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11 TITLE

12 **Heat stress reveals the existence of a specialized variant of the pachytene**
13 **checkpoint in meiosis of *Arabidopsis thaliana***

14

15 SHORT TITLE

16 Pachytene checkpoint at high temperature

17

18 ABSTRACT

19 Plant growth and fertility strongly depend on environmental conditions such as
20 temperature. Remarkably, temperature also influences meiotic recombination and
21 thus, the current climate change will affect the genetic make-up of plants. To further
22 understand temperature effects on meiosis, we have followed male meiocytes of
23 *Arabidopsis thaliana* by live cell imaging under three different temperature regimes,
24 at 21°C and at heat shock conditions of 30°C and 34°C as well as after an
25 acclimatization phase of one week at 30°C. This work led to a cytological framework
26 of meiotic progression at elevated temperature. We found that an increase to 30°C,
27 sped up meiotic progression with specific phases being more amenable to heat than
28 others. An acclimatization phase often moderated this effect. A sudden increase to
29 34°C promoted a faster progression of meiosis in early prophase compared to 21°C.
30 However, the phase in which cross-overs mature was found to be prolonged at
31 34°C. Interestingly, mutants involved in the recombination pathway did not show the
32 extension of this phase at 34°C demonstrating that the delay is recombination
33 dependent. Further analysis revealed the involvement of the ATM kinase in this
34 prolongation indicating the existence of a specialized variant of the pachytene
35 checkpoint in plants.

36 INTRODUCTION

37 The ambient temperature is one of the key environmental parameters that
38 determines plant growth and fertility and has been the focal interest of many plant
39 researchers. Understanding the plant response to temperature is further boosted by
40 the ongoing climate change (Anderson et al., 2016; Collins, 2014; Couteau et al.,
41 1999), during which crops are expected to be exposed to very high temperatures in
42 the near future, threatening a sharp reduction in crop yield (Hatfield and Prueger,
43 2015; Yue et al., 2019). For example, a yield decrease of up to 22% for corn (*Zea*
44 *mays*) could be observed with a 1°C increase in temperature (Kukal and Irmak,
45 2018). To rebuttal these detrimental effects and adjust breeding programs, it is vital
46 to understand the changes temperature stress imposes on yield-related traits at the
47 cellular and molecular level.

48 Central for sexual reproduction and fertility is meiosis and previous work has
49 demonstrated its sensitivity to changes in environmental conditions, especially
50 temperature as reviewed by (Bomblies et al., 2015; Morgan et al., 2017). Meiosis is a
51 specialized cell division in which DNA replication is followed by two rounds of
52 chromosome segregation (meiosis I and meiosis II), resulting in the reduction of DNA
53 content by half as a prerequisite for a subsequent fusion of gametes and restoration
54 of the full genome size. Furthermore, meiosis I plays an important role for the
55 generation of genetic diversity via cross-over (CO) formation during prophase I and
56 the new assortment of chromosome sets. COs are not only important for the
57 generation of new allelic combinations but also ensure physical connections between
58 homologous chromosomes (homologs) that are needed for their balanced
59 segregation. COs are visible as chiasmata, that connect two homologs in the form of
60 a bivalent.

61 The control and execution of meiotic recombination is highly conserved and
62 like in other eukaryotes, meiotic recombination in plants is initiated by a conserved
63 topoisomerase-like protein SPORULATION 11-1 (SPO11-1), and together with
64 associated proteins catalyzes DNA double stranded breaks (DSBs) in early meiosis
65 (Grelon et al., 2001; Hartung et al., 2007; Keeney et al., 1997; Stacey et al., 2006).
66 Subsequently, DSBs are processed by the MRE11, RAD50 and NBS1 (MRN) protein
67 complex and recognized by the recombinases DISRUPTED MEIOTIC cDNA1
68 (DMC1) (Bishop et al., 1992; Couteau et al., 1999) and RECA HOMOLOG
69 RADIATION SENSITIVE 51 (RAD51) (Jachymczyk et al., 1981; Li et al., 2004). They

70 mediate the invasion of the processed single stranded DNA into the DNA double
71 strand of the homolog. In the absence of DMC1, DSBs are repaired by inter-sister
72 recombination resulting in the absence of COs and hence, causing the formation of
73 unconnected homologs, called univalents. In *rad51* mutants, DSBs are not repaired
74 resulting in severely fragmented chromosomes and complete sterility of the mutant
75 plants.

76 Towards the end of prophase I, all DSBs are resolved into either non-
77 crossovers (NCOs) or COs. COs fall in one of the two classes: Type I COs rely on
78 the ZMM proteins (acronym from the *Saccharomyces cerevisiae* Zip, Mer and Msh
79 proteins), including MUTS HOMOLOG 4 (MSH4), and are formed at a distance to
80 each other (CO interference) (Higgins et al., 2004; Su and Modrich, 1986). Type II
81 COs are catalyzed by a protein called MMS AND UV SENSITIVE 81 (MUS81) and
82 are not subjected to interference (Berchowitz et al., 2007; Interthal and Heyer, 2000).
83 Homologs remain connected to each other until cohesin, a proteinaceous ring that
84 embraces the sister chromatids of each homolog, is opened by cleavage of its alpha
85 kleisin subunit, RECOMBINANT PROTEIN (REC8), along the chromosome arms in
86 anaphase I paving the road for the separation of the homologs, each to opposite cell
87 poles and hence, completing meiosis I (Cai et al., 2003).

88 The successful execution of meiotic recombination as means to equally
89 segregate homologs and ensure genetic diversity is controlled by the pachytene
90 checkpoint or meiotic recombination checkpoint in animals and yeast (Roeder and
91 Bailis, 2000). This checkpoint delays meiotic progression until recombination defects
92 are resolved. Consequently, several mutants, especially in the recombination
93 pathway, e.g. *dmc1* mutants, trigger this checkpoint and a prolonged arrest, which
94 can even lead to apoptosis in several species, including mouse (Barchi et al., 2005;
95 Bishop et al., 1992; Lange et al., 2011; Rockmill et al., 1995).

96 A master regulator of the pachytene checkpoint is ATAXIA
97 TELANGIECTASIA MUTATED (ATM), a kinase activated by DNA damage, that
98 triggers checkpoint signaling, promotes DSB repair and in addition, controls the DSB
99 number by regulating SPO11-1 activity via a negative feedback loop (Lange et al.,
100 2011). While ATM is present in plants and fulfills several important functions during
101 meiosis, it was thought so far that a pachytene checkpoint in plants did not exist
102 since mutants, like *dmc1*, do not arrest at pachytene and instead complete meiosis

103 leading to aneuploid gametes (Caryl et al., 2003; Couteau et al., 1999; Jackson et
104 al., 2006; Jones and Franklin, 2008; Muylt et al., 2009).

105 Meiosis and in particular meiotic recombination are highly sensitive to
106 environmental conditions, leading to meiotic failure in many different organisms,
107 such as *Caenorhabditis elegans* (Bilgir et al., 2013), mouse (Nebel and Hackett,
108 1961), wheat (Pao and Li, 1948) and rose (Pecrix et al., 2011). Elevated
109 temperatures affect the meiotic microtubule cytoskeleton, resulting in irregular
110 spindle orientation, aberrant cytokinesis and the production of unreduced gametes,
111 polyads and micronuclei in *Populus*, *Rosa* and *Arabidopsis thaliana* (*Arabidopsis*)
112 (De Storme and Geelen, 2020; Hedhly et al., 2020; Pecrix et al., 2011; Wang et al.,
113 2017).

114 Further, although the DSB numbers are reported to be unaffected at elevated
115 temperatures in several organisms, e.g. yeast and *Arabidopsis* (Brown et al., 2020;
116 Modliszewski et al., 2018), other aspects of the recombination pathway were found
117 to be altered by temperature leading to diverse effects, which differ depending on the
118 environmental conditions and species. Chiasma frequency was shown to be highly
119 sensitive to environmental conditions. Upon high temperatures, the chiasma
120 frequency was reduced in some species, such as female barley, *Tradescantia*
121 *bracteata*, *Uvularia perfoliata* and wild garlic (Dowrick, 1957; Lloyd et al., 2018; Loidl,
122 1989; Modliszewski et al., 2018; Phillips et al., 2015), while it increased in other
123 species, for instance in male barley, *Arabidopsis* and *Sordaria fimicola* (Lamb, 1969;
124 Lloyd et al., 2018; Modliszewski et al., 2018; Phillips et al., 2015). In *Arabidopsis*, it
125 was shown that the increase in CO frequency upon high temperatures is regulated
126 via the Type I CO pathway (Lloyd et al., 2018; Modliszewski et al., 2018). In addition,
127 CO distribution is also altered by heat stress (Dowrick, 1957; Higgins et al., 2012). In
128 barley, high temperatures cause an increase in chiasmata at the interstitial/proximal
129 region of chromosomes but an overall decrease in chiasmata per cell (Higgins et al.,
130 2012). At very high temperatures, in many species, such as wheat, barley, wild garlic
131 and *Cynops pyrrhogaster*, synapsis of the homologs fails resulting in the formation of
132 univalents (Higgins et al., 2012; Loidl, 1989; Pao and Li, 1948; Yazawa et al., 2003).

133 To obtain further insights into temperature effects on meiosis, we followed in
134 this study *Arabidopsis* male meiocytes under three different temperature regimes via
135 live cell imaging. This led to a detailed picture of the meiotic progression under heat
136 stress. A key discovery was that the length of pachytene/diakinesis is prolonged at

137 34°C, while in general, meiocytes progressed through meiosis much faster than at
138 21°C. An extension of pachytene/diakinesis was not observed when recombination
139 was abolished. Since this extension was also eradicated in *atm* mutants, we
140 conclude that Arabidopsis and likely other plants have a specialized form of the
141 pachytene checkpoint that is only triggered by recombination intermediates but not
142 the complete absence of recombination.

143

144

145 RESULTS

146 **A cytological sensor of heat stress in meiocytes**

147 To analyze the effects of increased temperatures on meiosis, we decided to apply
148 three different heat conditions reflecting possible environmental stress scenarios in
149 the future and matching conditions used in previous studies. Arabidopsis is typically
150 grown between 18 and 24°C (our standard growth conditions being 21°C). As a first
151 stress condition, we used an immediately applied heat shock of 30°C (HS30°C). In
152 parallel, we allowed plants to acclimatize to 30°C for one week (long-term, LT30°C)
153 before analyzing meiosis. The third condition was a greater heat stress of 34°C
154 (HS34°C) that was also applied immediately.

155 However, the proper and reliable application of heat stress to multicellular
156 structures, such as anthers, can be challenging when the focus is on particular cells,
157 like meiocytes, which are surrounded by many different cell layers, such as the
158 tapetum layer and the epidermis. The multicellular environment and the size of these
159 structures have the capacity to buffer temperatures, hence making it difficult to
160 exactly time the moment when the heat stress will reach the cells of interest.

161 To approach this problem, we made use of the observation that stress
162 granules (SGs) are formed at elevated temperatures in different plant tissues, e.g.
163 roots, leaves and hypocotyls (Chodasiewicz et al., 2020; Dubiel et al., 2020; Hamada
164 et al., 2018; Kosmacz et al., 2019; Modliszewski et al., 2018). It was previously
165 shown that these SGs in Arabidopsis seedlings contain the cell cycle regulator
166 CYCLIN-DEPENDENT KINASE A;1 (CDKA;1)(Kosmacz et al., 2019). CDKA;1 is a
167 major regulator of meiotic progression as well as recombination and is highly
168 expressed in male meiocytes of Arabidopsis (Bulankova et al., 2010; Dissmeyer et
169 al., 2007; Sofroni et al., 2020; Wijnker et al., 2019; Yang et al., 2020; Zhao et al.,
170 2017; Zhao et al., 2012). To test whether CDKA;1 would change its homogenous
171 cytosolic and nuclear localization pattern in meiosis upon heat stress, we applied
172 different temperature regimes to male meiocytes from plants carrying the
173 *CDKA;1:mVenus* and the *TagRFP:TUA5* reporters, and followed the localization
174 pattern of CDKA;1:mVenus during meiosis (Sofroni et al., 2020).

