Physiological and anatomical differentiation of two sympatric weed populations

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In the vineyards of Rhineland-Palatinate (Germany), two different types of Shepherd's Purse coexist: (1) the common type called 'wild type', and (2) the decandric type called *Capsella apetala* or '*Spe*' with four stamens in place of petals. In this study, we compare the anatomical and physiological characters of rosette leaves of the respectice types. Progeny of individual plants was cultivated in growth chambers under low- and high-light conditions. Under low-light conditions, the stomata densities of the adaxial and abaxial epidermis did not differ between the two types. When grown under high-light conditions, wild type and *Spe*, both exhibited increased stomata densities compared to low-light conditions, but *Spe* to a lesser extent than the wild type. The maximal photosynthetic capacity of *Spe* was lower in both, low-light and high-light conditions compared to wild-type plants. Even under ambient and sub-ambient CO_2 concentrations, *Spe* seemed to be less productive. The less effective CO_2 assimilation of the *Spe* mutant *C. apetala* was accompanied by later flowering. This fact prolonged the vegetative phase of *Spe* by about two weeks and was sufficient for the maintenance of both populations stably over years.

1 **Research article**

3 Physiological and anatomical differentiation of two sympatric weed

4 populations

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23 This publication is dedicated to Prof. Herbert Hurka's 80th birthday

24

25

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Abstract

In the vineyards of Rhineland-Palatinate (Germany), two different types of Shepherd's Purse coexist: (1) the common type called 'wild type', and (2) the decandric type called *Capsella apetala* or '*Spe'* with four stamens in place of petals. In this study, we compare the anatomical and physiological characters of rosette leaves of the respectice types. Progeny of individual plants was cultivated in growth chambers under low- and high-light conditions. Under low-light conditions, the stomata densities of the adaxial and abaxial epidermis did not differ between the two types. When grown under high-light conditions, wild type and *Spe*, both exhibited increased stomata densities compared to low-light conditions, but *Spe* to a lesser extent than the wild type. The maximal photosynthetic capacity of *Spe* was lower in both, low-light and high-light conditions compared to wild-type plants. Even under ambient and sub-ambient CO_2 concentrations, *Spe* seemed to be less productive. The less effective CO_2 assimilation of the *Spe* mutant *C. apetala* was accompanied by later flowering. This fact prolonged the vegetative phase of *Spe* by about two weeks and was sufficient for the maintenance of both populations stably over years.

43

Introduction

In the vineyards of southwestern Germany (Rhineland-Palatinate) two morphologically distinct types of the common annual weed Shepherd's Purse (*Capsella bursa-pastoris* (L.) Medik., Brassicaceae) occur side by side (Reichert 1998, **Fig. 1**).

48   The two populations can readily be differentiated by their characteristic flower structure.

49   Wild type (wt) *C. bursa-pastoris* flowers exhibit the conserved body plan of

50   Brassicaceae: it comprises four sepals in the first, four petals in the second, six stamens

51   in the third, and two fused carpels in the fourth, the innermost whorl (**Fig. 1**).

52   In contrast, the flowers of the floral variant, also referred to as *Spe* (Hameister

53   and Neuffer 2017, Nutt et al. 2006), can easily be identified by four additional stamens

54   replacing the wild-type petals thus giving it its unique decandric phenotype (**Fig. 1**). This

55   floral variant of *C. bursa-pastoris* has initially been described as *C. apetala* Opiz (Opiz

56   1821), a terminology which we follow here to differentiate between both types. Notably,

57   the decandric flower shape does not affect floral symmetry, but the lack of petals might

58   have an impact on pollination success (Hameister and Neuffer 2017). Recent field

59   surveys in vineyards revealed that the wild type is the predominant taxon with tens of

60   thousands of individuals, and the *Spe* occurs with a stable frequency of about 10%

61   (Hameister et al. 2009). Intriguingly, potential pollinators, especially wild bees, and

62   hoverflies, tend to prefer one over the other type, probably due to differing volatile

63   emissions of the two flower types (Ziermann et al. 2009, Hameister and Neuffer 2017,

64   **Fig. 1**). Recent investigations by Hameister and Neuffer (2017) demonstrated that

65   flower induction is delayed in *Spe* when compared with wild type in both, field and

66   controlled greenhouse conditions. Notably, the authors could show that in an

67   overlapping period both types are fertile. However, both taxa exhibit a high "selfing" rate

68   and a low crossing rate between the two flower types has been found (Hameister and

69   Neuffer 2017, **Fig. 1**). Furthermore, pollen tubes of *C. apetala* self-pollen grew faster

70   compared with *C. apetala* crossed with wild-type pollen (*spe* x *wt*, Neuffer and Paetsch

71 2013). Field experiments in the Botanical Garden of the University of Osnabrueck

72 revealed that the two types differ in the physiological investments into their respective

73 offspring. Wild-type individuals produced more siliques per plant, whereas the *Spe*

74 generated a higher number of seeds in the siliques (Hameister and Neuffer 2017). The

75 total seed output per plant was balanced among the two flower types (Hameister and

76 Neuffer 2017) indicating that the fitness-related factor seed production remains

77 unaffected.

78   Taken together, the previous investigations lead to the assumption that *C. bursa-*

79 *pastoris* and *C. apetala* are separated by several smooth to strong isolation barriers.

