**Running title:** Sex Lability and Dimorphism

# Sex Lability and Dimorphism in Dioecious Palmer Amaranth (*Amaranthus palmeri*)

Mohsen B. Mesgaran[1, †], Maor Matzrafi[2, 3, †] and Sara Ohadi[4]

[1]Assistant Professor (ORCID 0000-0002-0841-6188), Department of Plant Sciences, University of California, Davis, One Shields Avenue, Davis, CA 95616

[2]Former Postdoctoral Research Associate (ORCID 0000-0002-4867-0850), Department of Plant Sciences, University of California, Davis, One Shields Avenue, Davis, CA 95616

[3]Current address: Department of Plant Pathology and Weed Research, Agricultural Research Organization, Newe Ya'ar Research Center, Israel.

[4]Postdoctrol Research Associate, Department of Plant Sciences, University of California, Davis, One Shields Avenue, Davis, CA 95616

Author for correspondence: Correspondence: Mohsen B. Mesgaran, Assistant Professor, Department of Plant Sciences, University of California, Davis, One Shields Avenue, Davis, CA 95616

(E-mail: mbmesgaran@ucdavis.edu)

[†]These authors have contributed equally to this work

## Abstract

Dioecious weeds (separate sexes) may benefit from a maximized outcrossing and optimal sex-specific resource allocation but there are costs associated with the evolution of this breeding system which can be exploited for long-term management of dioecious weeds. That is, seed

production in dioecious species is contingent upon the co-occurrence and co-flowering of the two genders and can be further complicated by biases in sex ratio. We therefore explored the sex ratio and dimorphism in secondary sex characters in three populations of Palmer amaranth (*Amaranthus palmeri*) from California, Kansas and Texas and tested if water stress can change the sex expression and/or the synchrony of flowering (anthesis) between male and female plants. Sex ratio (proportion of males) was balanced and did not deviate from 1:1 in all experiments and populations (California, Kansas, Texas) when plants received normal watering. Male and female plants of *A. palmeri* did not differ in timing of emergence, plant height and relative growth rate. While the initiation of flowering (emergence of inflorescence) occurred earlier in males than females, females preceded males in timing of anthesis. Water stress delayed anthesis in males to a larger extent than females giving rise to an anthesis mismatch as large as seven days between the two sexes. Water stress induced a female sex expression in Kansas population giving rise to a female to male ratio of 1.78 which significantly differed from the equal 1:1 sex ratio. Our data provide the first evidence of sex lability and environment sex determination (ESD) in *A. palmeri,* suggesting manipulation of sex expression and phenological asynchrony as novel tools for ecological management of dioecious weeds.

**Key words:** Sex ratio, environmental sex determination (ESD), secondary sex characters, flowering overlap, gender specialization, environmental stress

42 **Introduction**

43 Hermaphroditism represents the most common mode of sexual reproduction in weeds whilst
44 dioecy (separate male and female individuals) is rare and have been observed in about 5-7% of
45 weed species (Sutherland 2004). Whereas a selfing breeding system is often associated with a
46 weedy character (Baker 1955; Razanajatovo et al. 2016), it might come as a surprise that some
47 dioecious weeds such as palmer amaranth (*Amaranthus palmeri* S. Watson) and common
48 waterhemp (*A. tuberculatus* (Moq.) J. D. Sauer) have been reported amongst the most the
49 troublesome weeds in US (Van Wychen 2017). Contrary to hermaphroditism (particularly
50 selfing), dioecy maximizes outcrossing and thereby reduces the likelihood of inbreeding
51 depression (Thomson and Barrett 1981,1990; Charlesworth 1999). Dioecy also has the benefit of
52 optimizing the allocation of resources between male and female functions by minimizing the
53 competition between these two functions through sexual specialization, i.e. the so-call "division
54 of labor" theory (Maynard Smith 1978; Charnov 1982).

55 Despite these advantages, a dioecious breeding system is subject to several demographic
56 handicaps. First, the two sexes must co-occur in the same habitat and, unlike hermaphrodite
57 weeds, dioecious plants cannot propagate from a single individual. Second, the two sexes need to
58 flower at the same time. The smaller the degree of temporal overlapping between male and
59 female flowering, the lower the chance of successful fertilization and hence seed production.
60 Third, the fecundity of a dioecious population can be affected by the sex ratio (the proportion of
61 males) and is likely to decline if the population becomes skewed towards one sex: a male-biased
62 population may not achieve its maximum seed production because of the ovule limitation, whilst
63 in a female-biased population, limited pollen availability may reduce the seed output. Therefore,
64 it seems that there exists an optimal sex ratio at which the population-level seed production is

65 maximized, but beyond that optimal point, the seed output is likely to decline due to pollen or
66 ovule limitations (Mulcahy 1967).

