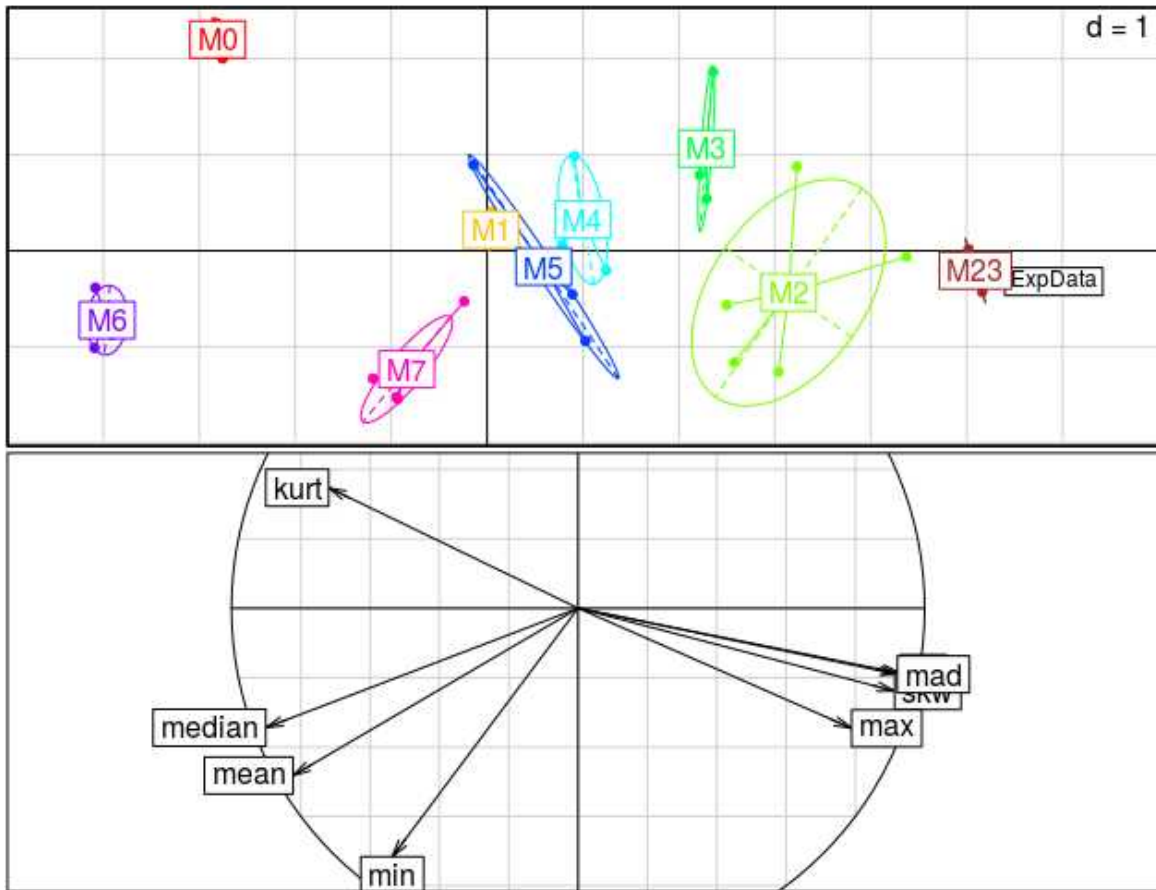
910

911

912

913

914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935



937

938

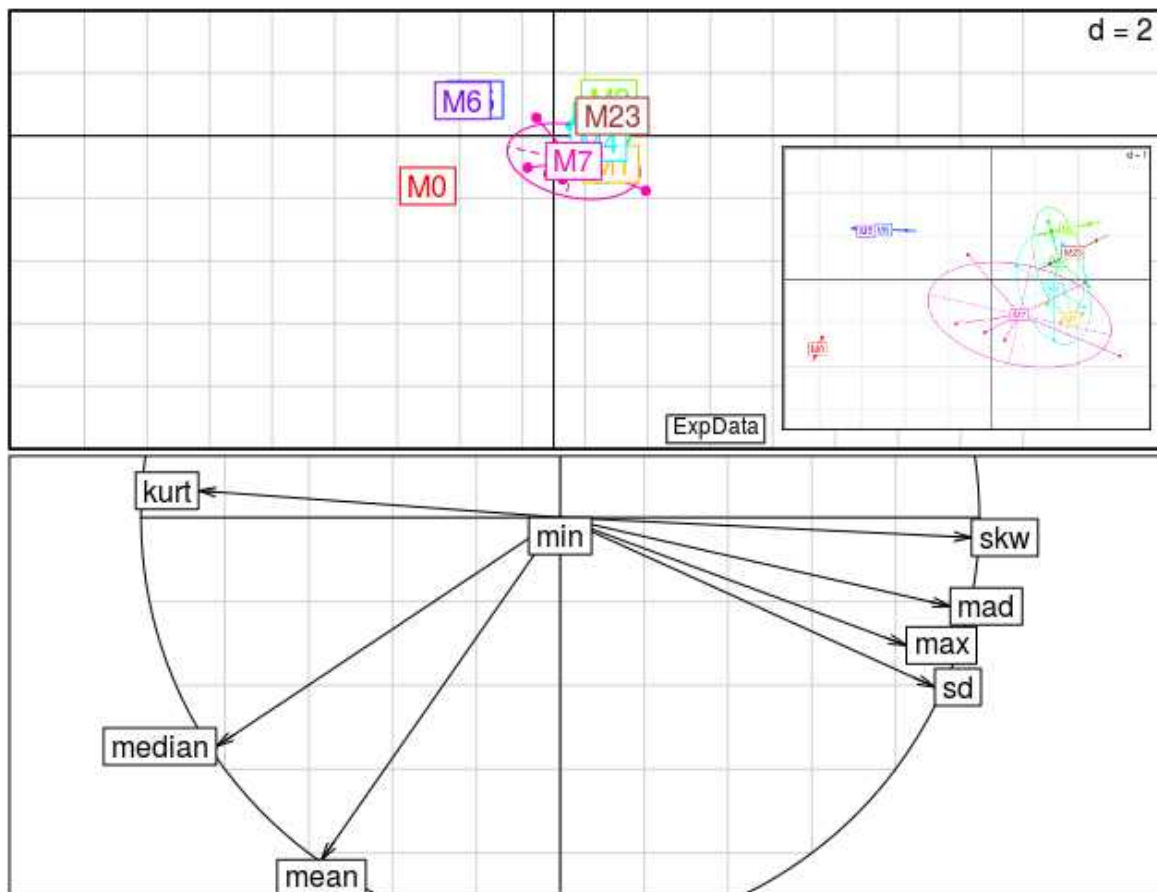
939Figure 3: Principal component analysis (PCA) cell size distributions obtained for the different
940estimations of models M0-M7 on Cervil genotype. 8 statistical descriptors are used as variables to
941characterize cell distribution. Measured cell size distribution results of model M23 are projected
942as a supplementary observation. *Top*: Projection of individual distributions on the PC1-PC2 plane.
943Model variants are tagged with different colors. *Bottom*: Correlation of the variables with the first
944two principal components.

945

946

947

948



950

951Figure 4: Principal component analysis (PCA) cell size distributions obtained for the different
952estimations of models M0-M7 on Levovil genotype. 8 statistical descriptors are used as variables
953to characterize cell distribution. Measured cell size distribution and results of model M23 are
954projected as a supplementary observation. *Top*: Projection of individual distributions on the PC1-
955PC2 plane. Model variants are tagged with different colors. *Bottom*: Correlation of the variables
956with the first two principal components.

957
 958
 959
 960
 961
 962
 963
 964
 965
 966
 967
 968
 969
 970
 971
 972
 973
 974
 975
 976
 977
 978
 979
 980
 981

Figure 5: Predicted cell area distribution at fruit maturity. On the left side, Cervil genotype: A: model M0, B: model M2, C: model M3. On the right side, Levovil genotype: D: model M0, E: model M2, F: model M3.