80 Thus, they form two morphologically distinct types that grow sympatrically in the

81 vineyards of southwestern Germany (Reichert 1998). Further support for this hypothesis

82 can be extracted from genetic work by Hameister et al. (2009). Here, the authors

83 showed that wild type and *Spe* differed in their isozyme genotype. Furthermore, the

84 genetic difference was confirmed by creating a mapping population between a wild-type

85 individual and a *Spe* individual followed by comparative linkage analysis (Hameister et

86 al. 2013).

87   In this study, we focus on the properties of the leaves of the two types in the

88 vegetative stage when the plant accumulates assimilates which then are used for

89 building up the inflorescence and production of seeds. Rosette leaves provide the basis

90 for an individual to withstand harsh environmental conditions, e.g., cold temperatures,

91 high irradiation, and drought stress. When the apical meristem switches from the

92 vegetative to the reproductive state, no further rosette leaves are generated. The

93 question was: what is the contribution of leaf differentiation to the ecotypic variation

94   between sympatrically growing individuals with normal (wild-type) flowers in contrast to

95   individuals with decandric (*Spe*) flowers? Here, we studied the ecotypic differentiation of

96   the rosette leaf after growth under stress (high-light) and no stress (low-light) conditions

97   using anatomical and physiological methods.

98

99   **Material and Methods**

100   *Plant material and cultivation of plants*

101   Seeds of individual plants of *Capsella bursa-pastoris* (wild type) and *Capsella*

102   *apetala* Opiz (*Spe*) have been collected in the vineyards located near to Gau-

103   Odernheim (Rhineland-Palatinate, 25 km southwest of Mainz, 49.7847 N, 8.1942 E,

104   elevation 150 m, Germany) and subsequently stored at minus 20°C in the seed gene

105   bank of the Botanical Garden of Osnabrueck. The material refers to the gene bank

106   numbers 1951/19 and 1960/25 for the wild type and 1956/5 and 1961/4 for the

107   decandric type. Note that plant material subjected to photophysiological and anatomic

108   characterization in this publication is derived material from seeds of two independent

109   population subgroups of each flower morphological type. Plants were initially cultivated

110   under controlled conditions programmed with a 12-h photoperiod per day, resulting in

111   four subgroups with six individuals each. The temperature was adjusted to 15°C during

112   day time and 5°C during the night phase (initial growth conditions for seedlings). After

113   six weeks, the plants were divided into two experimental groups. Three individuals of

114   each subgroup were transferred into two different light conditions in an 8-h light period

115   at 20°C: (i) high-light setting (600 µmol quanta m^{-2}s^{-1}); (ii) low-light setting

116 (100 µmol quanta m^{-2}s^{-1}) (**Fig. 2**). Later on sister individuals have been planted in the

117 experimental field of the Botanical garden.

118

119 *Morphology and anatomy*

120 Leaf characterization was performed on three-month-old leaves. Epidermal leaf

121 tissue was prepared for microscopic examination (**Fig. 3**) by a customized technique

122 applying nail polish. After solidification of the nail polish, the epidermal cell layer was

123 removed by transparent adhesive tape (Tesafilm). The nail polish imprint, including

124 adhesive tape, was observed with a microscope. Subsequently, stomata were

125 quantified in a representative area of 400 x 400 µm^2 on one rosette leaf of every

126 individual plant (**Fig. 4**).

127

128 *Photosynthetic characterization*

129 All measurements were performed using one fully expanded rosette leaf from

130 plants two months after sowing when they did not yet flower. The CO_2 gas exchange

131 was measured using the LI-6400/XT Portable Photosynthesis System (LI-COR

132 Environmental, Lincoln, NE, U.S.A.). All measurements were conducted under

133 saturating light intensities of 800 µmol quanta m^{-2} s^{-1} at 20°C. The relative humidity was

134 adjusted to approximately 50%. The rate of CO_2 assimilation (A) was measured as a

135 function of sub-stomatal CO_2 concentration (C_i). C_i values were applied in the following

136 order: 400 ppm; 200 ppm; 100 ppm; 50 ppm; 0 ppm; 400 ppm; 600 ppm; 1000 ppm;

137 1750 ppm. Here, the initial decrease in C_i concentration was chosen to ensure a

138 sufficient leaf conductance. The leaf conductance is widely considered as an indicator

139 of effective gas exchange of the leaf with the surrounding environment and is

140 predominantly determined by the extent to which the stomata are opened. By applying

141 decreasing CO_2 concentrations in the first half of a photosynthesis measurement, a

142 conductance of at least 0.2 was maintained throughout the entire measurement

143 (**Fig. S1**). In this way, a possible limitation of CO_2 assimilation due to inefficient gas

144 exchange was avoided. Data points were logged within 2 min after the start of each C_i

145 concentration when infrared gas analyzer (IRGA) parameters reached a steady-state

146 value (raw data for 22 individuals **Suppl. Tab. S2**).

147

148 *Determination of flowering time under field conditions*

149 Seventy days after sowing sister plants were planted into the experimental field of the

150 Botanical Garden Osnabrueck and flowering time was determined by observation of the

151 emerging inflorescence bud.