67 We believe that novel weed management strategies can be developed by better understanding of
68 the above "cracks" in the evolution of a dioicous mating system. Two potential strategies
69 envisioned involve shifting the sex ratio towards one sex (sub-optimal sex ratio strategy) and
70 reducing the flowering overlap between the two sexes (phenological isolation strategy). The
71 proposed strategies rely on the assumptions that the sex is labile–hence sex ratio can be
72 changed–and that we can manipulate the flowering phenology of male and female individuals
73 differentially. For long sex was posited to be a fixed trait of the plant throughout its life cycle
74 and genetic sex determination (GSD), where genes/chromosomes specify the gender of an
75 individual, was considered as the sole mechanism by which the sex of an individual (dioecious)
76 plant is determined (Fuller and Ritchie 1967). Evidence, however, has mounted over the past half
77 century showing that sex can be labile and the genetic control of sex can be overridden by the
78 environment (reviewed in Freeman et al. 1980; Korpelainen 1998; Vega-Frutis et al. 2014):
79 Environmental sex determination (ESD) has been found in many plants where the expression of
80 sex has changed in response to the environmental factors such as soil moisture (Freeman and
81 Vitale 1985), nutrients (Dorken and Barrett 2004), light intensity (Zimmerman 1991),
82 photoperiod (Thompson 1956), plant density (Onyekwelu and Harper 1979), and exogenous
83 application of plant hormones (Chailakhyan 1979). Environmental stresses such as water stress
84 (drought) are more likely to induce maleness, while conditions conducive to growth e.g. high soil
85 nutrition favor a female sexuality (Freeman et al., 1980), perhaps because reproduction is more
86 costly to females than males (Delph, 1999; Meagher, 1988).

87 In many dioicous species, male and female individuals have shown to differ in secondary sex
88 characters (i.e. traits other than the sex itself) related to morphology, physiology, life history and
89 phenology (reviewed in Geber et al. 1999; Juvany and Munné-Bosc 2015; Lloyd and Webb
90 1977). Differences in progression of phenological events (e.g. timing of germination) can result
91 in temporal segregation of males and females, ultimately reducing the chance of successful
92 cross-pollination in a dioecious population. In fact, most dioecious species seems to be
93 protandrous i.e. males flower earlier than females (Forero-Montaña and Zimmerman 2010;
94 Forest 2014). As female's investment in reproduction is greater than males, it has been
95 hypothesized that females postpone flowering (reproduction) for longer period of resource
96 accumulation (Lloyd and Webb 1977; Purrington and Schmitt 1998). This hypothesis has direct
97 implications for manipulative separation of flowering timing between the two sexes. That is, if
98 requirement for resource accumulation delays flowering in females, then environmental stresses
99 can further isolate the females, exacerbating the flowering mismatch between the two sexes.

100 In this study, we attempted to test the viability of exploiting the demographic handicap
101 associated with a unisexual breeding system (i.e. shift in sex ratio and phenological isolation) for
102 novel management of dioecious weeds. We used palmer amaranth (*Amaranthus palmeri* S.
103 Watson) as our dioecious model system. The species has been ranked as the worst weed in US
104 corn fields in a recent survey (Van Wychen 2017) and evolved resistance to six classes of
105 herbicides with 56 herbicide-resistant biotypes found in the US (Heap 2018). Most of these
106 biotypes were found to be resistant to the widely used herbicide, glyphosate (Roundup®),
107 requiring the development of non-chemical control methods. The objectives of this study were
108 threefold: 1) determine if the male and female individuals of *A. palmeri* differ in secondary sex
109 characters related to growth and development, 2) examine sex lability (shift in sex ratio) in *A.*

110 *palmeri* in response to environmental stress (drought), and 3) test if the degree of flowering
111 synchrony between *A. palmeri* male and female plants can be affected by the environmental
112 stress (drought). As a proof of concept study, we used water stress for the sex change experiment
113 as this environmental factor has been found to affect sex expression in several plant species
114 (Freeman et al., 1980; Korpelainen, 1998).

115 **Materials and Methods**

116 Seeds of *A. palmeri* from California (CA) and Kansas (KS) were cordially provided by Dr. Anil
117 Shrestha (California State University, Fresno, California) and Dr. Dallas E. Peterson (Kansas
118 State University, Manhattan, Kansas), respectively. Seeds of a third population were collected
119 from mature plants (about 30 plants) from a set-aside field in College Station, Texas (TX), in the
120 summer of 2017. Seeds were stored at 4ºC before use. Two sets of experiments were conducted
121 in the greenhouse facility (Orchard Parks) of University of California, Davis, during the spring
122 and summer of 2018.

123

124 *Experiment 1. Natural sex ratio and dimorphism in secondary sex characters*

125 The aim of this experiment was to examine whether *A. palmeri* has undergone divergent
126 evolution in secondary sex traits related to growth and development. We therefore compared
127 male and female plants of *A. palmeri* for potential differences in emergence pattern, timing of
128 flowering and anthesis, plant height, and relative growth rate (RGR). By using three populations
129 (CA, KS, and TX) and large number of plants (~250), *Experiment 1* also served as a platform for
130 a robust estimate of baseline sex ratio in *A. palmeri*, which was the major trait to be examined in
131 the second experiment (*Experiment 2*, see below). In July 2018, we sow 200 seeds from each
132 population, into 5 by 5 by 5 cm plastic pots (one seed per pot) filled with a soil mix (1:1:1:3