175 Under our standard Arabidopsis growth conditions (21°C) and consistent with
176 previous analyses, CDKA;1:mVenus is uniformly localized in both the cytoplasm and
177 the nucleus. The localization shifts from preferential cytosolic to predominantly
178 nuclear in late leptotene till early pachytene followed by increased cytosolic

179 accumulation in pachytene and diakinesis. After anaphase I and anaphase II,
180 CDKA;1:mVenus accumulates again in the reforming nuclei (Yang et al.,
181 2020)(**Figure 1A**).

182 At elevated temperatures, 30°C and 34°C, we found the same cytosolic-
183 nuclear localization dynamics of CDKA;1 (**Figure 1B,C**). At 34°C, no SGs were
184 observed in early meiotic stages (n=0/89 in G2-early leptotene; n=0/105 from late
185 leptotene till early pachytene), when CDKA;1 is preferentially localized to the nucleus
186 of meiocytes (**Figure 1C,D**). Notably, SGs were readily formed at 34°C in all
187 meiocytes from pachytene till diakinesis (n=81/81), from metaphase I till interkinesis
188 (n=82/82), and from metaphase II till telophase II (n=72/72), *i.e.* the period when
189 CDKA;1 starts to locate predominantly in the cytoplasm. These granules were
190 immediately visible after the heat stress was applied, *i.e.* after 15 min, the time
191 required to set up the acquisition for live cell imaging at the microscope. Thus, the
192 formation of SGs occurs within the first 15 min of heat stress.

193 In contrast, CDKA;1 granules were rarely formed at 30°C, *i.e.* in only 9% and
194 14% of the meiocytes in pachytene/diakinesis (n=16/165) and from metaphase I till
195 interkinesis (n=4/24), respectively (**Figure 1B,D**). In addition, the number of SGs per
196 meiocyte was also lower at 30°C compared to granule-containing meiocytes at 34°C.

197 Taken together, monitoring of SG formation allows a visual discrimination
198 between 30°C and 34°C consistent with the previous observation that the
199 temperature threshold for the formation of SGs is 34°C (Hamada et al., 2018).
200 Importantly, this optical marker indicated that the ambient temperature reaches
201 meiocytes in short time, *i.e.* less than 15 min, paving the road for the faithful
202 application of different heat treatments and comparison by live cell imaging.

203

204 **Heat stress affects microtubule configurations in meiosis in a quantitative but** 205 **not qualitative manner**

206 After having confirmed that the applied temperature regime reached male meiocytes
207 fast and faithfully, we turned towards addressing the general aim of this study, *i.e.*
208 the question how increased temperature affects the dynamics of meiosis. To address
209 this, we aimed to use a previously established live cell imaging method for meiosis
210 (Prusicki et al., 2019). A crucial finding in this approach was the observation that
211 meiosis can be dissected by so-called landmarks that occur in a predictable order
212 and that reflect highly defined cytological stages, for instance using fluorescently

213 labeled microtubules (MT, TagRFP:TUA5). Thus, these landmarks not only allow the
214 staging of meiocytes but also provide a mean to reveal the dynamics of meiosis by
215 determining the time between landmarks.

216 In brief, MT have the following dynamics during male meiosis: During G2-
217 early leptotene, MT are first homogenously distributed in meiocytes with the nucleus
218 in the center, called MT array state 1 (**Supplemental Figure 1A**). Then, they will
219 gradually polarize into a half moon-like structure on one side of the nucleus, MT
220 array state 2-3-4, from late leptotene till early pachytene (**Figure 2A, Supplemental**
221 **Figure 1B**). This develops further into a full moon-like structure entirely surrounding
222 the nucleus, MT array state 5-6, during pachytene, diplotene and diakinesis (**Figure**
223 **2B, Supplemental Figure 1C**). After nuclear envelope breakdown (NEB), the pre-
224 spindle transforms into the first meiotic spindle, MT array state 7-8-9, from
225 metaphase I till anaphase I (**Figure 2C, Supplemental Figure 1D**). Next, MT
226 reorganize around the two newly formed nuclei and central MT form a phragmoplast-
227 like structure, MT array state 10-11, at telophase I and interkinesis (**Figure 2D,**
228 **Supplemental Figure 1E**). The second division is characterized by the formation of
229 two pre-spindles, followed by two spindles, MT array state 12-13, from metaphase II
230 till anaphase II (**Figure 2E, Supplemental Figure 1F**). Phragmoplast-like structures,
231 MT array state 14 are visible at telophase II (**Figure 2F, Supplemental Figure 1G**)
232 until cytokinesis, resulting in tetrads, the four meiotic products.

233 Analyzing meiosis at HS30°C, HS34°C and LT30°C, we confirmed that
234 meiosis does not arrest upon exposure to these temperature regimes, consistent
235 with previous studies (De Storme and Geelen, 2020; Lei et al., 2020). Importantly, in
236 all movies taken at higher temperature (in total 46), the meiocytes progressed
237 through the same MT array states as previously found for 21°C (**Supplemental**
238 **Figure 1, Supplemental Movies 1-4**, (Prusicki et al., 2019)).

239 MT stability and polymerization are known to be temperature-sensitive,
240 (Bannigan et al., 2007; Li et al., 2009a; Liu et al., 2017; Song et al., 2020; Wu et al.,
241 2010). Consistently, we observed quantitative changes in some MT structures
242 confirming that meiocytes were exposed to elevated temperatures. As revealed by
243 pixel intensity quantification of meiocytes in MT array state 6, in which MT are fully
244 surrounding the nucleus (**Figure 3A,B**), we found that the measured intensity of the
245 fluorescence signal of TagRFP:TUA5 dropped upon both HS30°C and HS34°C in
246 comparison to 21°C, indicating that MT density is reduced. Notably, this reduction is

247 reverted at LT30°C, implying the existence of an adaptation mechanism for meiosis
248 to heat. In addition, irregular spindle structures were observed at 34°C but not at
249 lower temperatures (**Figure 3C**), consistent with previous analyses (De Storme and
250 Geelen, 2020; Lei et al., 2020).

251 Taken together, the quantitative but not qualitative changes of the typical
252 meiotic MT configurations allow using the adoption of characteristic MT arrays for
253 staging of meiosis during live cell imaging. At the same time, the quantitative effects
254 on the MT arrays corroborate the previous finding that meiocytes successfully
255 received the heat treatment in our experimental set up.

256

257 **Duration of meiosis under heat stress**

258 The next challenge to overcome for the evaluation of meiotic progression at elevated
259 temperatures was the question how the MT-based dissection of the different heat
260 stress experiments could be statistically compared with the control growth
261 conditions. This is not a trivial question since the analyses of meiocytes within one
262 anther-sac cannot be regarded as statistically independent measurements but
263 represent clustered data. In addition, the above-mentioned nature of defined meiotic
264 stages gives rise to a multi-state nature of our dataset. Moreover, our measurements
265 occasionally did not capture the exact start and/or end point (left, right and/or interval
266 censored data) of a MT array state since the observed anthers sometimes move out
267 the focal plane (but also occasionally move into focus again).

268 The combination of the three characteristics of our data, *i.e.* clustering data,
269 left/right and/or interval censoring, as well as having a multistate nature, in one
270 statistical model was not possible. Therefore, we reduced the complexity of the
271 analysis and built one separate model for each state. This also allowed us to simplify
272 the mixture of left/right and/or interval censored data with respect to the duration of
273 each state to interval (and right) censoring.

274 We applied parametric models for interval-censored survival time data with a
275 clustered sandwich estimator of variance to address the clustering of meiocytes
276 within anther-sacs, including effects of the heat treatment, genotype and their
277 interactions. The underlying distribution of the parametric model was chosen based
278 on the Akaike Information Criterion (AIC) with exponential, Gompertz, log-logistic,
279 Weibull and log-normal distribution as candidates.

280 The models used information from all cells which had at least one observation
281 in the respective stage. The event of interest is the transition of a cell from one stage
282 to the next. Each cell for which the exact beginning and end of the stage were known
283 was modelled as having an event with the event time as the difference between the
284 start of the next stage and the end of the previous stage. Cells where the exact time
285 points of either the transition from the previous stage to the stage of interest or to the
286 next stage were not known were modelled as interval-censored data points with the
287 lower limit of the interval being the time where the cell was observed in this specific
288 state and the upper limit of the interval being one time unit before when the cell was
289 observed in the previous or next stage, respectively. If for a cell a certain stage either
290 at the beginning or end was not observed the cell was modelled as right-censored
291 with the censoring time being the minimum observed time for this cell in the stage of
292 interest.

293 With the imaging and evaluation system in hand, we then addressed what the
294 effect of HS30°C, HS34°C and LT30°C has on the total length of meiosis. To this
295 end, plants were grown under long-day conditions in highly controlled growth
296 chambers (see material and methods). For the long-term heat treatment, plants were
297 transferred to constant 30°C around bolting time. At flowering stage, flower buds
298 were dissected for live cell imaging of male meiocytes, as described in (Prusicki et
299 al., 2020). For the heat shock treatments, only flower buds which were in MT array
300 state 1 were used for the prediction of the duration of the different meiotic states
301 upon heat shock. The determination of the meiotic duration relies on defined start
302 and end points of MT states (called events). Since this is not possible for MT state 1
303 (no start point), the first stage that could be temporally evaluated was MT state 2-3-
304 4.

305 From a total of 59 movies, we first aimed for movies that covered all MT array
306 states (2-14) under the four temperature regimes. Unfortunately for HS34°C, we
307 were not able to reliably determine the end point of MT array state 14 as the
308 fluorescent signal of the MT became very poor, possibly due the fact that MT are
309 more defusedly organized at high temperature versus control conditions (described
310 above, **Figure 3**) and photo-bleaching after long time lapses.

311 To compare the overall meiotic duration at all heat conditions, we excluded
312 MT array state 14 for this analysis and only considered movies capturing MT array
313 states 2-13 (23 movies). Then, we built a separate parametric model for the

314 complete duration (as described above), resulting in the total predicted median time,
315 together with the 95% confidence interval (CI).

316 The duration of MT array states 2-13 at 21°C was determined to have a
317 predicted median time of 1271 min (*i.e.* 21.2 h, CI 1151-1390 min, **Figure 2G**,
318 **Supplemental Table 1**), This value matched very well the previous analyses of the
319 duration of male meiosis in Arabidopsis by pulse-chase experiments and live cell
320 imaging, underlining the robustness of our analysis and the reproducibility of meiotic
321 progression at 21°C (Armstrong et al., 2003; Prusicki et al., 2019; Sanchez-Moran et
322 al., 2007; Stronghill et al., 2014).

323 Next, the duration of meiosis under the heat conditions was analyzed,
324 resulting in a predicted median time of 966 min (*i.e.* 16.1 h, CI 876-1056 min) upon
325 HS30°C, 1086 min (*i.e.* 18.1 h, CI 1048-1124 min) upon HS34°C and 1086 min (*i.e.*
326 18.1 h, CI 1050-1122 min) upon LT30°C (**Figure 2G**, **Supplemental Table 1**). These
327 data confirm previous observations that meiosis progresses faster under elevated
328 temperatures in comparison to control conditions, also demonstrating that our
329 experimental system can be faithfully used to study the effect of heat on meiosis
330 (Bennett et al., 1972; Draeger and Moore, 2017; Stefani and Colonna, 1996; Wilson,
331 1959).

332

333 **Duration of individual meiotic phases under heat stress**

334 The live cell imaging approach together with the model based estimation of the
335 duration of the MT array states, allowed us then to target the main aim of this study,
336 *i.e.* to obtain a detailed and meiotic-phase specific assessment of the meiotic
337 progression under elevated temperatures.