152

153 **Results**

154 *Stomata density on both sides of rosette leaves*

155 After growth for three months in the climate chambers, epidermal peel from upper

156 and lower leaf surfaces was prepared and inspected by light microscopy (**Fig. 3**). The

157 number of stomata was determined for wild type and *Spe* after growth in low light and

158 high light. As expected, the stomata density of the lower epidermis was found to be

159 higher compared to the upper surface for both types in all light conditions (**Fig. 4**). When

160 grown under low-light conditions, no obvious differences could be detected between the

161 two flower types. In contrast, both types after growth under high-light conditions

162   exhibited a significantly increased stomata density on both leaf surfaces (**Fig. 3 and 4,**

163   **Suppl. Table S1**). Interestingly, the number of stomata on the adaxial (upper) surface of

164   the leaves of *Spe* was significantly less increased when compared with wild-type

165   individuals after growth in high-light conditions (**Fig. 4**). Notably, this trend could not be

166   observed for the abaxial (lower) epidermis under the same conditions.

167

168   *Photosynthetic characteristics of rosette leaves*

169   To further elucidate the impact of the differentially increased stomata density observed

170   in plants grown under high-light conditions, the photosynthetic efficiency was

171   determined under saturating light using gas-exchange measurements. The rate of

172   carbon assimilation (A) in response to various externally applied CO_2 concentrations

173   given as internal CO_2 (C_i) was monitored for wild type and *Spe* plants. The resulting

174   A/C_i plots show the typical saturation behavior for all plants grown under both light

175   conditions (**Fig. 5**).

176   Photosynthesis was limited by the availability of CO_2 until a calculated internal CO_2

177   concentration (C_i) of approximately 350 ppm was reached. The initial linear increase of

178   CO_2 assimilation reflects the maximal carboxylation rate of RubisCO (**Fig. 5**, **C** and **D**).

179   Above 350 ppm, the curves enter saturation as net photosynthesis becomes limited by

180   other factors such as the electron transport and, at higher CO_2 concentrations, the

181   utilization of triose phosphate. At saturating CO_2 concentrations, maximum net-

182   photosynthesis rates of *Spe* were always lower than of the wild type when cultivated

183   under both, low- and high-light conditions (**Fig. 5**, **A** and **B**). However, the initial

184   carboxylation rate was rather similar for the two types. At ambient CO_2 concentrations of

185 approximately 400 ppm which more accurately reflect the photosynthetic efficiency of

186 the plants the rates of CO_2 assimilation are higher when the plants have been grown

187 under high light, and significantly lower when grown under low-light conditions (**Fig. 5**, **C**

188 and **D**). No premature stomatal closure had limited gas exchange as shown by plotting

189 the stomatal conductance (gs) against the C_i values for each measurement (**Suppl. Fig.**

190 **S1**).

191

192 *Flowering time*

193 In order to identify traits of *Spe*, which explain the observed stable co-existence in the

194 field even with a lower photosynthetic performance, the flowering time was analysed.

195 After 70 days sister plants (wt 72 individuals, *Spe* 63 individuals) were transferred from

196 the initial growth conditions for seedlings to the experimental field in the Botanical

197 Garden of Osnabrueck. Under these field conditions, wild-type individuals in average

198 flowered about two weeks earlier (x = 77.64 +/- 3.562 days after sowing) than *Spe*

199 individuals (x = 91.75 +/-12.618 days after sowing (**Fig. 6)**.

200

201 **Discussion**

202 Finely scaled ecotypic differentiation for Shepherd's Purse is well known and

203 quite substantial regarding germination, the onset of flowering, and growth forms (Linde

204 *et al*. 2001, Neuffer and Linde 1999, reviewed in Neuffer *et al.* 2011). Already at the

205 beginning of the 20th century, Almquist (1907) determined several elementary species

206 based on fruit and rosette-leaf morphology. This variable leaf morphology attracted the

207 interest of the geneticist Shull (1909, 1911). Aksoy *et al*. (1999) included the system of

208   Shull in their attempt towards a simplified taxonomy of *C. bursa-pastoris* and Ianetta *et*

209   *al.* (2007) widened the system of Shull.

210   Combining anatomical and physiological traits provides the first insight into the

211   value of leaf differentiation other than the morphology of leaf types after Shull (Neuffer

212   *et al.* 2018). The ecotypic differentiation of the leaf in various habitats and conditions at

213   geographically and climatically different places of origin of many populations is an

214   obvious character that somehow serves adaptation and persistence in new growth

215   conditions. The occurrence of a specific flower morphology of *C. bursa-pastoris*

216   individuals in the vineyards south of Mainz in southwestern Germany is known for quite

217   some time (Reichert 1998). At first sight, this natural mutant is predominantly important

218   for studies of plant geneticists (Nutt *et al.* 2006, Ziermann *et al.* 2009, Hintz *et al.* 2006,

219   Hameister *et al.* 2013). With a more detailed analysis, several traits became apparent,

220   and population studies led to the insight that the wild type of *C. bursa-pastoris* and its

221   variant *C. apetala* even form two different ecotypes, sympatrically occurring at one

222   place over many years (Hameister and Neuffer 2017, Hameister et al. 2009). This

223   differentiation is also evident from genetic differences (Isozyme Genotypes, AFLPs, see

224   Hameister *et al.* 2009).

225   As a typical leaf characteristic, stomata density was determined in the

226   experimental populations (**Fig. 4**). In general, all leaves had developed more stomata

227   under high-light growth conditions than in low-light as was already observed for

228   *C. bursa-pastoris* provenances from Morocco and Norway, but not from Russia (Neuffer

229   et al. 2018). Furthermore, stomatal density on the abaxial epidermis was about twice as

230   high as in the adaxial leaf surface both in wild type and *Spe* after growth under high

231   light. However, as an exception, *Spe* had not increased its stomata density on the upper

232   leaf surface to the same extent as did the wild type. It had developed about 35% fewer

233   stomata per area unit on the upper surface of its rosette leaves as compared to the wild

234   type (**Fig. 4**). A possible explanation for this phenomenon might lie in the fact that the

235   incident light primarily impacts the adaxial surface of the rosette leaves and is exposed

236   to adverse conditions more than the abaxial surface.