133 sand:compost:peat:dolomite). We recorded the emergence time for each individual seed and then
134 transplanted the young plants (two-four leaf stage) into larger plastic pots (2.37 L) filled with a
135 soil mix (1:1 sand/peat) plus a controlled-release fertilizer (15–9–12, 150 g 75 $L^{-1}$; Scotts
136 Osmocote PLUS, Mississauga, ON). Pots were randomly placed in a net house under natural
137 California-summer conditions and watered twice a day through an automated drip irrigation.
138 Plant height was measured three times (11 July, 23 July, and 24 September) during the season.
139 We inspected plants daily and an individual was recorded to be at the flowering stage when the
140 tip of its inflorescence was visible either on the terminal or lateral buds. The onset of anthesis for
141 a flowering plant was then determined when the stigma (in pistillate flowers) or dehisced anther
142 (in staminate flower) become visible. At this stage, i.e. anthesis, we also recorded the sex of
143 plants.
144
145 *Experiment 2. Sex expression and flowering synchrony in response to water stress*
146 This experiment aimed at evaluating the effect of water stress on the sex ratio and flowering
147 synchrony between the male and female plants of *A. palmeri*. Because of space limitation we
148 only used two populations (CA and KS) in this experiment. The experiment was conducted twice
149 (April-July and July-September in 2018) in a completely randomized design with 50 replicates
150 (plants) per treatment (water stress *vs.* well-watered control). About 10 seeds were sown in each
151 round plastic pot (2.37 L), filled with a soil mix (1:1 sand/peat) plus a controlled-release
152 fertilizer (15 – 9 – 12, 150 g 75 $L^{-1}$; Scotts Osmocote PLUS, Mississauga, ON). Pots were placed
153 in a greenhouse set at a temperature of 32/22ºC (day/night) with a day length of 16 hours
154 provided through supplementary lighting between 5:00 to 9:00 am and 5:00 to 9:00 pm. Seedling
155 were thinned haphazardly several times to achieve one plant per pot by their four-leaf stage.

156 When plants reached six to seven true-leaf stage, 50 random plants from each population were
157 randomly assigned to well-watered or water deficit treatments. Well-watered plants received
158 water from four emitters inserted into the potting medium to deliver 65 mL of water $min^{-1}$ for
159 two mins and twice per day (7 am and 2:00 pm). Water deficit treatment was achieved by
160 removing three out of four drip emitters from designated water stress treatment pots, reducing
161 the amount of water by approximately 75%. Additional irrigation (~100 mL twice per week) was
162 added to water deficit plants when severe visual stress symptoms were observed. Plants were
163 sexed by examining flowers for the presence of anther (in males) or stigma (in females). For
164 each plant we also measured time to flowering (inflorescences) and time to anthesis as described
165 in *Experiment 1*.
166
167 *Statistical analysis*
168 *In Experiment 1*, a binomial test (two-tailed) was used to test if the observed sex ratio in each
169 population statistically deviates from the expected 1:1 sex ratio (i.e. 50% male). Binomial test
170 was conducted by `binom.test()` function in `stats` package of R (R Core Team, 2018). Using a
171 t-test, male and female plants were compared for the secondary sex characters including time to
172 flowering (inflorescence), time to anthesis, plant height, and early stage RGR. Function `t.test()`
173 of 'stats' package (R Core Team, 2018) was used for the t-test analysis. The early stage RGR
174 was calculated based on the height gain between the first and second height measurements using
175 the following formula (Radosevich et al. 2007):

176 $$RGR = \frac{\log(H_2) - \log(H_1)}{t_2 - t_1} \qquad [1]$$

177 where $H_1$ and $H_2$ are plant heights measured at time $t_1$ (11 July) and $t_2$ (23 July), respectively (log
178 stands for natural logarithm). To explore difference in seedling emergence pattern between the

179 sexes, we fitted the following two-parameter logistic equation to cumulative emergence data of

180 *A. palmeri* using the `drm()` function of `drc` package (Ritz et al. 2016):

181 $$E(t) = \frac{1}{1 + \left(b(\log(t) - \log(t_{50}))\right)} \qquad [2]$$

182 where *E(t)* is the cumulative emergence over time, *t*, $t_{50}$ is time to 50% emergence while *b*

183 denotes the steepness of emergence curve around $t_{50}$.

184 In *Experiment 2*, for each water treatment level we tested the deviation of sex ratio from 1:1 by

185 using a binomial test (two-tailed) as described above. Phenology data (time to inflorescence and

186 time to anthesis) were subject to analysis of variance, ANOVA, using the `lm()` and `Anova()`

187 functions of `stats` (R Core Team, 2018) and `car` (Fox et al. 2019) packages in R, respectively.

188 Inspection of residuals showed no predictable pattern when plotted against fitted values

189 suggesting that the ANOVA's assumption of homogeneity of variance has been met. The degree

190 of flowering/anthesis overlap between the two sexes was visually inspected by fitting a kernel

191 density smoother to time data (i.e. time to flowering and time to anthesis) using the

192 `geom_density_ridges()` function of `ggplot2` R package (Wickham et al. 2019).

193

194 **Results and Discussion**

195 *Experiment 1. Natural sex ratio and dimorphism in secondary sex characters*

196 **Sex Ratio.** The recorded sex ratio was balanced in all three tested populations and did not

197 deviate from the expected 1:1 male/female ratio as indicated by a binomial test (Figure 1).