338 At 21°C, a total of 206 meiocytes were observed and the predicted median
339 time per MT array state was calculated from those cells for which we had at least
340 one observation in that specific state (**Table 1**, **Supplemental Table 1**). The median
341 time in MT array state 2-3-4 was predicted to be 845 min (CI 746-944 min, **Figure**
342 **2A'**). Followed by MT array state 5-6, which had a predicted median time of 360 min
343 (CI 309-412 min, **Figure 2B'**), MT array state 7-8-9 takes 47 min (CI 44-49 min,
344 **Figure 2C'**) and MT array state 10-11 spans 52 min (CI 47-57 min, **Figure 2D'**).
345 After that, the second meiotic division follows with a predicted median time of 46 min
346 for MT array state 12-13 (CI 44-49 min, **Figure 2E'**), finishing the meiotic division
347 with 219 min for MT array state 14 (CI 205-234 min, **Figure 2F'**).

348 Next, male meiosis subjected to the three different temperature regimes was
349 analyzed in the same way. A total of 133, 188 and 211 meiocytes were observed for
350 HS30°C, HS34°C and LT30°C, respectively and the predicted median time per state
351 was calculated from those cells for which we had at least one observation in that
352 specific state (**Table 1, Supplemental Table 1**). The duration of MT array state 2-3-
353 4 upon higher temperature was decreased compared to 21°C (845 min), with a
354 predicted median time of 556 min upon HS30°C (CI 485-628 min), 428 min upon
355 HS34°C (CI 403-453 min) and 609 min upon LT30°C (CI 550-667 min, **Figure 2A'**).
356 This shows that the increase in temperature generally decreases the duration of this
357 phase.

358 A strikingly different behavior was revealed for the next phase, *i.e.* MT array
359 state 5-6. While upon exposure to HS30°C and LT30°C the predicted median time
360 was 365 min (CI 319-411 min) and 378 min (CI 340-416 min), respectively, HS34°C
361 resulted in a median of 522 min (CI 498-546 min, **Figure 2B'**). This was a much
362 longer duration of this phase compared to 21°C (360 min), presenting a prolongation
363 of ~2.7 h.

364 After NEB, the meiocytes undergo the first round of chromosome segregation,
365 *i.e.* MT array state 7-8-9, with a predicted median time of 32 min (CI 28-36 min) upon
366 HS30°C, 34 min (CI 32-36 min) upon HS34°C and 39 min (CI 35-44 min) upon
367 LT30°C, which is decreased compared to 21°C (47 min, **Figure 2C'**).

368 The following MT array state 10-11 spanned 47 min (CI 41-53 min) upon
369 HS30°C, 59 min (CI 55-63 min) upon HS34°C and 45 min (CI 38-51 min) upon
370 LT30°C (**Figure 2D'**). Whether the differences between HS34°C and 21°C (52 min)
371 is biologically relevant is to be resolved.

372 Upon HS30°C, HS34°C and LT30°C, the second round of chromosome
373 segregation, MT array state 12-13, spanned 29 min (CI 27-31 min, n=77), 24 min (CI
374 22-25 min, n=175) and 37 min (CI 32-43 min, n=150), respectively (**Figure 2E'**).
375 Although statistically different compared to 21°C (46 min), the biological relevance is
376 not clear at this moment and needs to be investigated in future.

377 The end of the meiotic division upon heat treatment was predicted using MT
378 array state 14, which spanned 209 min (CI 185-233 min, n=65) upon HS30°C and
379 256 min (CI 230-282 min, n=144) upon LT30°C (**Figure 2F'**). The pairwise
380 comparison of 21°C and LT30°C showed a statistical difference which needs to be

381 investigated in future. Last, as described before, for HS34°C we were not able to
382 predict the duration of MT array state 14.

383

384 **Exposure to high temperature causes chromosomal defects**

385 To investigate the unexpected prolongation of late prophase at 34°C (**Figure 2B'**) in
386 more detail, we first performed chromosome spreads from fixed flower buds at the
387 different temperature regimes to investigate chromosomal behavior in our heat
388 conditions and which could be confirmed in previous studies (De Storme and
389 Geelen, 2020; Hedhly et al., 2020; Higgins et al., 2012).

390 At control growth conditions, decondensed chromatin becomes organized into
391 chromosomes which will gradually condense during early prophase I and reach a
392 fully paired state at pachytene. The paired homologs condense further, where
393 chiasmata hold homologs together, finally reaching the highest condensed state at
394 diakinesis with the formation of five bivalents that align at the metaphase plate during
395 metaphase I (**Supplemental Figure 2A**). At both HS30°C and LT30°C, homologs
396 condense and fully pair. Occasionally, two or more bivalents appear to be entangled
397 at diakinesis and metaphase I forming chromosome bridges, suggesting
398 interconnected non-homologous chromosomes. In addition, chromosome fragments
399 and univalents were infrequently observed (**Supplemental Figure 2B,C**). In contrast
400 to 21°C and 30°C, fully paired homologs could not be found at 34°C. Further,
401 chromosome spreads of cells in diakinesis and metaphase I at 34°C revealed the
402 formation of mainly 10 univalents. In addition, chromosome bridges were visible
403 between both homologs and non-homologous chromosomes (**Supplemental Figure**
404 **2D**).

405 Thus, consistent with previous data, we find that high temperature causes
406 recombination defects, which increase with rising temperatures (Bomblies et al.,
407 2015; Brown et al., 2020; De Storme and Geelen, 2020; Higgins et al., 2012;
408 Modliszewski et al., 2018; Morgan et al., 2017; Phillips et al., 2015).

409

410 **Synaptonemal complex formation is defective at 34°C**

411 Given the central role of the formation of the chromosome axis for pairing and
412 meiotic recombination, we next analyzed the localization of the previously generated
413 reporters ASY1:RFP and ZYP1b:GFP upon 30°C and 34°C (Yang et al., 2019; Yang
414 et al., 2020). ASYNAPTIC 1 (ASY1) is a chromosome axis-associated protein, which

415 plays a major role in the initiation of synapsis and recombination (Armstrong et al.,
416 2002; Caryl et al., 2000; Sanchez-Moran et al., 2007). ZIPPER 1 (ZYP1) encodes for
417 a component of the transversal element of the synaptonemal complex (SC), which is
418 thought to be crucial for the maturation of COs and CO interference (Capilla-Perez et
419 al., 2021; France et al., 2021; Higgins et al., 2005; Osman et al., 2006).

420 At standard conditions, 21°C, ASY1 localizes to the chromosome axis from
421 early leptotene till pachytene. During zygotene, when the formation of the SC is
422 initiated, ASY1 gets largely depleted from the chromosome axis and ZYP1b signal
423 starts to appear on chromosomes from where it gradually forms into a linear
424 structure, resulting in the labeling of the complete chromosome axis at pachytene
425 (**Figure 4A**).

426 Under high temperatures, 30°C and 34°C, the localization of ASY1 at the
427 chromosome axis was unaffected and ZYP1b started to form short linear stretches at
428 the chromosome axis during zygotene (**Figure 4B,C**). At 30°C, ZYP1b continues to
429 label the full length of the axis, in contrast to 34°C, at which only small stretches of
430 ZYP1b signal could be observed, suggesting that ZYP1b loading is initiated properly
431 but discontinues (**Figure 4B,C**). This result is in accordance with previous findings
432 showing that synapsis is obstructed upon very high temperature leading to the
433 formation of abnormal structures, called polycomplexes (Bilgir et al., 2013; Higgins et
434 al., 2012; Loidl, 1989).

435

436 **Late heat shock 34°C does not cause an elongation of pachytene/diakinesis**

437 Seeing defective SC formation at 34°C, we asked whether events between zygotene
438 and pachytene are particularly sensitive to heat stress and hence, responsible for the
439 delay of NEB. Therefore, we specifically applied heat stress only from MT state 2-3-4
440 (zygotene) onward and compared the effect of this treatment with the previously
441 applied heat shock before MT state 1, *i.e.* from pre-meiosis-leptotene onwards, by
442 live cell imaging. Since we showed above that male meiocytes perceive heat stress
443 in less than 15 min, we were confident that a late heat shock can allow distinguishing
444 the temperature effects on early versus late prophase faithfully.

445 The predicted median time of MT array state 5-6 was calculated as described
446 before and the comparison of flower buds in MT array state 1 and MT array state 2-
447 3-4 at the onset of the heat stress was performed. We did not observe a statistical
448 difference between early and late applied HS30°C, with a predicted median time of

449 313 min for MT array state 5-6 at late HS30°C (CI 270-355 min, **Figure 5A,**
450 **Supplemental Table 1**). Remarkably, the extension of MT state 5-6 seen at early
451 HS34°C (median time of 522 min) did not take place when we applied a late
452 HS34°C, with a median time of 393 min (CI 349-437 min, **Figure 5B, Supplemental**
453 **Table 1**). This suggests that the prolongation of MT state 5-6 is most probably not
454 due to temperature effects on regulatory processes that take place at the moment of
455 SC formation or on the SC itself. Rather early steps in prophase I, e.g. the initiation
456 of meiotic recombination, might be temperature sensitive and subsequently affect the
457 duration of pachytene/diakinesis.

458

459 **Loss of recombination *per se* does not cause the elongation of** 460 **pachytene/diakinesis**

461 To address to what degree a failure of recombination, which is initiated in early
462 prophase, causes a pachytene/diakinesis delay, we first made use of the well-
463 characterized *spo11-1* mutant (Grelon et al., 2001; Hartung et al., 2007), in which
464 recombination is completely abolished due to a failure to form DSBs. The
465 *TagRFP:TUA5* reporter was introduced in *spo11-1* allowing us to follow the meiotic
466 progression by using live cell imaging and MT state based determination of meiotic
467 phases from a total of 224 observed meiocytes (**Supplemental Movie 5, Table 1,**
468 **Supplemental Table 1**).

469 Interestingly and not previously recognized, *spo11-1* mutants spent much
470 longer in early prophase (MT array state 2-3-4, late leptotene till early pachytene)
471 compared to the wildtype (845 min, **Table 1, Figure 2A'**), with a predicted median
472 time of 1119 min (CI 1031-1206 min, **Figure 6A**). The underlying molecular reason
473 for this is not clear but interesting to study in the future. Important for this study, in
474 *spo11-1* the duration of MT array state 5-6 had no statistical difference compared to
475 the wildtype (360 min, **Table 1, Figure 2D'**), with a predicted median time of 374 min
476 (CI 349-399 min, **Figure 6D**). This suggests that the complete loss of recombination
477 caused by the absence of DSBs in the *spo11-1* mutant does not lead to the
478 prolongation of MT array state 5-6 at 21°C.

479 After prophase I, the meiotic division in *spo11-1* mutants continues with a
480 predicted median time of 72 min (CI 67-76 min) for MT array state 7-8-9, followed by
481 MT array state 10-11 for 63 min (CI 58-67 min), MT array state 12-13 for 48 min (CI
482 45-52 min) and finally MT array state 14 for 356 min (CI 326-385 min)

483 **(Supplemental Figure 4)**. Interestingly and unexpectedly, the durations of MT array
484 state 7-8-9, MT array state 10-11 and MT array state 14 of *spo11-1* mutants show a
485 statistical difference to the wildtype (**Figure 2C', D', F'**).