237   In our study, wild type individuals compared to *Spe* exhibited a higher maximal

238   photosynthetic capacity both when grown under low light as well as high light. At

239   saturating CO_2 and light, the rates of CO_2 assimilation were always higher for the wild

240   type compared to *C. apetala* (**Fig. 5**, **A** and **B**). This points to a limitation at the level of

241   electron transport and would cause a disadvantage for *Spe*. However, as can be taken

242   from the initial slopes of the A/C_i curves, under ambient conditions with limiting CO_2

243   concentrations up to 400 ppm, only slight differences were apparent when both types

244   were compared (**Fig. 5**, **C** and **D**).

245   One way to explain the coexistence of wild type and *Spe* in the same habitat is

246   based on the fact that the flowering phenology of both types differs. Wild-type plants

247   flower earlier by several days after sowing under greenhouse conditions (Hameister *et*

248   *al.* 2009, 2013) as well as under field conditions at the Botanical Garden of Osnabrueck

249   (Hameister and Neuffer 2017). The appearance of the inflorescences was monitored

250   also for sister plants after transfer to field conditions in the Botanical Garden in

251   Osnabrück (**Fig. 6**). On average flowering started two weeks later in *Spe* individuals as

252   compared to wild type. This might be explained by the disadvantage of *Spe* over the

253   total growth period due to somewhat lower photosynthetic capacity and consequently a

254 lower growth rate resulting in a delay of biomass production in the vegetative stage.

255 Either due to the difference in the nutritional status or due to environmental signals,

256 flowering induction occurs significantly later in *Spe* thus leading to a temporal niche for

257 successful pollination and seed set. A small but stable population of *C. apetala* can

258 apparently coexist in the presence of the large wild-type population. Both types might be

259 adapted to slight differences in their growth properties and the environment by growing

260 in the rosette stage for a longer or shorter time resulting in a difference in flowering time

261 of about two weeks. Taken together, the selection pressure for both types is apparently

262 similar leading to stable populations growing side by side.

263

264 **Conclusions**

265 In this study, we were able to analyse two independent sympatrically occurring

266 populations of Shepherd's Purse. These two populations (wild type and Spe) occur

267 intermingled with each other in stable frequencies of 9:1 over the years. This situation is

268 rather specific and the question arose: What are the characters enabling these

269 populations to coexist as they actually do? Both populations are isolated by strong

270 (selfing) and smooth (flowering, reduced pollen growth activity between taxa) barriers.

271 We have been able here to quantify anatomical and physiological traits which, with all

272 probability, stay under selection pressure. Of course, many more characters are

273 included in the ecotypic differentiation and already have been studied elsewhere (e.g.

274 seed production). Here, in Spe, photosynthetic capacity appears to be lower, while

275 growing for a longer time in the vegetative stage and later flowering induction. As both

276 populations occur in stable frequencies, the selection pressure on single traits might be

277   different, however, when combined, they seem to be neutralized. The specific

278   combination of characters in each taxon is linked due to predominantly selfing.

279

280   **Acknowledgements**

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283

284   **References**

285   **Aksoy A, Hale WHG, Dixon JM. 1999.** Towards a simplified taxonomy of

286   *Capsella bursa-pastoris* (L.) Medik. (Brassicaceae). *Watsonia* **22**: 243-250.

287   **Almquist E. 1907.** Studien über die *Capsella bursa pastoris* (L.). *Acta Horti*

288   *Bergiani* **4** No. 6: 1-92.

289   **Hameister S, Neuffer B, Bleeker W. 2009.** Genetic differentiation and

290   reproductive isolation of a naturally occurring floral homeotic mutant within a wild-type

291   population of *Capsella bursa-pastoris* (Brassicaceae). *Molecular Ecology* **18**: 2659–

292   2667.

293   **Hameister S, Neuffer B. 2017.** Establishment of a natural floral variant of

294   Shepherd's Purse in the wild: analysis of life-history traits in '*Capsella apetala*'

295   (Brassicaceae). *Plant Ecology and Evolution* **150**: 67-75.

296   **Hameister S, Nutt P, Theißen G, Neuffer B. 2013.** Mapping a floral trait in

297   Shepherd's Purse – ‚Stamenoid petals' in natural populations of *Capsella bursa-pastoris*

298   (L.) Medik. *FLORA* **208**: 641-647.

299   **Hintz M, Bartholmes C, Nutt P, Ziermann J, Hameister S, Neuffer B,**

300   **Theissen G. 2006.** Catching a 'hopeful monster': Sheperd's Purse (*Capsella bursa-*

301   *pastoris*) as a model system to study the evolution of flower development. *Journal of*

302   *Experimental Botany* **57**: 3531-3542.

303   **Ianetta PPM, Begg G, Hawes C, Young M, Russell J, Squire GR. 2007.**

304   Variation in *Capsella* (shepherd's purse): an example of intraspecific functional diversity.

305   *Physiologia Plantarum* **129**: 542-554.