198 Although California population had slightly higher proportion of males (57%) than females, the

199 departure from 50% sex ratio was not significant (P = 0.306) (Figure 1).

200 An equal sex ratio was found in *A. palmeri* plants grown under greenhouse conditions with no

201 water or nutrient limitations (Giacomini et al., 2014). A balanced sex ratio of 55% (22 males vs.

202 18 females) also was reported by Lemen (1980); however, he does not provide any information
203 about method of measuring this parameter e.g. whether sex has been determined in plants grown
204 from the seed or sex ratio has been measured under field conditions. Keeley et al. (1987) also
205 reported a male:female ratio of 47%:53%—which shows a balanced sex ratio—for a field grown
206 *A. palmeri*. However, the source of seed for this test population was collected from one single
207 plant suggesting that their reported sex ratio could best represent the sex ratio of that individual
208 and not the population. Of 300 pot-grown *A. palmeri* plants—for a pollen mediate gene flow
209 study—172 (~60%) and 162 (%54) plants became male in the first (2005) and second year
210 (2006) of experiment (Sosnoskie et al. 2012). The sex ratio has clearly (and statistically) been
211 male-based in the first year according to a binomial test (*P-vale* = 0.0009), though the sex ratio
212 data were not analyzed in the original article.
213 **Seedling Emergence.** Total emergence for California, Kansas, and Texas populations were 31%,
214 55%, and 35% (n=200 for each individual population), respectively. Logistic model (Eq.2) fitted
215 the emergence data adequately as evidenced in Figure 2 and small root mean square errors
216 (RMSE ≤ 4%) in all populations. Female and male plants of *A. palmeri* exhibited almost
217 identical emergence pattern and in none of the tested populations the emergence curves differed
218 between the sexes. Populations, however, differed in emergence rate (rapidity) with Kansas
219 being the fastest emerging population ($t_{50}$ = 2.5±0.10 days) while California was the slowest ($t_{50}$
220 = 4.3±0.12 days) irrespective of the gender (see inset in Figure 2).
221 **Time to Flowering and Anthesis.** In all three populations, the initiation of flowering (i.e. tip of
222 inflorescence becoming visible) occurred earlier in male than female plants of *A. palmeri* (Figure
223 3). The difference in timing of flowering was particularly profound in Texas population where
224 males, on average, flowered ~9 days before females. Variability in flowering time seemed to be

225 similar between males and females as illustrated by the violin plots. The only exception was
226 observed with California population where the flowering bulge occurred close to the earliest
227 flowering times (i.e. at the bottom of the violin ) as opposed to females that had their bulge
228 above the median time of flowering. Note that a bulge on a violin plot represents the value (here
229 flowering time) with the highest probability of occurrence. The male's earlier flowering may
230 suggest a protandrous flowering habit in *A. plameri*; however, when we investigated the timing
231 of anthesis, which might be regard as the effective flowering event, we arrived at an opposite
232 conclusion.
233 In both, California and Texas populations, anthesis happened four days earlier in females than
234 males (Figure 3). For Texas population, although the median time to anthesis was the same for
235 male and females, the most probable anthesis time (i.e. the bulge) was at an earlier time in
236 females compared to males. While male plants of *A. palmeri* began to flower earlier than female
237 plants, it took them a much longer time to mature as shown in Figure 4. For females, regardless
238 of the population, the length of time from the appearance of inflorescence to anthesis (visible
239 stigma) was no more than 4-5 days compared with a 11-day time period needed for males to
240 develop mature anthers following the initiation of flowering. This observation suggests that *A.*
241 *palmeri* should be classified as a protogynous species as its pistillate flowers mature earlier than
242 the staminate ones (Figure 4). Korres et al. (2017) also showed that female plants of *A. palmeri*
243 flowered 6 to 8 d earlier than male plants, a flowering pattern that is very similar to our result from
244 Kansan and California population. However, Korres et al. (2017) do not explicitly define "flowering"
245 stage and we are not sure if our "anthesis" represents the same reproductive stage as with the
246 "flowering" stage in Korres et al. (2017). A synchronous anthesis between male and female plants
247 observed with Texas population resembles the results of Giacomini et al. (2014) who found no
248 differences in days to first flowering (anthesis) between male and female plants of *A. palmeri*.

249

250 **Plant Height and Relative Growth Rate (RGR).** We observed no sex-specific differences in
251 final plant height for the three tested populations (Figure 5). The only significant sexual
252 dimorphism in growth was related to the height-based RGR in Texas population. At the early
253 stage of growth, males of this population grew tall at a rate that was ~13% faster than females.
254 However, the male's faster initial growth did not translate into significant larger plants as males
255 attained a shorter stature (125.3 ± 3.78 cm) than females (133.3 ± 4.69 cm) at the end of growing
256 season. In the study of Webster and Grey (2015) plant height did not differ between *A. palmeri*
257 sexes either grown alone or in competition with cotton, but sex differential growth was detected
258 in other traits e.g. females had wider canopy and produced more than double the biomass of
259 males. Several other studies have shown that female *A. palmeri* plants tend to be taller and grow
260 faster than males (e.g. Keeley et al., 1987; Korres et al., 2017). Whereas *A. palmeri* populations
261 originating from ten contrasting cropping systems differed in many morphological and growth
262 traits, no sexual dimorphism was reported in these populations (Bravo et al. 2017).