486 Next, we asked whether a prolongation of MT state 5-6 could be dependent
487 on homologous recombination repair by following meiosis in *dmc1* mutants in which
488 we introduced the *TagRFP:TUA5* reporter and observed a total of 157 meocytes
489 (**Supplemental Movie 6, Table 1, Supplemental Table 1**). In *dmc1* mutants, DSBs
490 are repaired through the sister chromatid of the same chromosome in an HR-
491 dependent manner (Kurzbaue et al., 2012). The predicted median time per state
492 was calculated and resulted in a duration of 1056 min for MT array state 2-3-4 (CI
493 929-1184 min, **Figure 6A**), showing a statistical difference to the wildtype (845 min,
494 **Figure 2A'**) and resembling the extension of this phase seen in *spo11-1*. Thus, loss
495 of early recombination steps appears to trigger a prolongation of early meiosis. For
496 MT array state 5-6 in *dmc1* we determined a similar duration of 343 min (CI 331-355
497 min, **Figure 6D**) compared to wildtype (360 min, **Figure 2B'**), hence, for *dmc1*
498 mutants we also do not observe a temporal extension of MT state 5-6. The meiotic
499 division continued with a median time of 67 min (CI 63-71 min) for MT array state 7-
500 8-9. MT state 10-11 takes 63 min (CI 59-67 min), MT array state 12-13 lasts 47 min
501 (CI 45-49 min) and MT array state 14 spans 281 min (CI 262-301 min,
502 **Supplemental Figure 4**). All these subsequent phases are similar to its duration in
503 *spo11-1*, which is interesting to investigate in the future.

504 Finally, we tested whether a failure to resolve recombination intermediates as
505 Type I COs could be responsible for the delayed onset of NEB, using *msh4* mutants
506 that contain the *TagRFP:TUA5* reporter (**Supplemental Movie 7**). A total of 193
507 meocytes were and for every stage, the predicted median time was calculated
508 (**Table 1, Supplemental Table 1**). In *msh4*, the MT array state 2-3-4 takes 951 min
509 (CI 861-1040 min). This duration is not statistically different from wildtype (845min,
510 **Figure 2A'**) but lies in between the CI for the wildtype on the one hand and the CI
511 for *spo11-1* and *dmc1* on the other hand. Hence, it is difficult to judge from this
512 dataset so far, whether this extension is relevant in comparison to the wildtype and
513 resembles the situation found in the other two recombination mutants.

514 Subsequently, in *msh4*, a duration of 314 min (CI 299-329 min) was
515 determined for MT array state 5-6 (**Figure 6A,D**). The meiotic division continued with
516 extended MT array state 7-8-9 for 67 min (CI 65-72 min) compared to wildtype (47

517 min, **Figure 2C'**), which is similar as seen for *spo11-1* and *dmc1*. Next, MT array
518 state 10-11 lasts 59 min (CI 56-63 min), MT array state 12-13 takes 49 min (CI 46-52
519 min) and finally, MT array state 14 spans 274 min (CI 253-294 min, **Supplemental**
520 **Figure 3**). Thus, all recombination mutants tested have a similar duration of MT
521 array states 7-8-9, 10-11, 12-13 and 14, compared to the wildtype (**Figure 2**). Yet,
522 *msh4* mutants progressed through pachytene/diakinesis as wild-type plants at 21°C.
523 Previously, 5'-bromo-2'-deoxyuridine (BrdU) labeling experiments showed a delay of
524 S-phase till the end of prophase I in *msh4* mutants of 8 h, which could not be
525 confirmed here, most probably because our time predictions did not include meiotic
526 S-phase and early leptotene, where MSH4 is known to start appearing as numerous
527 foci on the axes (Higgins et al., 2004).

528 Taken together, these results indicate that the loss of recombination *per se*
529 does not cause the elongation of the MT array state 5-6 seen in wild-type meiocytes
530 at 34°C.

531

532 **Prolongation of MT array state 5-6 at very high temperature is recombination-** 533 **dependent**

534 To further investigate the role of the recombination pathway on the elongation of MT
535 array state 5-6 upon very high temperature heat stress, we observed a total of 198
536 meiocytes and analyzed the duration of this phase in *spo11-1* mutants at 34°C
537 (**Supplemental Movie 8, Table 1, Supplemental Table 1**). Identically to the heat
538 shock treatments of wild-type meiocytes, only flower buds which were in MT array
539 state 1 were used for the modelling of the duration of the different meiotic states at
540 HS34°C.

541 The MT array state 2-3-4 had a predicted median time of 626 min (CI 572-681
542 min), which is a decrease in duration compared to *spo11-1* at 21°C (1119 min,
543 **Figure 6A**), showing a similar reduction as described for wild-type meiocytes
544 (**Figure 6B**). Notably, the elongation of MT state 5-6 seen in the wildtype at HS34°C
545 (522 min, a delay of 126 min compare to wildtype 21°C, **Figure 2B'**) was not found
546 in *spo11-1* mutant at HS34°C, with a predicted median time of 412 min (CI 393-431
547 min, **Figure 6E**), compared to *spo11-1* at 21°C (374 min, **Figure 6D**). Further, MT
548 array state 7-8-9 takes 35 min (CI 32-38 min), MT array state 10-11 spans 54 min
549 (CI 49-58 min) and MT array state 12-13 lasts 23 min (CI 21-26 min, **Supplemental**
550 **Figure 4**). All these states, except MT array state 5-6, had no statistical difference

551 compared to wildtype at HS34°C (**Figure 2**), showing a similar reduction as
552 described for wildtype at 21°C and HS34°C. For MT array state 14 of *spo11-1* at
553 HS34°C, we could not provide the median time, identical to wildtype at HS34°C.

554 This suggested that the delay in the wildtype at very high temperature is not
555 due to the absence of recombination but likely due to aberrant recombination
556 intermediates and when they cannot be formed, as in *spo11-1* mutants, meiosis
557 progresses without delay. To corroborate this, we next observed a total of 160 *dmc1*
558 and 116 *msh4* meiocytes and measured the duration of MT state 5-6 at HS34°C
559 (**Supplemental Movies 9-10, Table 1, Supplemental Table 1**). The predicted
560 median time of MT array state 2-3-4 of *dmc1* upon HS34°C was 565 min (CI 526-
561 605 min) and for the *msh4* mutant 571 min (CI 536-606 min, **Figure 6B**), which is a
562 decrease in median time compared to these mutants at 21°C (**Figure 6A**). The
563 predicted median time of MT array state 5-6 of *dmc1* and *msh4* at HS34°C was 383
564 min (CI 362-403 min) and 398 min (CI 346-450 min), respectively (**Figure 6E**).
565 Compared to these mutants at 21°C, the duration was slightly longer, yet statistically
566 different, but not to the degree of the elongation seen in wild-type meiocytes at 34°C
567 (**Figure 2B'**).

568 These mutants at 34°C continued the meiotic division with MT array state 7-8-
569 9 for 30 min (CI 28-32 min) and 32 min (CI 30-34 min), MT array state 10-11 for 57
570 min (CI 54-60 min) and 49 min (CI 43-55 min) and MT array state 12-13 for 22 min
571 (CI 21-23 min) and 24 min (CI 22-26 min), respectively (**Supplemental Figure 3**).
572 Similar to wildtype and *spo11-1* at HS34°C, we could not provide a predicted median
573 time for the MT array state 14.

574 Taken together, the absence of the 2.7 h prolonged duration of MT array state
575 5-6 in the recombination mutants, *spo11-1*, *dmc1* and *msh4*, upon HS34°C leads to
576 the hypothesis that the extension of the MT array state 5-6 upon 34°C is
577 recombination dependent.

578

579 **High temperature reveals the presence of a pachytene checkpoint in** 580 **Arabidopsis**

581 The here observed prolongation of pachytene/diakinesis is reminiscent of the
582 pachytene checkpoint in animals and yeast. However, the observation that mutants
583 devoid of recombination progress through meiosis in plants, as quantified above, has
584 previously raised the hypothesis that plants do not have a pachytene checkpoint

585 (Caryl et al., 2003; Jackson et al., 2006; Li et al., 2009b). A central executor of the
586 pachytene checkpoint in yeast and animals is the checkpoint kinase ATM (Lange et
587 al., 2011; Pacheco et al., 2015; Penedos et al., 2015). ATM is highly conserved and
588 also plays a major role in meiosis in Arabidopsis, for instance for the repair of DSBs
589 (Garcia et al., 2003; Kurzbauer et al., 2021; Lange et al., 2011; Li et al., 2004; Yao et
590 al., 2020).

591 To test an involvement of ATM in the prolongation of pachytene/diakinesis
592 upon heat stress, we introduced the *TagRFP:TUA5* reporter in the *atm* mutant and
593 followed meiotic progression at 21°C and HS34°C using live cell imaging and
594 observed a total of 228 and 172 meiocytes, respectively, and determined the
595 duration of the MT array states as described before (**Supplemental Movies 11-12,**
596 **Table 1, Supplemental Table 1**). At 21°C, the MT array state 2-3-4 lasts 834 min
597 (CI 761-908 min) and MT array state 5-6 takes 295 min (CI 270-321, **Figure 6C,F**).
598 Thus, *atm* meiocytes progressed even faster than the wildtype through MT array
599 state 5-6 (360 min, **Figure 2B'**), hinting at a possible role in prolonging this phase
600 even under control conditions.

601 Next, MT array state 7-8-9 takes 45 min (CI 42-49 min), MT array state 10-11
602 lasts 60 min (CI 55-66 min), MT array state 12-13 persists 43 min (CI 40-46 min) and
603 MT array state 14 spans 245 min (CI 230-260 min, **Supplemental Figure 3**). The
604 biological relevance of the statistical difference of MT array state 10-11 and 14,
605 compared to wildtype, needs to be further investigated.

606 Under HS34°C, the MT array state 2-3-4 had a predicted median time of 702
607 min (CI 640-764 min) and surprisingly the prolongation of MT array state 5-6 seen in
608 the wildtype was abolished, *i.e.* 350 min (CI 330-370 min) in *atm* mutants versus 522
609 min in the wildtype (**Table 1, Figure 6C,F**). Thus, *atm* meiocytes progressed through
610 this phase with a similar speed as meiocytes in which recombination is abolished.

611 All other MT array states were not found to be obviously different in duration
612 when compared to the wildtype at 34°C, *i.e.* MT array state 7-8-9 lasts 31 min (CI 29-
613 33 min), MT array state 10-11 takes 55 min (CI 50-60 min) and MT array state 12-13
614 spans 26 min (CI 23-28 min, **Figure 6C,F, Supplemental Figure 3**). Once again,
615 the duration of MT array state 14 at HS34°C could not be predicted.

616 These results suggest the involvement of ATM in the prolongation of
617 pachytene/diakinesis upon 34°C. Given the similarities in extension of
618 pachytene/diakinesis and the involvement of ATM, we conclude that Arabidopsis and

619 likely other plants do have a specialized variant of the pachytene checkpoint that
620 relies on the action of ATM and possibly other regulators to monitor aberrant
621 recombination intermediates at high temperatures but, in contrast to animals, not the
622 absence of recombination.

623

624

625 DISCUSSION

626 More than 50 years ago, the consequences of high temperature on plant
627 development in general and on meiosis in particular were already studied (Dowrick,
628 1957; Pao and Li, 1948; Wilson, 1959). Due to the latest insights into climate
629 change, research on the influence of temperature on meiosis has been revived.
630 Previous and current studies have relied on the analysis of fixed samples and
631 obtained important insights into the duration of meiosis and meiotic recombination
632 patterns at elevated temperatures. Here, we have followed a complementary
633 approach by following meiosis by time-lapse live cell imaging. This has allowed us to
634 obtain a highly temporally resolved dissection of meiotic progression in which we
635 have compared the effects of three heat stress treatments, *i.e.* a heat shock at 30°C
636 and 34°C and a long-term (one week) treatment at 30°C in comparison to the control
637 temperature of 21°C. Notably, this work has provided novel insights into the effects
638 of temperature on recombination as well as meiotic progression and has set the
639 stage for revising of a dogma in the field.