306   **Linde M, Diel S, Neuffer B. 2001.** Flowering ecotypes of *Capsella bursa-pastoris*

307   (L.) Medik. (Brassicaceae) analysed by a cosegregation of phenotypic characters (QTL)

308   and molecular markers. *Annals of Botany* **87**: 91-99.

309   **Neuffer B, Bernhardt KG, Hurka H, Kropf M. 2011**. Monitoring population and

310   gene pool dynamics of the annual species *Capsella bursa-pastoris* (Brassicaceae) –

311   initiation of a long-term genetic monitoring and a review of relevant species traits.

312   *Biodiversity and Conservation* **20**: 309-323.

313   **Neuffer B, Linde M. 1999.** *Capsella bursa-pastoris* – Colonization and

314   adaptation; a globe-trotter conquers the world. In: van Raamsdonk LWD, den Nijs JCM,

315   eds. *Plant Evolution in Man-Made Habitats*. Proceedings of the VIIth International IOPB

316   Symposium 1998, pp. 49-72.

317   **Neuffer B, Paetsch M. 2013.** Flower morphology and pollen germination in the

318   genus *Capsella* (Brassicaceae). *FLORA* **208**: 626-640.

319   **Neuffer B, Wesse C, Voss I, Scheibe R. 2018.** The role of ecotypic variation in

320   driving worldwide colonization by a cosmopolitan plant. *AoB Plants* **10**: 1-20.

321   **Nutt P, Ziermann J, Hintz M, Neuffer B, Theißen G. 2006.** *Capsella* as a model

322   system to study the evolutionary relevance of floral homeotic mutants. *Plant*

323   *Systematics and Evolution* **259**: 217-235.

324   **Opiz PM. 1821.** 2. *Capsella apetala* Opiz. Eine neue merkwürdige Pflanze.

325   *FLORA* **4** (II): 436-443.

326   **Reichert H. 1998.** Eine kronblattlose Sippe des Hirtentäschels (*Capsella bursa-*

327   *pastoris*) seit Jahren bestandsbildend bei Gau-Odernheim/Rheinhessen. *Hessische*

328   *Floristische Rundbriefe* **47**: 53-55.

329   **Shull H. 1909.** *Bursa bursa-pastoris* and *Bursa heegeri*: biotypes and hybrids.

330   *Carnegy Institution of Washington Publication* **112**: 3-56.

331   **Shull H. 1911.** Defective inheritance-ratios in Bursa hybrids. *Verhandlungen des*

332   *Naturhistorischen Vereins in Brünn* **49**: 156-168.

333   **Ziermann J, Ritz M, Hameister S, Abel C, Hoffmann M, Neuffer B, Theißen G.**

334   **2009.** Floral visitation and reproductive traits of stamenoid petals, a natural occurring

335   homeotic floral homeotic variant of *Capsella bursa-pastoris* (Brassicaceae). *Planta* **230**:

336   1239-1249.

337

Figure 1

Flower morphology

Figure 1 Typical wild-type flower (**A** arrow points to the petal) and its typical pollinator, a wild bee (**D**). Typical decandric flower (**C**) and its typical pollinator, a hoverfly (**E**). Proof for a rare hybrid between both flower types with a developing petal and pollen sacs below (**B**). In the field, both types are growing in mixed populations (**F**). Fotos: Gitta Schüttler, Birgit von Höveling.
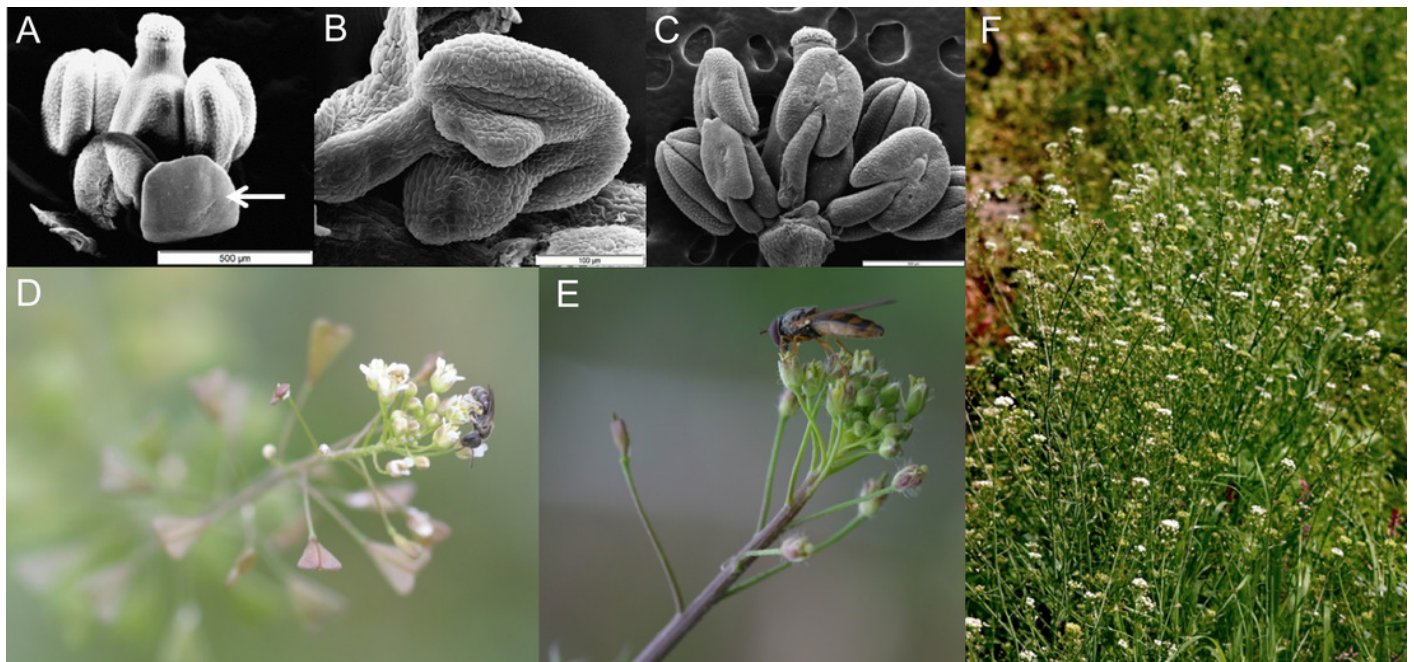
Figure 2