263

264 *Experiment 2. Sex expression and flowering synchrony in response to water stress*

265 **Sex Ratio.** While sex ratio remained balanced across water treatments in California population,
266 exposure to drought produced a heavily female-biased sex ratio (~1.78 times more female than
267 males) in Kansas population. Sex ratio significantly deviated from 50% (P-value = 0.009, n = 99)
268 in water stress treatment whilst it was balanced (P-value = 0.547, n = 99) when *A. palmeri* plants
269 received normal irrigation (Figure 6). Our data provide the first evidence of sex lability and
270 environment sex determination (ESD) in *A. palmeri:* water stress induced a female sex
271 expression in Kansas population.

272 To the best of our knowledge, our study provides the first case of a feminizing effect for water
273 stress: almost all previous work has reported a masculinizing effect for this stressor. Since
274 reproduction is more costly to females than males (Delph 1999; Meagher 1988), sex allocation
275 theory predicts that the environmental conditions conducive to plant growth should favor a
276 female sex expression while resource-poor environments should induce maleness (Charnov
277 1982). Empirical data support the prediction of sex allocation theory. Reviews by Freeman et al.
278 (1980) and Korpelainen (1998) show that water stress has induced maleness in all 11 species
279 (five dioecious and six monoecious) that have been tested for potential sex change in response to
280 draught. Limited field surveys have also recorded overrepresentation of males in resource-poor
281 habitats whereas females were more abundant in benign habitats (Policansky, 1981; Lovett
282 Doust and Cavers 1982). The observed overrepresentation of males under stressful environments
283 may be due to a sex differential mortality rather than being the result of an actual sex change.
284 Most studies on sex ratio, however, lack the capacity to disentangle these two processes i.e. sex
285 lability *vs.* sex-differential survival. By randomly thinning seedlings (with no bias towards
286 selection of vigorous seedlings), our study was able to rule out differential mortality as the
287 potential driver of shift in sex ratio. In our experiments, only two plants (one per water treatment
288 in Kansas population) died over the course of experiment.
289 **Time to Flowering and Anthesis.** Flowering phenology was recorded for plants from California
290 and Kansas populations grown under different irrigation regimes. Similar to *Experiment 1*, the
291 initiation of flowering happened earlier in males than females with water stress having no effects
292 on the flowering sequence between the two sexes (Figure 7). However, water stress slightly
293 delayed flowering, almost to the same extent, in both male and female plants giving rise to a
294 more temporally staggered flowering pattern as compared with the control. Consistent with

295 *Experiment 1*, *A. palmeri* female plants preceded males in timing of anthesis in the water stress
296 experiment (*Experiment 2*; Figure 8). Sex-differential responses to water stress were observed
297 with the timing of anthesis: water stress distorted the frequency distribution of anthesis timing to
298 a larger extent in males than females (Figure 8). Time to anthesis nearly followed a unimodal
299 distribution in both sexes when plants received normal irrigation but became multimodal and
300 "wavy" when plants were exposed to water stress. Anthesis was much synchronized between
301 male and female plants of *A. palmeri* under normal watering but the two sexes became more
302 separate (i.e. less overlapping in anthesis) with water stress. In both populations, the difference
303 between males and females in median time to anthesis (arrows in Figure 8) was only two days in
304 control but extended to seven days in water-stressed plants. The increased asynchrony between
305 the two sexes was mainly driven by a differential response of the sexes to water stress. While
306 water stress delayed anthesis in both sexes, the delay in males was much longer than females
307 (Figure 8). In California population, for example, water stress postponed anthesis (median time)
308 by seven days in male plants compared with a 2-day delay in females.
309 Decreasing the level of irradiance (i.e. greater stress) also postponed *A. palmeri* flowering in the
310 study of Korres et al. (2017). Plants experiencing stressful environments often accelerate their
311 transition to reproductive stage; perhaps as a potential adaptive strategy to produce seed before
312 they die due to stress (Wada and Takeno 2010). However, depending on the intensity and
313 duration of stress and plant development stage, stress may produce an opposite response (i.e.
314 delay the flowering) by slowing the metabolism in plant (Cho et al. 2017). The delay in
315 flowering of *A. palmeri* observed in Korres et al. (2017) and our study might have resulted from
316 a slowed metabolism. However, contrary to our initial hypothesis, the timing of
317 flowering/anthesis in females was less sensitive to stress than males. The prevalence of protandry