640

641 **Formation of stress granules in meiosis**

642 Heat stress induces a multitude of cellular responses, including the inhibition of
643 general translation and the formation of SGs, which are proposed to function as
644 transient places for both storage and degradation of proteins and mRNAs during
645 stress resulting in a re-programming of translation. This is thought to be especially
646 important for the re-initiation of translation upon recovery from the stress condition,
647 as reviewed by (Anderson and Kedersha, 2002, 2008; Buchan and Parker, 2009). In
648 mice spermatocytes, SGs have been previously found to be formed after heat
649 treatment (42°C) and these SGs contain for instance DAZL, an RNA-binding protein,
650 which interacts with the SC, is involved in mRNA transport and is proposed to
651 function as a translational activator (Kim et al., 2012).

652 Labelling the major cell cycle regulator of Arabidopsis, CDKA;1, we have
653 shown here that meiocytes in Arabidopsis also form SGs at 30°C and 34°C. CDKA;1
654 has been previously demonstrated along with several other proteins, like MPK3 and
655 TORC1, to be present in SGs of heat-stressed seedlings (Kosmacz et al., 2019).
656 Why and how CDKA;1 is recruited to the SGs and which other proteins and RNAs
657 are present in SGs during meiosis remains to be investigated. It has been previously
658 hypothesized that the presence of CDKA;1 in SGs would allow a cell to resume cell

659 division activity in Arabidopsis after attenuation of the stress (Kosmacz et al., 2019).
660 CDKs typically require a co-factor, called cyclin, for their activity and in budding
661 yeast, WHI8, an RNA-binding protein, was shown to bind to and recruit the mRNA of
662 the cyclin CLN3 to SGs upon heat stress causing the inhibition of CLN3 translation
663 (Yahya et al., 2021). Interestingly, CDC28, the homolog of CDKA;1 in budding yeast,
664 itself is also recruited to SGs by WHI8 and has been found to play an important role
665 in SG dissolution and the translation of SG-recruited mRNAs, such as the one of
666 CLN3, upon release from stress.

667 This raises the question whether in Arabidopsis CDKA;1 is also a mediator of
668 SG dissolution and subsequent re-initiation of translation. Interestingly, many
669 proteins related to translation have been previously identified as putative CDKA;1
670 substrates (Pusch et al., 2011). A pivotal role of translational control for the
671 abundance of proteins in meiosis has been established in several organisms
672 including budding yeast (Brar et al., 2012). This gives rise to the speculation that
673 translational regulation of meiosis in Arabidopsis is also present and likely controlled
674 by CDK activity.

675 The appearance of CDKA;1 in SGs has allowed us to faithfully confirm the
676 application of the heat stress in meiocytes. On the one hand, we could show that the
677 heat stress reaches meiocytes relatively fast, *i.e.* in less than 15 min. Thus, our
678 imaging starts when meiocytes are already exposed to the desired temperature
679 applied in our set-up. On the other hand, we observed that SGs are not regularly
680 found at 30°C. Thus, the appearance of SGs is also a sensor of the applied
681 temperature itself. Since SGs are formed rapidly at 34°C, we assumed that the heat
682 stress at 30°C also reaches meiocytes in a similar time frame providing confidence
683 that we have been looking at an immediate effect of the high temperature rather than
684 a ramping effect over a long period. We anticipate that the formation of CDKA;1-
685 containing SGs could be used as a general readout to study heat stress in other
686 plant tissues and possibly other plant species as well.

687 Interestingly, the localization of CDKA;1 to SGs is stage-specific and its SG
688 localization could only be observed from pachytene onwards but not earlier in
689 meiosis. Notably, DAZL also shows a stage-specific localization to SGs in mice
690 spermatocytes and is recruited to SGs only during pachytene in response to heat,
691 coinciding with its highest accumulation level (Kim et al., 2012). In comparison,
692 CDKA;1 is dynamically localized in the nucleus and the cytoplasm and the formation

693 of CDKA;1-positive SGs appears when its cytoplasmic portion is the highest.
694 Whether the formation of CDKA;1-positive SGs is a hence of function of its
695 cytoplasmic concentration or whether this relies on other stage specific parameters
696 needs to be determined. Conversely, it is also not clear whether non-CDKA;1-
697 containing SGs are formed prior to pachytene.

698

699 **Heat and meiotic progression**

700 The durational changes of meiosis upon high temperatures were studied in several
701 plant species including Arabidopsis, barley, wheat, *Dasypyrum villosum* (L.) P.
702 *candargy* and bluebell (Bennett et al., 1972; Draeger and Moore, 2017; Higgins et
703 al., 2012; Stefani and Colonna, 1996; Wilson, 1959). These studies have relied on
704 static analysis of fixed material, e.g. anther fixation and staging before and after a
705 certain time interval or BrdU pulse labelling followed by the analysis of meiotic
706 chromosome figures (Armstrong et al., 2003; Bennett et al., 1972). These studies
707 concluded that the duration of meiosis at high temperatures is decreased. Here, we
708 have confirmed the general trend of increased speed of meiosis at high
709 temperatures. However, our live cell imaging approach allowed us to follow meiotic
710 progression with unprecedented depth generating quantitative data that can be
711 statistically analyzed. This led to the finding not all meiotic phases respond equally to
712 an increase in temperature. Most strikingly, we found that pachytene/diakinesis were
713 substantially extended at 34°C when compared to control conditions at 21°C, as
714 seen by a considerable prolongation of the time of NEB.

715 This opens the door to study which regulators and/or processes are sensitive
716 to heat. For instance, it is well-established that NEB in animals is under full control of
717 CDK activity and nuclear envelope components, like lamins are bona fide CDK
718 substrates (Adhikari et al., 2012; Gong et al., 2007; Zuela and Gruenbaum, 2016).
719 How NEB is controlled in plants is still an enigma, especially also since lamins do not
720 appear to be conserved in plants (Ciska and Moreno Diaz de la Espina, 2013;
721 Fiserova and Goldberg, 2010). However, it is tempting to speculate that NEB is also
722 under the control of CDK activity. Hence, the here-observed delay in NEB might be
723 directly or indirectly mediated by a repression of CDK activity.

724 NEB also likely represents a gate in meiotic progression. Chromosomes are
725 strong microtubule organizing structures in plants (Lee and Liu, 2019), and once the
726 nuclear envelope is broken down, the MT array that is enriched around the nucleus

727 quickly connects to the chromosomes and organizes itself into a spindle (Prusicki et
728 al., 2019). Thus, a delay of NEB represents a physical barrier that provides
729 additional time to complete and/or correct processes in the reaction environment of
730 the nucleus before chromosomes start to be moved in the cell.

731

732 **Heat and meiotic recombination**

733 To further explore the extension of pachytene/diakinesis under heat stress, we have
734 genetically and temporally dissected this effect. An obvious cause for the observed
735 prolongation was altered meiotic recombination, supported by our study and
736 previous analyses of meiotic chromosome configurations (De Storme and Geelen,
737 2020; Hedhly et al., 2020; Higgins et al., 2012). Using then mutants of genes that
738 control different steps in the meiotic recombination process, like *spo11-1*, *dmc1*, and
739 *msh4*, we have shown that the extension of pachytene/diakinesis is recombination
740 dependent, *i.e.* the extension of pachytene/diakinesis was lost in these mutants at
741 34°C. Notably, these mutants, when grown under non-stress conditions at 21°C, do
742 not display a relevant prolongation of meiosis in a way that we could detect with our
743 assays. This stands in contrast to animals where loss of recombination, *e.g.* in *dmc1*
744 mice mutants, triggers meiotic arrest and subsequently induces cell death (Barchi et
745 al., 2005; de Rooij and de Boer, 2003; Roeder and Bailis, 2000).

746 To further narrow down the origin of the elongation of pachytene/diakinesis,
747 we applied heat stress only around zygotene, *i.e.* up to 17 h later than in our first
748 sets of experiments. Notably, this late heat stress did not cause a prolongation and
749 hence, recombination appears to be affected prior to SC formation. This is
750 interesting since earlier work indicated that the SC is severely affected by heat
751 leading to so-called polycomplexes in which transverse filaments become laterally
752 connected and a study in *C. elegans* suggests that ZYP1 aggregation upon high
753 temperature primarily reflects SC assembly failure (Bilgir et al., 2013; Higgins et al.,
754 2012; Loidl, 1989). In addition, temporal dissection of heat stress on grasshopper
755 spermatocytes revealed that heat-induced chiasma frequency changes are most
756 likely the consequence of the completeness or efficiency of pairing (Henderson,
757 1988). Thus, we conclude that already very early recombination processes, such as
758 pairing of homologs, are affected by heat and that likely these aberrant processes
759 cause the formation of polycomplexes. However, it is still possible that aberrant SC
760 configuration ZYP1 can serve as a signal to cause a delay in NEB.

761 From our mutant analysis and chromosome spreads at elevated
762 temperatures, it is likely that recombination intermediates cause this delay. What the
763 structure of these intermediates is and how they cause a delay needs to be
764 investigated in the future. It is possible that the delay is triggered by non-homologous
765 recombination caused by mispairing and hence partially interconnected
766 chromosomes. A *zmm* mutant analysis in yeast revealed that a specific block in
767 progression of CO formation occurs at high temperatures, resulting in the formation
768 of intermediates and/or interactions with sister chromatids (Borner et al., 2004).
769 Further, it is well known from yeast that unresolved recombination intermediates can
770 cause nuclear division defects (Kaur et al., 2015; Kaur et al., 2019; Tang et al.,
771 2015).

772 At high temperature (reported up to 33°C), DSB formation remains unaffected
773 in yeast and Arabidopsis, whereas from *C. elegans* spermatocytes it is known that
774 high temperature (above or at the threshold of 34°C) induces SPO11-1-independent
775 DSBs, which are recognized by the CO repair machinery (Brown et al., 2020;
776 Kurhanewicz et al., 2020; Modliszewski et al., 2018). Whether Arabidopsis has a
777 different recombination effect/response below and above a temperature threshold
778 and if there is a different molecular mechanism remains to be investigated in the
779 future.

780

781 **A specialized pachytene checkpoint in Arabidopsis**

782 Aberrant recombination structures and the absence of recombination trigger meiotic
783 arrest in animals and yeast, this arrest is controlled by the so-called pachytene
784 checkpoint causing meiotic arrest in early pachytene (Barchi et al., 2005; Bishop et
785 al., 1992; Rockmill et al., 1995). Since in plants mutants in which recombination is
786 abolished, such as *dmc1*, are not arrested in meiosis, it was proposed that plants do
787 not have a pachytene checkpoint (Couteau et al., 1999; Grelon et al., 2001; Higgins
788 et al., 2004; Li et al., 2004).

789 A major regulator of the pachytene checkpoint in animals and yeast is the
790 checkpoint kinase ATM (Barchi et al., 2005; Lange et al., 2011; Pacheco et al., 2015;
791 Penedos et al., 2015; Roeder and Bailis, 2000). Removing ATM in mutants that
792 trigger the pachytene checkpoint, for instance in weak loss-of-function mutants for
793 Trip13/PCH2, promotes further progression through pachytene indicating that the
794 early arrest is under control of this checkpoint kinase (Pacheco et al., 2015).

795 In budding yeast, *atm* mutants undergo the first meiotic division before all
796 recombination events are complete (Lydall et al., 1996; Stuart and Wittenberg,
797 1998). Indeed, we found that the pachytene/diakinesis extension is lost in *atm*
798 mutants implicating ATM in this checkpoint and the execution of the observed
799 meiotic delay, e.g. by sensing of aberrant recombination structures. Taken together
800 with our finding that the prolongation of pachytene/diakinesis is recombination
801 dependent, we conclude that Arabidopsis and likely other plants do have a
802 pachytene checkpoint. However, this checkpoint is less stringent than in animals
803 since it does not respond to the absence of meiotic recombination. Moreover, the
804 extension is timely restricted and typically after 2.7 h meiosis continues. It needs to
805 be analyzed in the future, when the nature of the presumptive aberrant
806 recombination intermediates is understood, whether they are resolved during this
807 time or whether the checkpoint erodes, i.e. even though checkpoint conditions are
808 not fulfilled, meiosis progresses. An erosion has been found for another checkpoint
809 in plants, i.e. the spindle assembly checkpoint that assures that all chromosomes are
810 connected to microtubule fibers of the spindle. Triggering this checkpoint was only
811 able to delay anaphase onset by a maximum of less than 2 h (Komaki and
812 Schnittger, 2017).