Habitus of individual plants cultivated under different light conditions

Habitus of individual plants of the same age (8th to 9th week after sowing) that had been cultivated under different light conditions. The inflorescence shoot was distorted since the light source was mounted 20 cm above the rosettes.
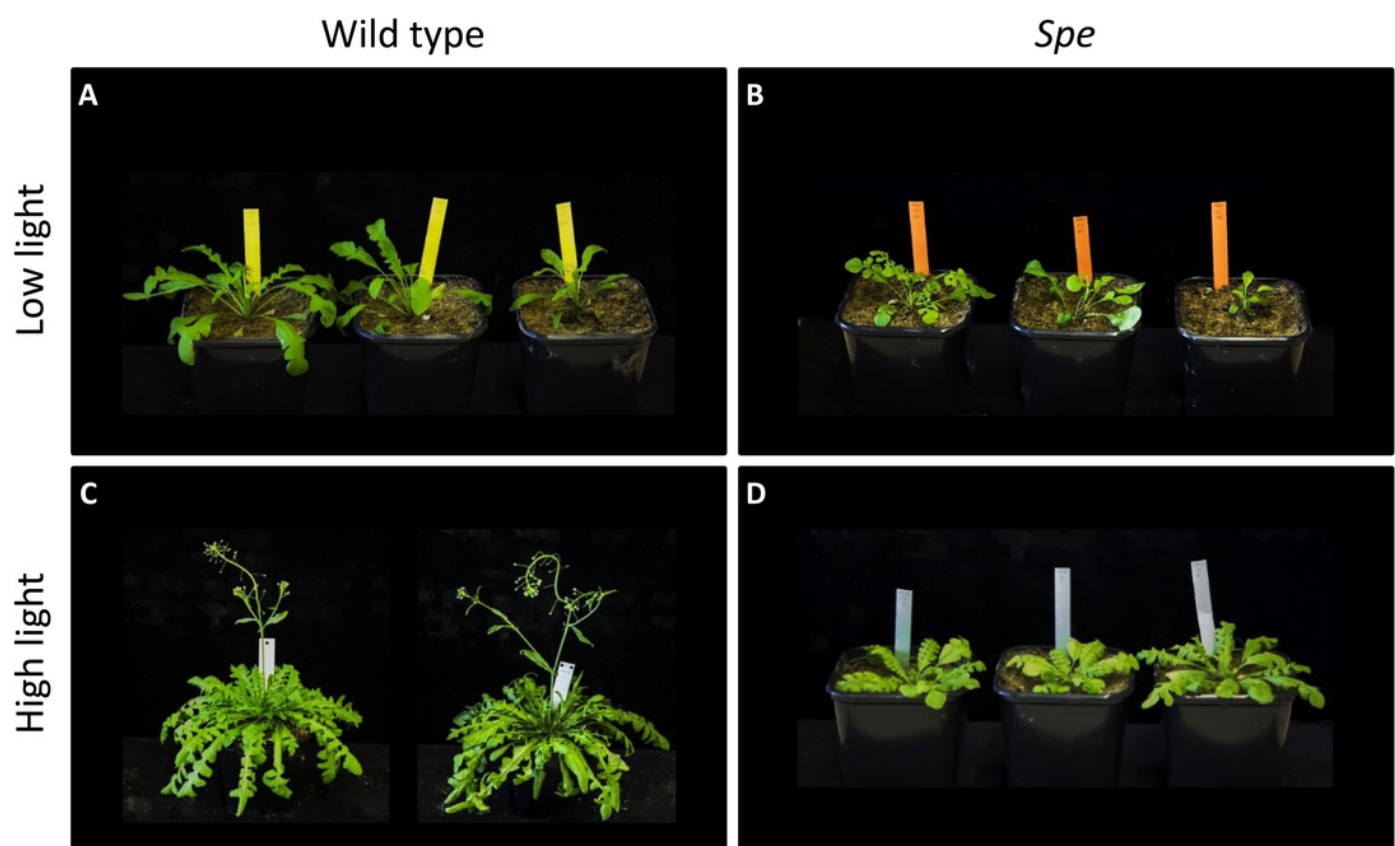
Figure 3

Stomata density of plants grown under high-light conditions.