318 in dioecious species has been attributed to the greater cost of reproduction incurred by females
319 (Lloyd and Webb 1977; Forest, 2014). As females need to produce seeds, they may undergo an
320 extended period of resources accumulation to ensure sufficient assimilates will be available for
321 allocation to seeds (Lloyd and Webb 1977; Purrington and Schmitt 1998). As a result, stress was
322 proposed to exert a stronger effect on the flowering phenology of females than that of males; a
323 hypothesis that was not supported by our data.
324
325 *Implications for weed management*
326 Our study showed that sex can be labile in *A. palmeri* and change in response to external
327 environmental conditions (here water stress). Sex lability can be exploited as new tool for long-
328 term management of *A. palmeri* (and other dioecious weeds): by shifting the sex ratio towards
329 the extremes we might be able to reduce seed production and hence suppress the population
330 growth. A variety of external factors can be used to shift sex towards the desirable direction (e.g.
331 plant hormones, nutrient and water management, light intensity, and herbicides) but currently we
332 have no information about the effects of these factors on the sex expression of *A. palmeri*.
333 Increasing the number of males might offer a more viable and safer management option as males
334 do not contribute to seed production. However, if the population is severely pollen limited,
335 increase in the share of females may also reduce the seed production capacity of population. A
336 quantitative understanding of the relation between thee sex ratio and seed output is needed to
337 determine the proper sex conversion strategy that can potentially disrupt seed production.
338 Our study clearly demonstrated that water stress can result in temporal separation of the two
339 sexes in *A. palmeri*, offering a potential means for ecological management of this weed and
340 perhaps other dioecious weeds. Asynchrony in timing of anthesis can reduce the chance of cross

pollination and hence reduce seed production in unisexual weeds such as *A. palmeri* and common waterhemp (*A. tuberculatus* (Moq.) J. D. Sauer). While the direct (negative) effect of water limitation on seed production is well understood, our study suggests a novel mechanism through which water stress (and perhaps other stressors) can reduce seed production indirectly by exacerbating the flowering/anthesis mismatch between the sexes in a dioecious weed. Future work should attempt to disentangle the direct effects of environmental stressors form their indirect effects on seed production, a subject which was out of the scope of the current study.

**Acknowledgments**

This research was funded by UC Davis New Research Initiatives and Interdisciplinary Research Grant. The authors would also like to thank Hannah Clifton and William Werner for greenhouse maintenance.

## References

Baker HG (1955) Self-compatibility and establishment after '"long-distance" dispersal. Evolution 9:347-349.

Bravo W, Leon RG, Ferrell JA, Mulvaney MJ, Wood CW (2017) Differentiation of life-history traits among Palmer amaranth populations (*Amaranthus palmeri*) and its relation to cropping systems and glyphosate sensitivity. Weed Sci 65: 339-349.

Chailakhyan MK (1979) Genetic and hormonal regulation of growth, flowering, and sex expression in plants. Am J Bot 66: 717–736.

Charlesworth D (1999) Theories of the evolution of dioecy. In Gender and sexual dimorphism in

363 flowering plants (pp. 33-60). Springer, Berlin, Heidelberg.

364 Charnov EL (1982) The Theory of Sex Allocation. Princeton University Press, Princeton, NJ.

365 Cho LH, Yoon J, An G (2017) The control of flowering time by environmental factors. Plant J
366 90:708-719.

367 Delph LF (1999) Sexual dimorphism in life history. *In* Gender and Sexual Dimorphism in
368 Flowering Plants (Geber, M. A., Dawson, T. E. & Delph, L. F. eds.), pp. 149–173, Springer-
369 Verlag, New York.

370 Dorken ME, Barrett SC (2004) Sex determination and the evolution of dioecy from monoecy in
371 *Sagittaria latifolia* (Alismataceae). Proc R Soc Lond [Biol] 271: 213-219.

372 Forero-Montaña J, Zimmerman JK (2010) Sexual dimorphism in the timing of flowering in two
373 dioecious trees in a subtropical wet forest, Puerto Rico. Caribbean J Sci 46: 88-95.

374 Forrest JR (2014) Plant size, sexual selection, and the evolution of protandry in dioecious plants.
375 Am Nat 184:338-351.

376 Fox J, Weisberg S, Adler D, Bates D, Baud-Bovy G, Ellison S, Firth D, Friendly M, Gorjanc G,
377 Graves S, Heiberger R (2019) Package 'car': Companion to applied regression. https://CRAN.R-
378 project.org/package=car

379 Freeman DC, Harper KT, Charnov EL (1980) Sex change in plants: old and new observations
380 and new hypotheses. Oecol 47:222-232.

381 Freeman DC, Vitale JJ (1985) The influence of environment on the sex ratio and fitness of
382 spinach. Botanical Gaz 146:137-142.

383 Fuller HJ, Ritchie DD (1967) General Botany, Barnes and Noble. Inc., New York. Geber MA
384 Dawson TE (1999) Gender and sexual dimorphism in flowering plants. Springer Science &
385 Business Media.

386 Giacomini D, Westra P, Ward SM (2014). Impact of genetic background in fitness cost studies:
387 an example from glyphosate-resistant Palmer amaranth. Weed Sci 62:29-37.

388 Heap I (2018) The International Survey of Herbicide Resistant Weeds. Online. Internet
389 Available at: www.weedscience.com (accessed Tuesday, June 17, 2019).

390 https://CRAN.R-project.org/package=car

391 Juvany M, Munné-Bosch S (2015) Sex-related differences in stress tolerance in dioecious
392 plants: a critical appraisal in a physiological context. J Exp Bot 66:6083-6092.

393 Keeley PE, Carter CH, Thullen RJ (1987) Influence of planting date on growth of Palmer
394 amaranth (*Amaranthus palmeri*). Weed Sci 35:199-204.