813 It is an interesting discussion point whether less stringent cell division
814 checkpoints (pachytene and spindle assembly checkpoint (SAC)) represent an
815 evolutionary strategy in plants. Genome mutations, especially polyploidization events
816 are more prominent in plants than in animals and are suspected to be a major driving
817 force of evolution in plants (Brownfield and Kohler, 2011; De Storme and Geelen,
818 2013; Li et al., 2009b; Wijnker and Schnittger, 2013). Moreover, hybridization events
819 are very frequent in plants. An alien genome would likely affect recombination by
820 either reducing it or causing aberrant recombination structures. Less stringent
821 checkpoints would pave the road for hybridization events since by chance viable
822 combinations of chromosomes are generated. Especially an interplay between a
823 relaxed pachytene checkpoint and a relaxed SAC would promote rapid genome
824 evolution.

825

826 MATERIAL AND METHODS

827 **Plant material and growth conditions**

828 All *Arabidopsis thaliana* plants used in this study were derived from the Columbia
829 (Col-0) ecotype. The *CDKA;1:mVenus-TagRFP:TUA5* double reporter line,
830 *KINGBIRD reporter line 2* (*PRO_{REC8}:REC8:GFP/PRO_{RPS5A}:TagRFP:TUA5*) and the
831 *ASY1:RFP-ZYP1b:GFP* double reporter line have been previously described
832 (Prusicki et al., 2019; Sofroni et al., 2020; Yang et al., 2019; Yang et al., 2020). The
833 T-DNA insertion lines for *DMC1* (GABI_918E07), *SPO11-1* (SALK_146172), *MSH4*
834 (SALK_136296) and *ATM* (SALK_006953) were obtained from GABI-Kat T-DNA
835 mutation collection via NASC (<http://arabidopsis.info/>) and the collection of T-DNA
836 mutants at the Salk Institute Genomic Analysis Laboratory ([http://signal.salk.edu/cgi-](http://signal.salk.edu/cgi-bin/tdnaexpress)
837 [bin/tdnaexpress](http://signal.salk.edu/cgi-bin/tdnaexpress)).

838 Seeds were surface-sterilized with chlorine gas and germinated on 1% agar
839 containing half-strength Murashige and Skoog (MS) salts and 1% sucrose, pH 5.8.
840 When required, antibiotics were added for seed selection. All plants were grown
841 under long-day conditions (16 h light at 21°C (+/- 0.5 °C)/ 8 h dark at 18°C (+/-
842 0.5 °C), with 60% humidity). For short-term heat treatment, plants were first grown
843 under standard long-day conditions until sample preparations for live cell imaging or
844 transferred to a climate chamber at long-day photoperiod with continuous
845 temperature (30°C/34°C (+/- 0.5°C)) for 16/24 h prior to observation/fixation. For
846 long-term heat treatment, healthy plants at bolting stage were transferred to a
847 climate chamber at long-day photoperiod with a continuous temperature of 30°C (+/-
848 0.5°C) with 60% humidity for 7 days.

849

850 **Plasmids and plant transformation**

851 The reporter constructs *PRO_{RPS5A}:TagRFP:TUA5* and *KINGBIRD reporter line 2*,
852 previously described (Prusicki et al., 2019), were transformed into the T-DNA
853 insertion plants by floral dipping and T1 seeds were selected on half strength MS
854 supplemented with antibiotics hygromycin, from T2 generation onwards plants were
855 used for observation.

856

857 **Confocal microscopy and intensity plot**

858 For protein localization experiments, healthy flower buds were dissected exposing 2
859 anthers and carefully positioned in a petri dish, with 0.8% agar with half-strength MS
860 salts, pH 5.8, and meiocytes of different meiotic stages were imaged using a Zeiss
861 LSM880 confocal microscope.

862 For the pixel intensity plot, flower buds were dissected and the anthers in MT
863 array state 6 were imaged using a Zeiss LSM880 confocal microscope with the exact
864 same settings for the different heat conditions. The pixel brightness was measured
865 through a region of interest using ImageJ and plotted against the X dimension, which
866 is the distance of the region of interest.

867

868 **Live cell imaging and data processing**

869 Live cell imaging was performed as described previously (Prusicki et al, 2019). In
870 short, up to 6 flower buds of 0.2-0.6 mm were carefully positioned in a petri dish with
871 0.8% agar with half-strength MS salts, pH 5.8. Time lapse was performed using an
872 upright Zeiss LSM 880 confocal microscope with ZEN 2.3 SP1 software (Carl Zeiss
873 AG, Oberkochen, Germany) and a W-plan Apochromat 40X/ 1.0 DIC objective (Carl
874 Zeiss AG, Oberkochen, Germany). GFP and TagRFP were excited at $\lambda = 488$ nm
875 and 561 nm, respectively, and detected between 498-560 nm and 520-650 nm,
876 respectively. Auto-fluorescence was detected between 680-750 nm. With a time
877 interval of 10 min, a series of 6 Z-stacks with 50 μ m distance was acquired under a
878 thermally controlled environment (21°C/30°C/34°C (+/- 0.15%)) in an incubation
879 chamber. Due to sample movement, the Z-planes were manually selected using the
880 review multi-dimensional data function of the software Metamorph Version 7.8 and
881 the XY movement was corrected using the Stack Reg plugin of Fiji.

882

883 **Quantitative analysis of the meiotic phases**

884 The analysis of the duration is based on the *TagRFP:TUA5* reporter. Meioocytes were
885 manually assigned to defined MT states. The data were collected from a minimum of
886 3 independent set-ups, with a minimum of 8 anthers per genotype per heat
887 treatment. The durations of the meiotic phases were extracted from at least 65
888 meiocytes.

889

890 **Statistical methods**

891 The chosen distributions underlying our parametric model were log-normal for MT
892 array states 7-8-9, 10-11, 12-13 and 14, whereas for MT array states 2-3-4 and 5-6
893 and the model for the complete duration of MT array states 2-13 a Weibull
894 distribution was selected. Estimation results are presented as predicted marginal

895 median times, together with 95% confidence intervals. The statistical analysis was
896 performed with R version 3.5.1 and Stata SE version 16.1.

897

898 **Cytology**

899 The cytological analysis of the meiocytes under short and long heat treatment was
900 done by performing chromosome spreads, as previously described (Sofroni et al.,
901 2020). Briefly, healthy flower buds were fixated in 3:1 ethanol:acetic acid for a
902 minimum of 24 h at 4°C, following washing steps with 70% ethanol and stored at
903 4°C. Next, flower buds were washed in water and in 10 mM citrate buffer, pH 4.5 and
904 digested in an enzyme mix (10 mM citrate buffer containing 0.5% cellulase, 0.5%
905 pectolyase and 0.5% cytohelicase) for 2.5 h at 37°C. Digested flower buds were
906 squashed and spread onto a glass slide in 45% acetic acid on a 46°C hotplate.
907 Finally, the slides were washed in cold 3:1 ethanol:acetic acid and mounted in
908 Vectashield medium with DAPI (Vector Laboratories).

909

910 **Supplemental Data files**

911 Supplemental Table 1- Detailed overview of the sample size

912 Supplemental Figure 1- Microtubule array states upon heat stress

913 Supplemental Figure 2- Meiotic defects of wildtype upon heat stress

914 Supplemental Figure 3- Duration of meiotic phases in recombination mutants *spo11-1*,
915 *dmc1*, *msh4* and *atm* at 21°C and HS34°C

916 Supplemental Movie 1- The meiotic division of wild-type meiocytes at 21°C

917 Supplemental Movie 2- The meiotic division of wild-type meiocytes at HS30°C

918 Supplemental Movie 3- The meiotic division of wild-type meiocytes at HS34°C

919 Supplemental Movie 4- The meiotic division of wild-type meiocytes at LT30°C

920 Supplemental Movie 5- The meiotic division of *spo11-1* meiocytes at 21°C

921 Supplemental Movie 6- The meiotic division of *dmc1* meiocytes at 21°C

922 Supplemental Movie 7- The meiotic division of *msh4* meiocytes at 21°C

923 Supplemental Movie 8- The meiotic division of *spo11-1* meiocytes at HS34°C

924 Supplemental Movie 9- The meiotic division of *dmc1* meiocytes at HS34°C

925 Supplemental Movie 10- The meiotic division of *msh4* meiocytes at HS34°C

926 Supplemental Movie 11- The meiotic division of *atm* meiocytes at 21°C

927 Supplemental Movie 12- The meiotic division of *atm* meiocytes at HS34°C

928

929

930 AUTHOR CONTRIBUTIONS

931 J.D.J.-B. and A.S. designed the research; J.D.J.-B. performed the experiments; L.K.
932 and A.B. performed the statistical analysis; J.D.J.-B., L.K., A.B. and A.S. analyzed
933 and discussed the data; J.D.J.-B. and A.S. wrote the article; J.D.J.-B., L.K., A.B. and
934 A.S. revised and approved the article.

935

936 ACKNOWLEDGEMENTS

937 This research was funded by the University of Hamburg. We thank Lucas Lang
938 (University of Hamburg) and Konstantinos Lampou (University of Hamburg) for
939 critical reading and helpful comments on the manuscript. We are grateful to Chao
940 Yang, Shinichiro Komaki and Konstantinos Lampou for providing the reporter lines in
941 the mutant background.

942

943

944

MT array state	2-3-4 late leptotene- early pachytene			5-6 pachytene- diakinesis			7-8-9 metaphase I- anaphase I			10-11 telophase I- interkinesis			12-13 metaphase II- anaphase II			14 telophase II		
Meiotic stage																		
Predicted time (in min)	Median	95% Conf. Interval		Median	95% Conf. Interval		Median	95% Conf. Interval		Median	95% Conf. Interval		Median	95% Conf. Interval		Median	95% Conf. Interval	
Treatment (n)																		
21°C (206)	845	746	944	360	309	412	47	44	49	52	47	57	46	44	49	219	205	234
HS30°C (133)	556	485	628	365	319	411	32	28	36	47	41	53	29	27	31	209	185	233
HS34°C (188)	428	403	453	522	498	546	34	32	36	59	55	63	24	22	25	NA	NA	NA
LT30°C (211)	609	550	667	378	340	416	39	35	44	45	38	51	37	32	43	256	230	282
<i>spo11</i> 21°C (224)	1119	1031	1206	374	349	399	72	67	76	63	58	67	48	45	52	356	326	385
<i>dmc1</i> 21°C (157)	1056	929	1184	343	331	355	67	63	71	63	59	67	47	45	49	281	262	301
<i>msh4</i> 21°C (193)	951	861	1040	314	299	329	67	63	71	59	56	63	49	46	52	274	253	294
<i>spo11</i> HS34°C (198)	626	572	681	412	393	431	35	32	38	54	49	58	23	21	26	NA	NA	NA
<i>dmc1</i> HS34°C (160)	565	526	605	383	362	403	30	28	32	57	54	60	22	21	23	NA	NA	NA
<i>msh4</i> HS34°C (116)	571	536	606	398	346	450	32	30	34	49	43	55	24	22	26	NA	NA	NA
<i>atm</i> 21°C (228)	834	761	908	295	270	321	45	42	49	60	55	66	43	40	46	245	230	260
<i>atm</i> HS34°C (172)	702	640	764	350	330	370	31	29	33	55	50	60	26	23	28	NA	NA	NA

Table 1- Overview of the duration of the meiotic phases based on the MT array states. Predicted median times and 95% confidence intervals (in min) of MT array state 2-3-4 (late leptotene-early pachytene), MT array state 5-6 (pachytene- diakinesis), MT array state 7-8-9 (metaphase I- anaphase I), MT array state 10-11 (telophase I- interkinesis), MT array state 12-13 (metaphase II- anaphase II) and MT array state 14 (telophase II) of wildtype at 21°C, HS30°C, HS34°C, LT30°C; recombination mutants *spo11*, *dmc1* and *msh4* at 21°C and HS34°C and *atm* mutant at 21°C and HS34°C. (n= number of cells observed). NA: not analysed.