Microscopical views of stomata distribution of plants grown under high-light conditions. Nail polish tracks of upper and lower leaf surfaces were taken. Black scale bar to avoid different font sizes.
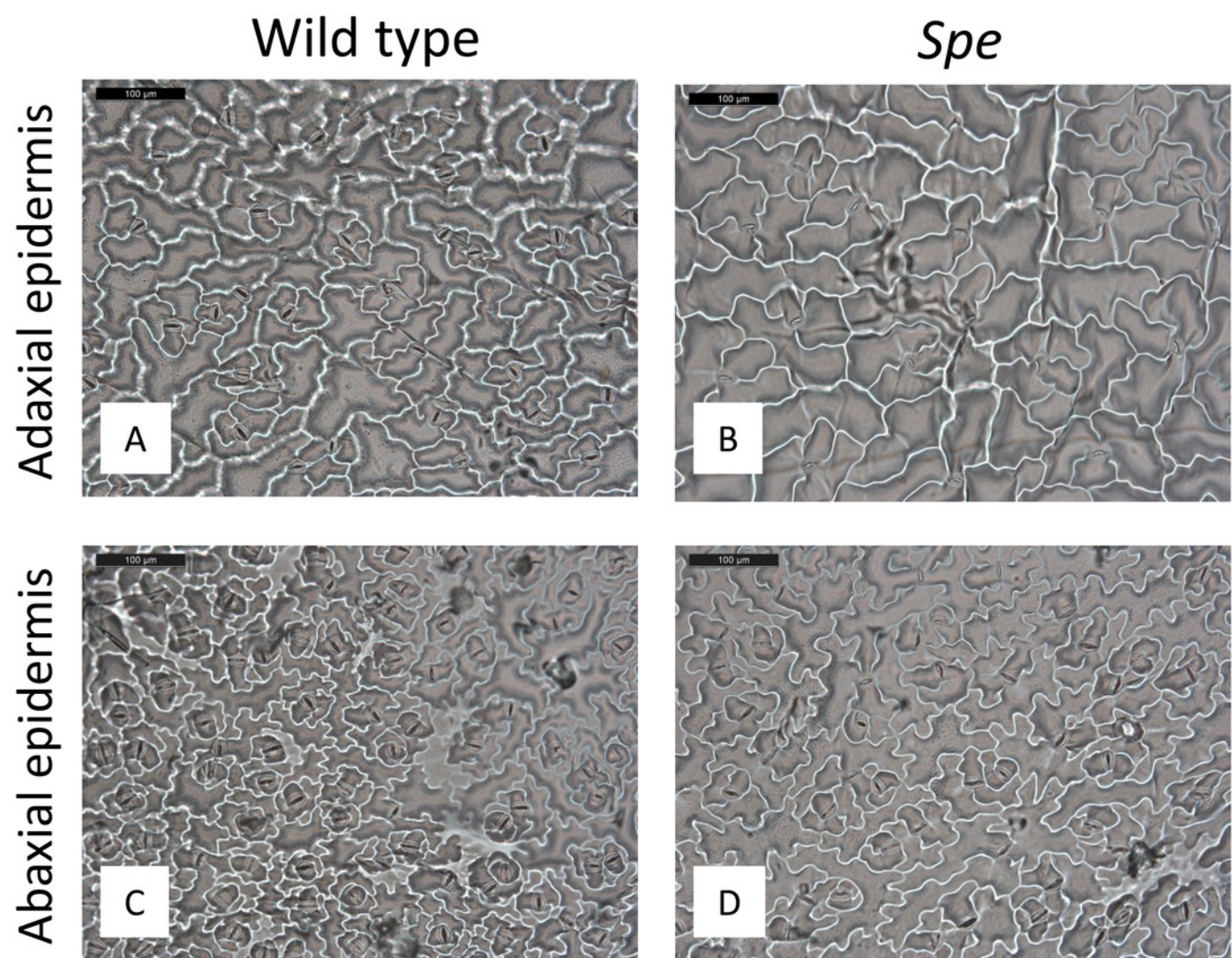
Figure 4

Stomata density

Stomata density of wild-type *C. bursa-pastoris* versus *C. apetala* (= *Spe*) grown under different light conditions. For significant differences between experimental groups see **Suppl. Table 1**.
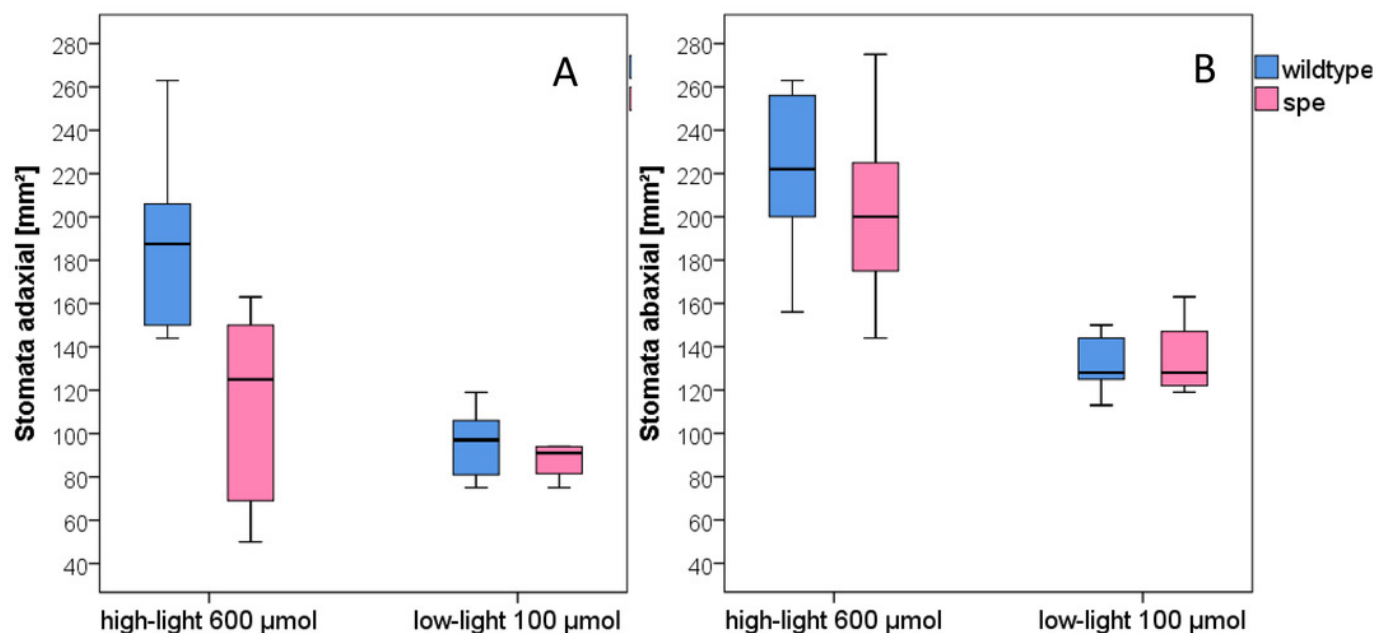
Figure 5