395 Khryanin VN (2002). Role of phytohormones in sex differentiation in plants. Russian J Plant
396 Physiol 49:545-551.

397 Korpelainen H (1998) Labile sex expression in plants. Biological Rev 73:157-180.

398 Korres NE, Norsworthy JK (2017) Palmer amaranth (*Amaranthus palmeri*) demographic and
399 biological characteristics in wide-row soybean Weed Sci 65:491-503.

400 Korres NE, Norsworthy JK, FitzSimons T, Roberts TL, Oosterhuis DM (2017) Differential
401 response of Palmer amaranth (*Amaranthus palmeri*) gender to abiotic stress. Weed Sci 65:213-
402 227.

403 Lemen C (1980) Allocation of reproductive effort to the male and female strategies in wind-
404 pollinated plants. Oecol 45:156-159.

405 Lloyd D G, Webb CJ (1977) Secondary sex characters in plants. Bot Rev 43:177-216.

406 Lovett Doust J, Cavers PB (1982) Sex and gender dynamics in jack-in-the-pulpit, *Arisaetna*
407 *triphyllum* (Araceae) Ecology 63:797-808.

408 Maynard Smith J (1978). The evolution of sex (No. 574.1 S5). Cambridge: Cambridge
409 University Press.

410 Meagher TR (1988). Sex determination in plants. *In* Plant reproductive ecology: patterns and
411 strategies. (Lovett Doust, J. & Lovett Doust, L. eds), pp. 125-138. Oxford University Press. New
412 York.

413 Morgan PW, Drew MC (1997) Ethylene and plant responses to stress. Physiol Plantarum,
414 100:620-630.

415 Mulcahy DL (1967) Optimal sex ratio in *Silene alba*. Heredity 22:411-423.

416 Onyekwelu SS, Harper JL (1979) Sex ratio and niche differentiation in spinach (*Spinacia*
417 *oleracea* L.). Nature 282:609-611.

418 Policansky D (1981) Sex choice and the size advantage model in jack-in-the-pulpit (*Arisaema*
419 *triphyllum*). PNAS 78:1306-1308.

420 Pospíšilová J, Synková H, Rulcová J (2000) Cytokinins and water stress. Biologia Plantarum
421 43:321-328.

422 Purrington CB, Schmitt J (1998) Consequences of sexually dimorphic timing of emergence and
423 flowering in *Silene latifolia*. J Ecol 86:397-404.

424 R Core Team (2018) R: A language and environment for statistical computing. R Foundation for
425 Statistical Computing, Vienna, Austria. http://www.R-project.org/.

426 Radosevich SR, Holt JS, Ghersa CM (2007) Ecology of weeds and invasive plants: relationship
427 to agriculture and natural resource management. John Wiley & Sons.

428 Razanajatovo M, Maurel N, Dawson W, Essl F, Kreft H, Pergl J, Pyšek P, Weigelt P, Winter M,
429 Van Kleunen M (2016). Plants capable of selfing are more likely to become naturalized. Nat
430 Comm 7:13313

431 Ritz C, Strebig JC, Ritz MC (2016) Package 'drc'. Analysis of dose-response curves.
432 http://www.bioassay.dk

433 Sosnoskie LM, Webster TM, Kichler JM, MacRae, AW, Grey TL, Culpepper AS (2012) Pollen-
434 mediated dispersal of glyphosate-resistance in Palmer amaranth under field conditions Weed Sci
435 60:366-373.

436 Sutherland S (2004) What makes a weed a weed: life history traits of native and exotic plants in

437 the USA. Oecol 141:24-39.

438 Thompson AE (1956) Methods of producing first-generation hybrid seed in spinach. Cornell
439 Univ Agr Exp Sta Mem 336:3-48.

440 Thompson FL, Eckert CG (2004) Trade-offs between sexual and clonal reproduction in an
441 aquatic plant: experimental manipulations vs. phenotypic correlations. J Evol Biol 17:581-592.

442 Thomson J D, Barrett SC (1981) Selection for outcrossing, sexual selection, and the evolution of
443 dioecy in plants. Am Nat 118:443-449.

444 Thomson J D, Brunet J (1990) Hypotheses for the evolution of dioecy in seed plants. Trends
445 Ecol Evol 5:11-16.

446 Van Wychen L (2017) Survey of the most common and troublesome weeds in grass crops,
447 pasture and turf in the United States and Canada. Weed Science Society of America National
448 Weed Survey Dataset. Available: http://wssa.net/wp-content/uploads/2017-Weed-Survey_Grass-
449 crops.xlsx.

450 Vega-Frutis R, Macías-Ordóñez R, Guevara R, Fromhage L (2014) Sex change in plants and
451 animals: a unified perspective. J Evol Biol 27:667-675.

452 Wada KC, Takeno K (2010) Stress-induced flowering. Plant Signal Behav 5:944-947.

453 Webster TM, Grey TL (2015) Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*)
454 morphology, growth, and seed production in Georgia. Weed Sci 63:264-272.

Wickham H, Chang W, Henry L, Pedersen TL, Takahashi K, Wilke C, Woo K, Yutani H (2019) Package 'ggplot2': Create elegant data visualisations using the grammar of graphics, http://ggplot2.tidyverse.org

Zimmerman JK (1991) Ecological correlates of labile sex expression in the orchid *Catasetum viridiflavum*. Ecology 72:597-608.