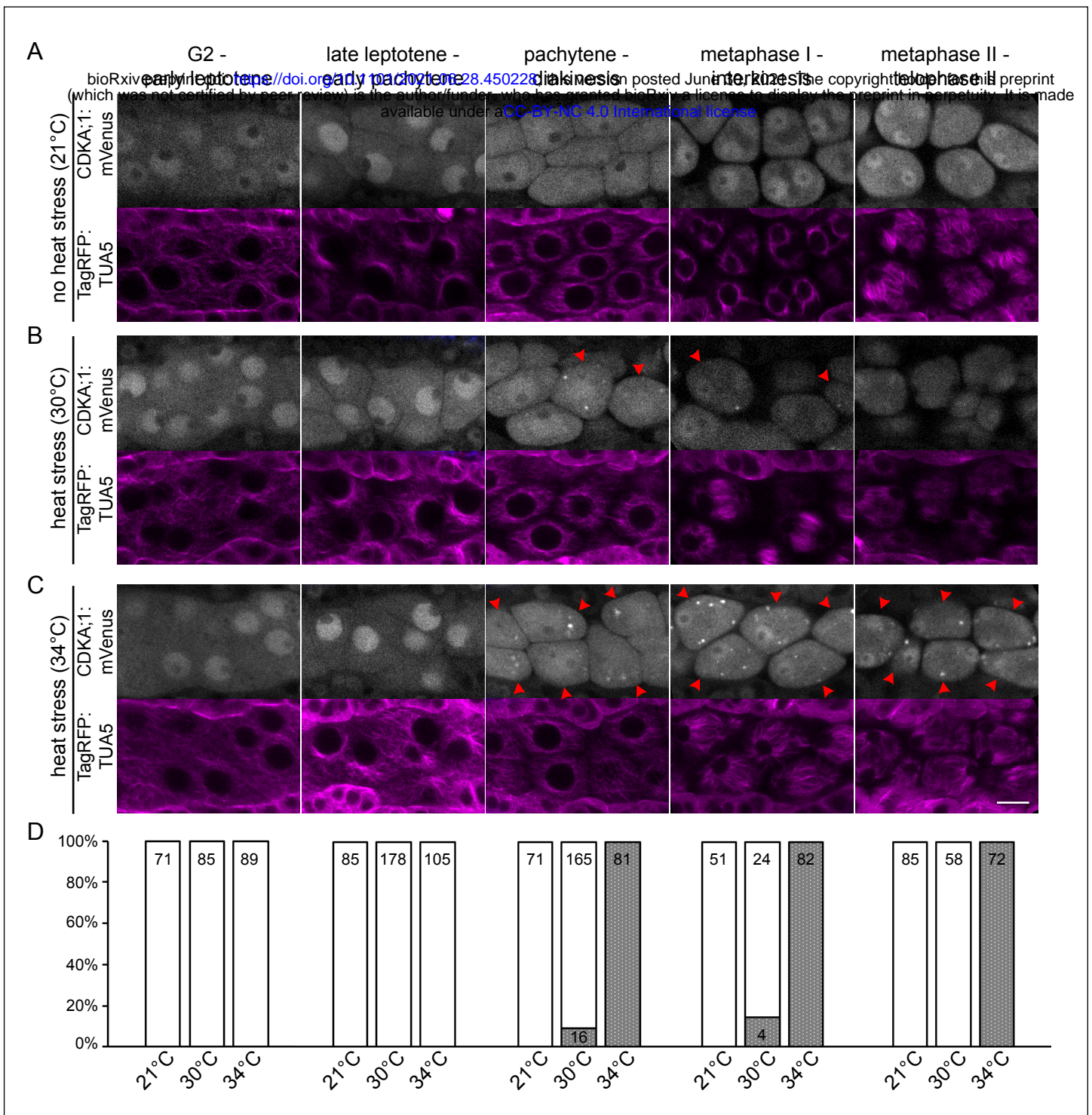


Figure 1- Localization of CDKA;1 in male meiocytes in control and stress conditions

CDKA;1:mVenus (first row; white) and TagRFP:TUA5 (second row; magenta) localization at control conditions of 21°C (A), heat stress of 30°C (B) and of 34°C (C) at different meiotic stages: G2-early leptotene (column 1), late leptotene-early pachytene (column 2), pachytene-diakinesis (column 3), metaphase I-interkinesis (column 4) and metaphase II-telophase II (column 5). Red arrowheads highlight cells with CDKA;1 localization at SGs. Scale bar: 10 μ m. Quantification of CDKA;1 SG formation on cellular level (D) per stage in percent; white bar: cells without SGs; grey bar: cells having at least one SG; the absolute sample size is given in the corresponding bar.

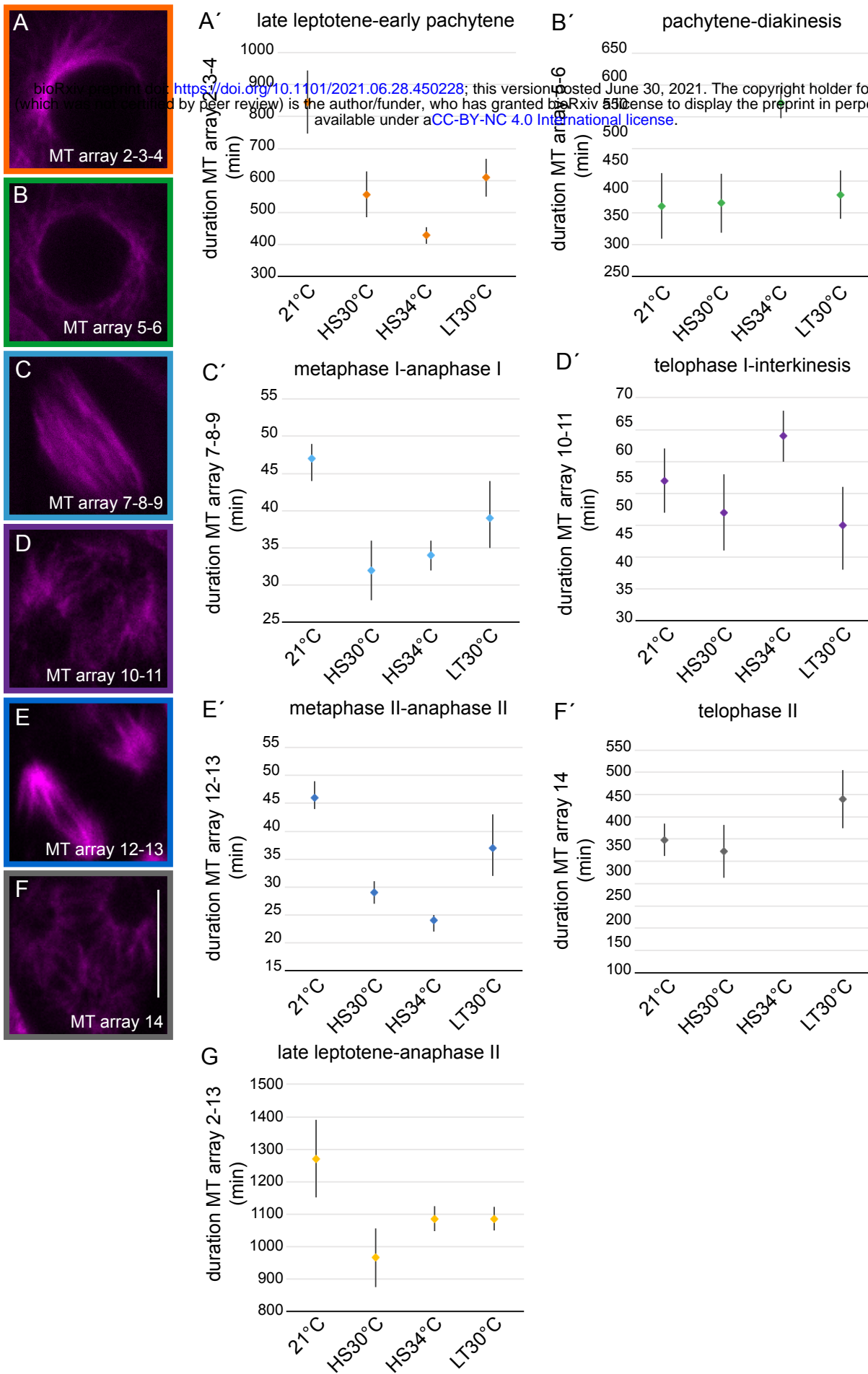


Figure 2- Duration of meiotic phases based on MT array states

Confocal images of the MT array states (A-F) and the corresponding predicted median times (in min) with 95% confidence intervals in control (21°C) and heat conditions (HS30°C, HS34°C and LT30°C) (A'-F'); (A,A'; orange) MT array state 2-3-4, late leptotene-early pachytene; (B,B'; green) MT array state 5-6, pachytene-diakinesis; (C,C'; light blue) MT array state 7-8-9, metaphase I-anaphase I; (D,D'; purple) MT array state 10-11, telophase I-interkinesis; (E,E'; dark blue) MT array state 12-13, metaphase II-anaphase II; (F,F'; grey) MT array state 14, telophase II. Scale bar: 10 μ m. Predicted median time (in min) of MT array states 2-13 with the 95% confidence interval in control (21°C) and heat conditions (HS30°C, HS34°C and LT30°C) (G; yellow).

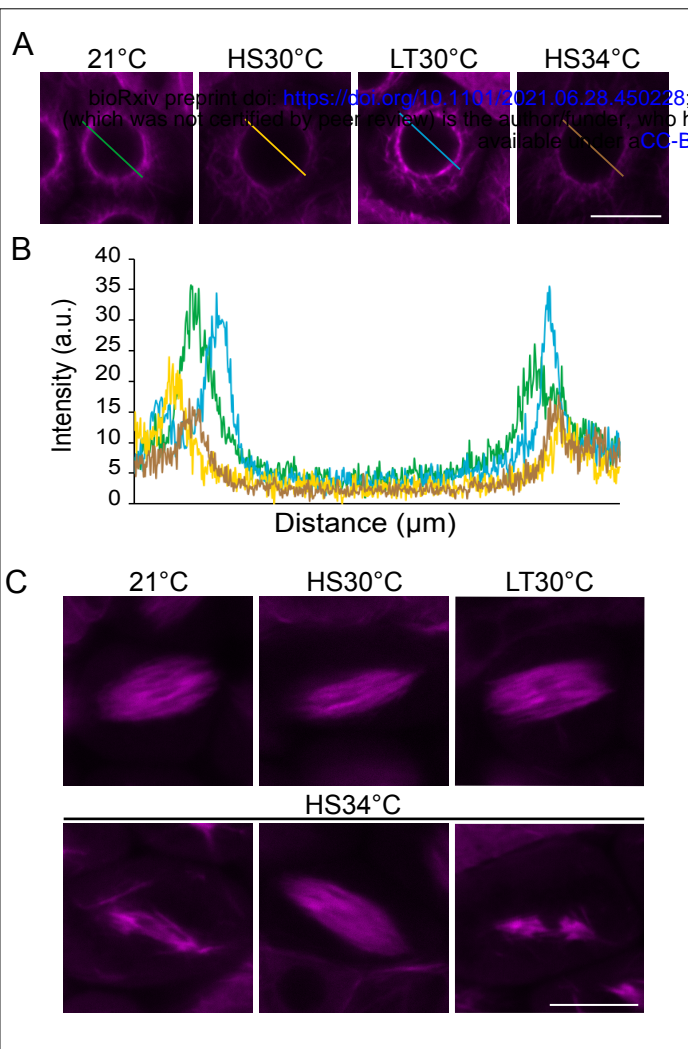


Figure 3- Microtubule array in wildtype in control and heat stress conditions

Confocal images of meicytes expressing TagRFP:TUA5 (magenta) at MT array state 6 in control conditions (21°C), heat shock conditions (HS30°C, LT30°C and HS34°C) (A). Pixel intensity plot of a section crossing through the middle of the cell (distance in μm) in MT array state 6 in 21°C (green), HS30 °C (yellow), LT30°C (blue) and HS34°C (brown) (B), section lines also highlighted in (A). Confocal images of meicytes expressing TagRFP:TUA5 (magenta) at MT state 8-9 in 21°C, HS30°C, LT30°C and HS34°C (C). Scale bar: 10 μm .