CO_2-concentration dependence of the rate of CO_2 assimilation

Figure 5 CO_2-concentration dependence of the rate of CO_2 assimilation of wild type (WT, five individuals low light, seven individuals high light) and *C. apetala* (*Spe*, three individuals low-light, seven individuals high light) grown under different light conditions. The plants had been grown either under low-light (**A** and **C**) or under high-light conditions (**B** and **D**), respectively. Determination of the rates of CO_2 assimilation was performed under saturating light using one leaf for each individual as described in Material and Methods. **A** and **B**: After application of descending CO_2 concentrations, the internal CO_2 concentration (C_i) was calculated in each case, and similar C_i values were clustered (horizontal error bars). Stomata aperture was recorded for each measurement and is shown in **Suppl. Fig. 1**. **B** and **D**: Single measurements in the linear range up to 350 ppm were plotted against C_i.
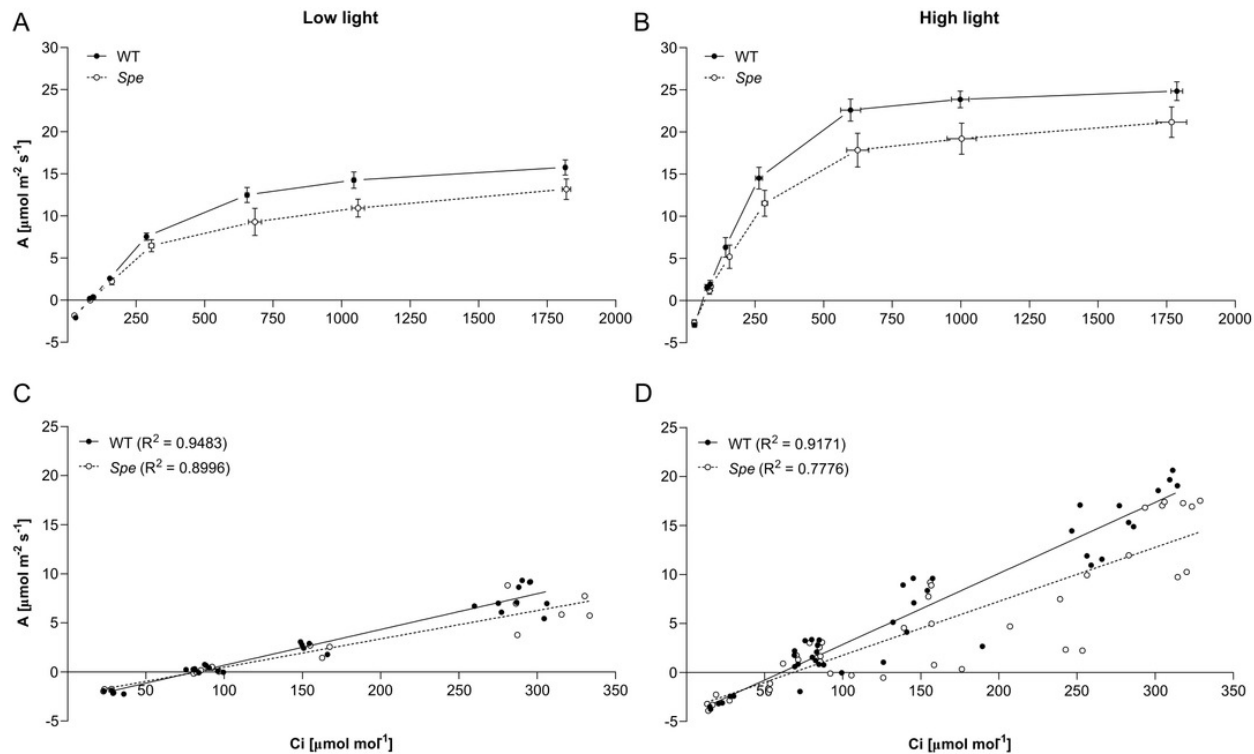
Figure 6

Onset of flowering in days after sowing.

Sister individuals of the plants that had been used for the physiological and anatomical analyses have been planted into the experimental field of the Botanical Garden Osnabrück on day 70 after sowing.