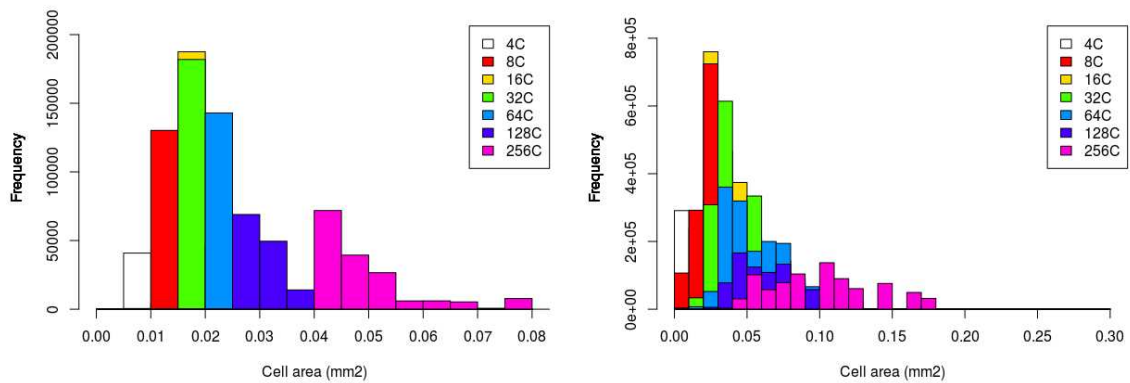
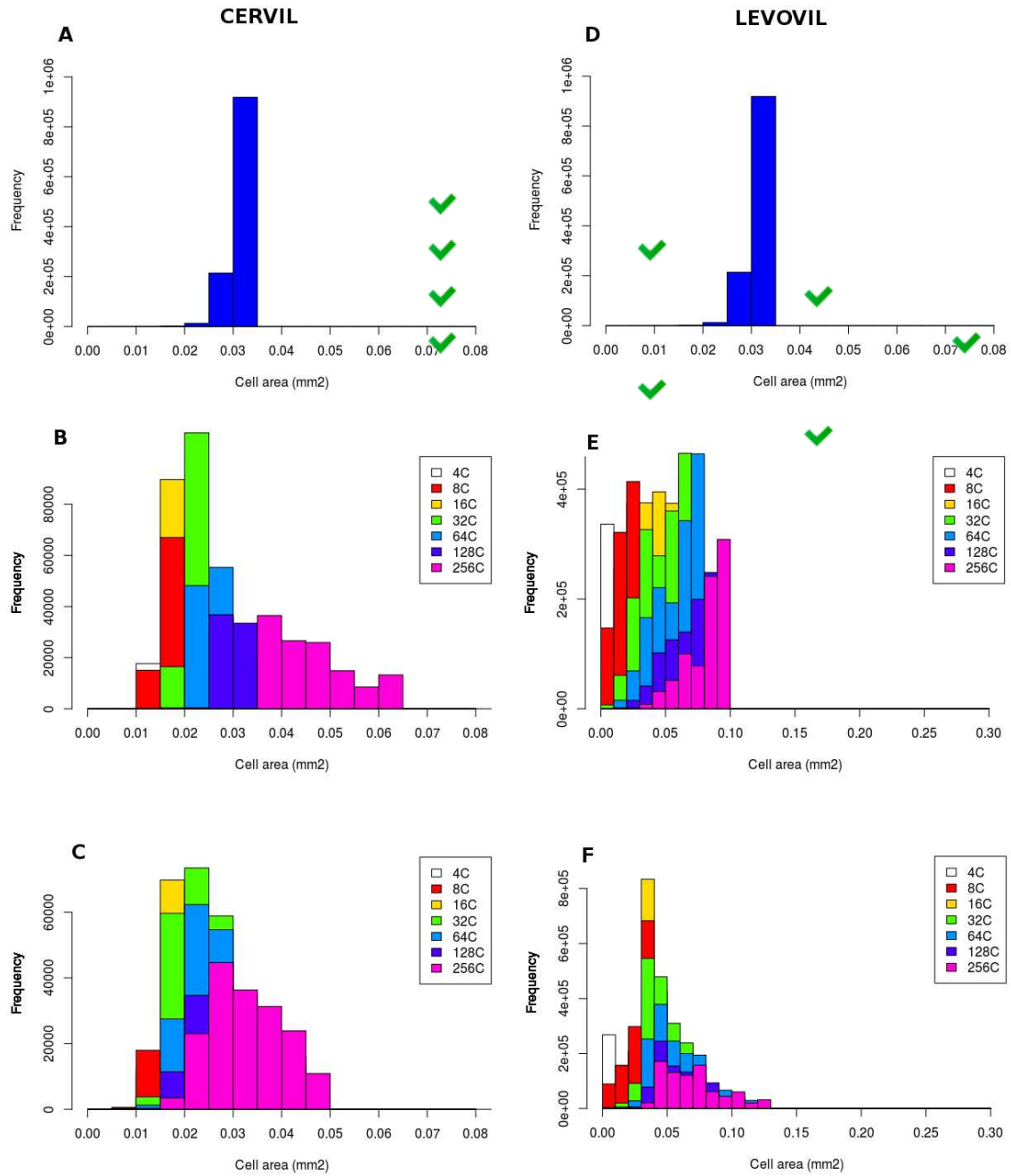


Figure 6: Predicted cell area distribution at fruit maturity for model M23, combining a ploidy-dependent effect on both carbon uptake and allocation. Left: Cervil genotype. Right: Levovil genotype.