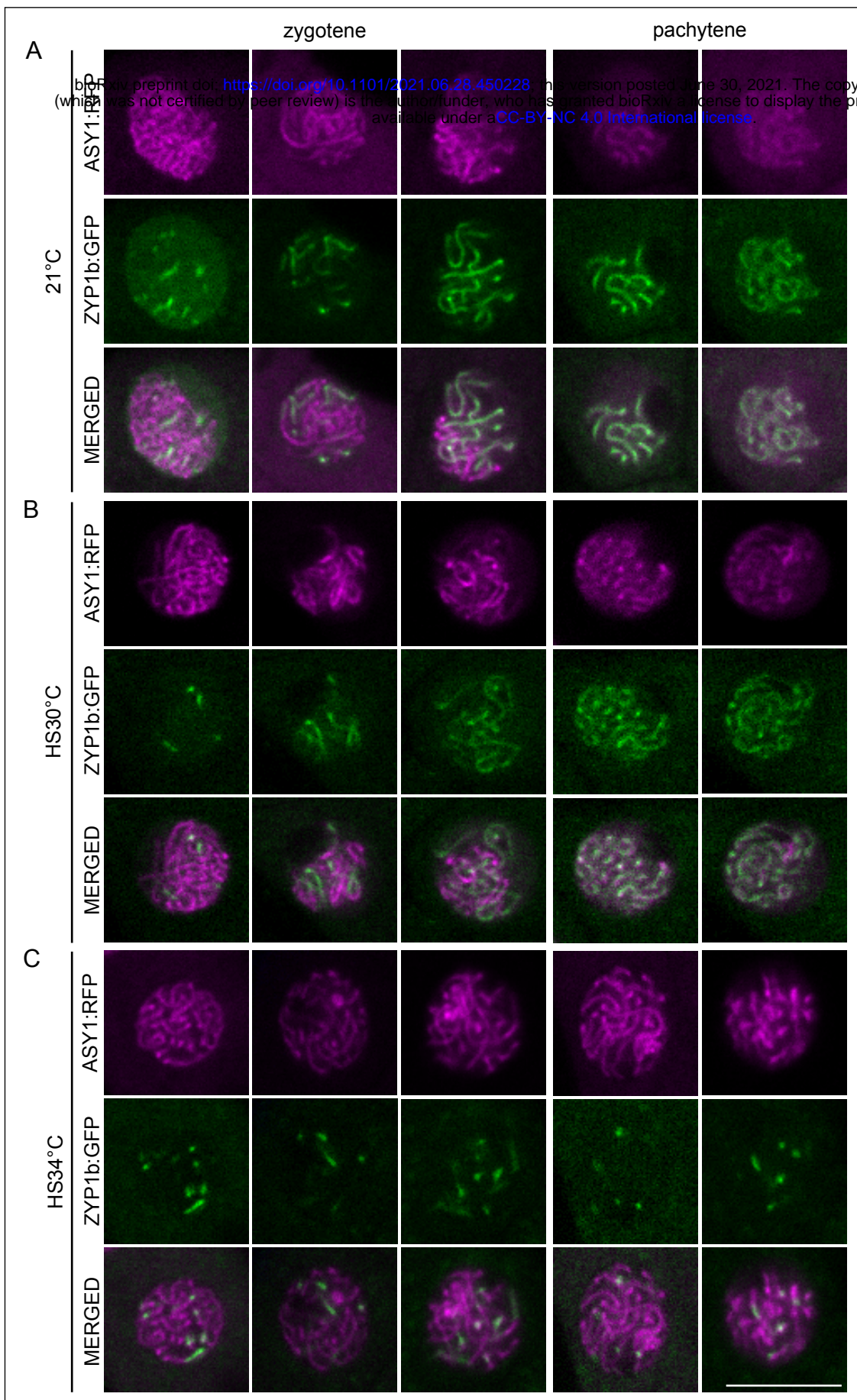


Figure 4- Synaptonemal complex elements ASY1 and ZYP1b localization upon heat stress
Confocal images of the nucleus of meiocytes at 21°C (A), HS30°C (B) and HS34°C (C) of SC elements ASY1:RFP (magenta, first row) and ZYP1b:GFP (green, second row) separately and merged (third row) at zygotene (columns 1-3) and pachytene (columns 4-5). Scale bar: 10 μ m.

pachytene-diakinesis

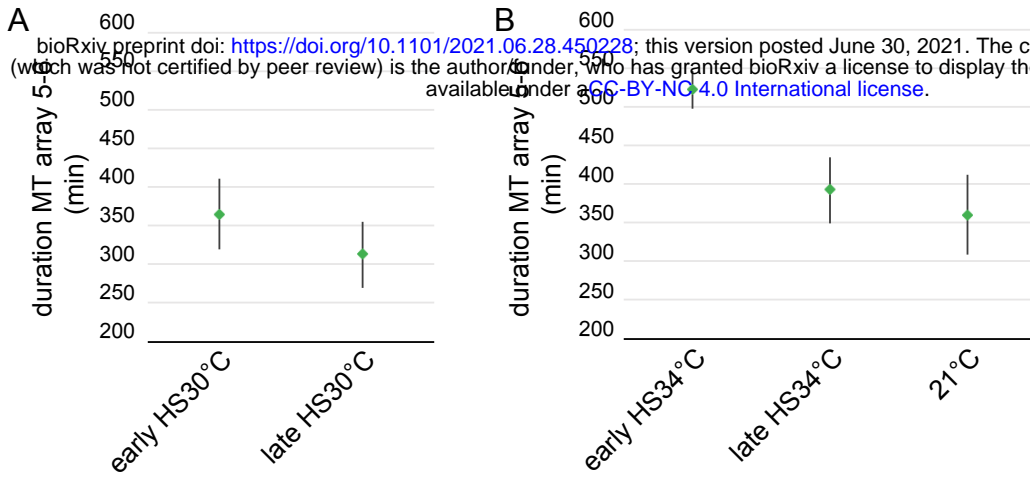
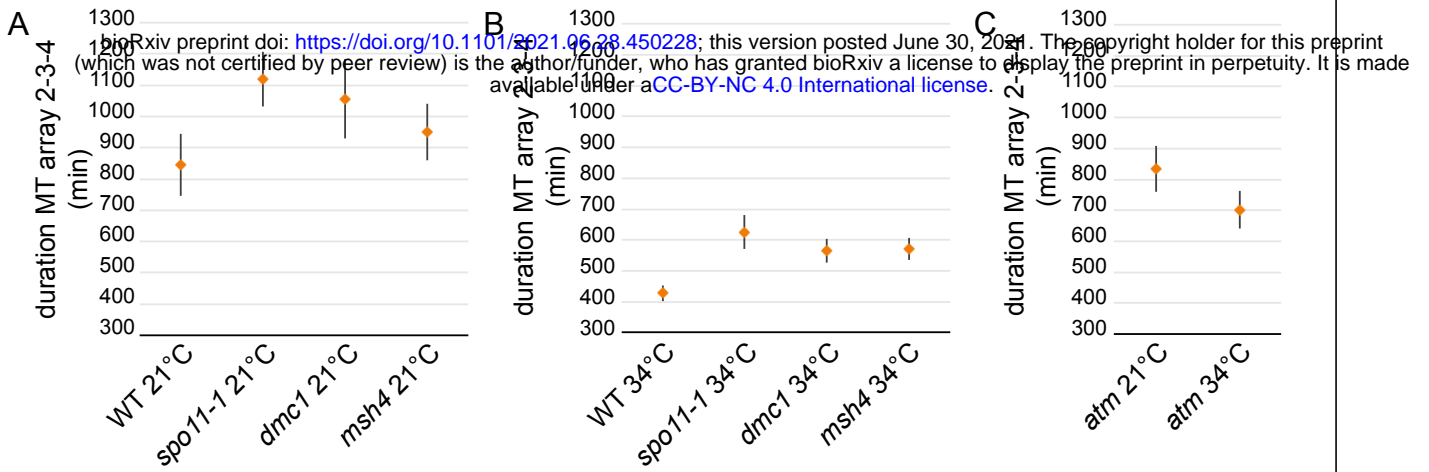


Figure 5- Effect of early and late heat shock on the duration of MT array state 5-6

The predicted median time (in min) with 95% confidence intervals of pachytene-diakinesis (MT array state 5-6; green) of early HS30°C versus late HS30°C (A) and early HS34°C versus late HS34°C and 21°C (B). Early HS30°C, early HS34°C and 21°C as shown in Figure 2B'.

late leptotene-early pachytene



pachytene-diakinesis

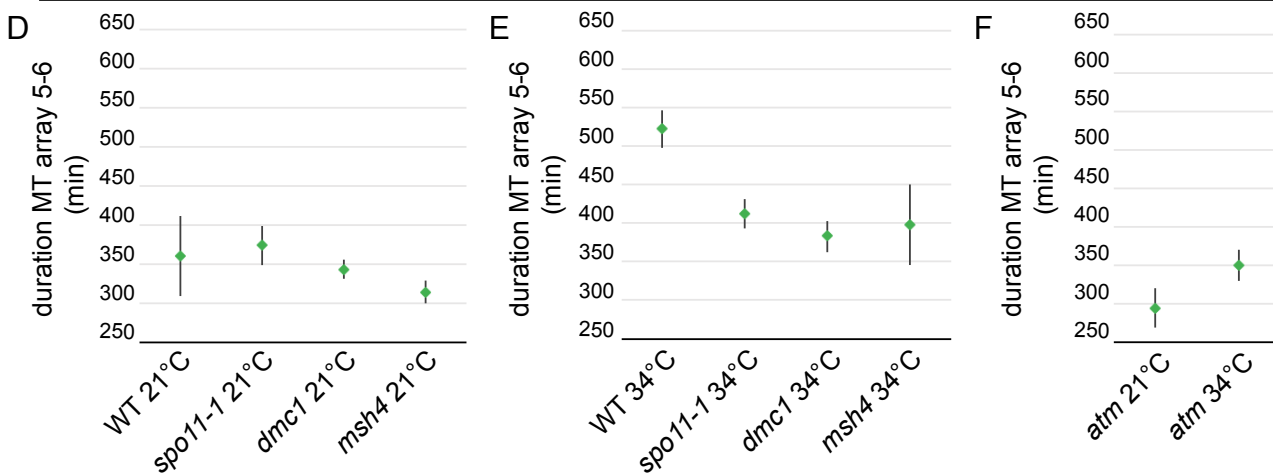


Figure 6- Duration of prophase in recombination mutants *spo11-1*, *dmc1*, *msh4* and *atm* at 21°C and HS34°C
 The predicted median times (in min) with 95% confidence intervals of late leptotene-early pachytene (MT array state 2-3-4; orange; A-C) and pachytene-diakinesis (MT array state 5-6; green; D-F) in wildtype (as shown in Figure 2A',B') and recombination mutants *spo11-1*, *dmc1*, *msh4* at 21°C (A, D) and HS34°C (B, E) and the *atm* mutant at 21°C and HS34°C (C, F).

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