985
 986
 987

988 Tables

989



991

992 Table 1: Experimental design showing the characteristics of the 8 model versions tested

993 in the paper.

994

995

996

997

998

999
 1000
 1001
 1002
 1003
 1004
 1005
 1006
 1007
 1008
 1009
 1010
 1011
 1012
 1013
 1014
 1015

MODEL	FRUIT MASS		CELL DISTRIBUTION							
	FIT QUALITY		SHAPE		POSITIONING				DISPERSION	
	NRMSE FM	NRMSE DM	Skewness	Kurtosis	Mean	Median	Min	Max	SD	MAD
Exp Data			0.97	3.13	0.026	0.019	0.0039	0.08	0.019	0.016
M0	31.49	31.34	-4.06	30.34	0.030	0.031	0.0042	0.033	0.0015	0.0005
M1	28.32	29.58	0.42	3.07	0.030	0.029	0.0044	0.041	0.0046	0.0042
M2	23	36	0.98	3.58	0.027	0.025	0.0035	0.062	0.011	0.0099
M3	20.18	31.24	0.53	2.58	0.026	0.025	0.0033	0.046	0.0077	0.008
M4	24.97	27.73	0.53	3.45	0.027	0.027	0.0040	0.045	0.005	0.005
M5	34.35	37.02	0.33	3.44	0.029	0.029	0.0040	0.037	0.0035	0.003
M6	34.21	33.81	-3.7	24.9	0.036	0.036	0.0051	0.039	0.002	0.0008
M7	29.53	32.47	0.24	3.34	0.034	0.033	0.0040	0.056	0.005	0.005
M23	23.33	33.05	2.0	8.9	0.026	0.021	0.0037	0.11	0.015	0.009

1016
 1017Table 2 Statistical descriptors for the measured and predicted cell area distribution for Cervil
 1018genotype. The NRMSE scores for predicted pericarp dry and fresh masses corresponding to the
 1019selected solution are reported under the columns "Fit Quality". For an easier interpretation, green
 1020boxes indicate an agreement within 30% with respect to experimental data, yellow an agreement
 1021between 30 and 40%, white between 40% and 70% and red a strong discrepancy (over 70%
 1022difference with respect to data).

1023
 1024
 1025
 1026

1027
 1028
 1029
 1030
 1031
 1032
 1033
 1034
 1035
 1036
 1037
 1038
 1039
 1040
 1041

MODEL	FRUIT MASS		CELL DISTRIBUTION							
	FIT QUALITY		SHAPE		POSITIONING				DISPERSION	
	NRMSE FM	NRMSE DM	Skewness	Kurtosis	Mean	Median	Min	Max	SD	MAD
Exp Data			0.99	3.01	0.092	0.074	0.0048	0.28	0.075	0.063
M0	23.3	25.27	-2.37	7.89	0.069	0.077	0.00049	0.078	0.017	0.0023
M1	25.79	25.32	0.26	2.13	0.059	0.052	0.00049	0.12	0.030	0.034
M2	25.39	25.59	-0.075	2.2	0.047	0.043	0.00049	0.12	0.022	0.017
M3	25.77	25.01	0.67	3.74	0.047	0.043	0.00049	0.13	0.022	0.017
M4	23.64	25.23	-0.15	2.8	0.053	0.054	0.00049	0.11	0.022	0.020
M5	23.36	27.47	-1.51	5.85	0.054	0.056	0.00049	0.077	0.014	0.008
M6	23.61	25.15	-2.22	7.31	0.055	0.060	0.00049	0.066	0.013	0.004
M7	23.74	25.42	-0.56	3.41	0.063	0.066	0.00049	0.10	0.020	0.018
M23	25.45	28.20	1.46	5.73	0.047	0.039	0.00049	0.018	0.028	0.021

1042
 1043 Table 3: Statistical descriptors for the measured and predicted cell area distribution for Levovil
 1044 genotype. The NRMSE scores for predicted pericarp dry and fresh masses corresponding to the
 1045 selected solution are reported under the columns “Fit Quality”. For an easier interpretation, green
 1046 boxes indicate an agreement within 30% with respect to experimental data, yellow an agreement
 1047 between 30 and 40%, white between 40% and 70% and red a strong discrepancy (over 70%
 1048 difference with respect to data).
 1049
 1050