472 **Figure 1.** Observed sex ratios in three *A. palmeri* populations grown in pots during the summer
473 of 2018 in California, Davis. Also reported are the P-values (shown as P) for testing the
474 deviation of sex ratio (% male) from 50% based on a binomial test. Total number of plants
475 examined for sex ratio estimation was 61, 110, and 76 for California, Kansas, and Texas.
476 Abbreviations: F: Female and M: Male.

477 **Figure 2.** Comparison of cumulative seedling emergence between female (F) and male (M)
478 plants in three populations *A. palmeri* originating. Inset tables show parameter estimates for the
479 logistic model (equation 2). Values in the parentheses indicate stand errors. Note that emergence
480 data were normalized relative to the final number of seedlings counted for each sex within the
481 population (see text for definition of model's parameters).

482 **Figure 3.** Days to first flowering (inflorescence visible; top row) and anthesis (bottom row) in
483 female (F) and male (M) plants of *A. palmeri* from three populations (California, Kansas, and
484 Texas). The width of violin plot represents the relative frequency of the event of interest (i.e.
485 flowering or anthesis time). Horizontal line within the boxplot shows the median, the box
486 includes the interquartile range, IQR, (i.e. $25^{th}$ to $75^{th}$ percentile range), whiskers extend 1.5
487 times the IQR, and filled circles represent extreme values which fall within > 1.5×IQR and <
488 3×IQR.

489 **Figure 4.** Length of time (day) from flowering (inflorescence visible) to anthesis in female (F)
490 and male (M) plants of *A. palmeri* from three populations (California, Kansas, and Texas). The
491 width of violin plot shows the relative frequency of event (inflorescence to anthesis time). The
492 horizontal line within the boxplot shows the median, the box includes the interquartile range,

493 IQR, (i.e. 25$^{th}$ to 75$^{th}$ percentile range), whiskers extend 1.5 times the IQR, and filled circles
494 represent extreme values which fall within > 1.5×IQR and < 3×IQR.

495 **Figure 5.** Compression of final plant height (top row) and relative growth rate (bottom row)
496 between female (F) and male (M) plants in three populations of *A. palmeri*. Vertical lines on bars
497 indicate 95% confidence interval.

498 **Figure 6.** Observed sex ratios in two *A. palmeri* populations grown under well-watered (control)
499 and water stress conditions. Also reported are the P-values (shown as P) for testing the deviation
500 of sex ratio (% male) from 50% based on a binomial test. Total number of plants (sum of two
501 experiments) examined for sex ratio estimation was ~100 plants per water treatment.
502 Abbreviations: F: Female and M: Male.

503 **Figure 7.** Frequency distribution of time to flowering (emergence of inflorescence) in female (F)
504 and male (M) plants of two *A. palmeri* populations grown under normal watering (control) or
505 water stress conditions. Vertical lines show the location of median time to flowering while
506 symbols indicate observed data. The differences in median flowering time between the two
507 sexes are shown with horizontal arrows.

508 **Figure 8.** Frequency distribution of time to anthesis in female (F) and male (M) plants of two *A.*
509 *palmeri* populations grown under normal watering (control) or water stress conditions. Vertical
510 lines show the location of median time to flowering while symbols indicate observed data. The
511 differences in median flowering time between the two sexes are shown with horizontal arrows.

512

513

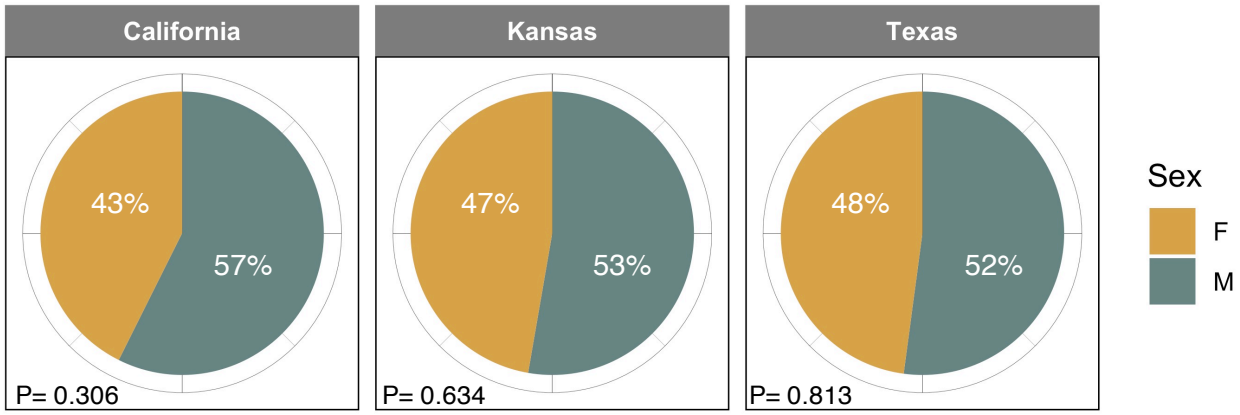

California
Kansas
Texas
43%
57%
47%
53%
48%
52%
P= 0.306
P= 0.634
P= 0.813
Sex
F
M

**Figure 1.**

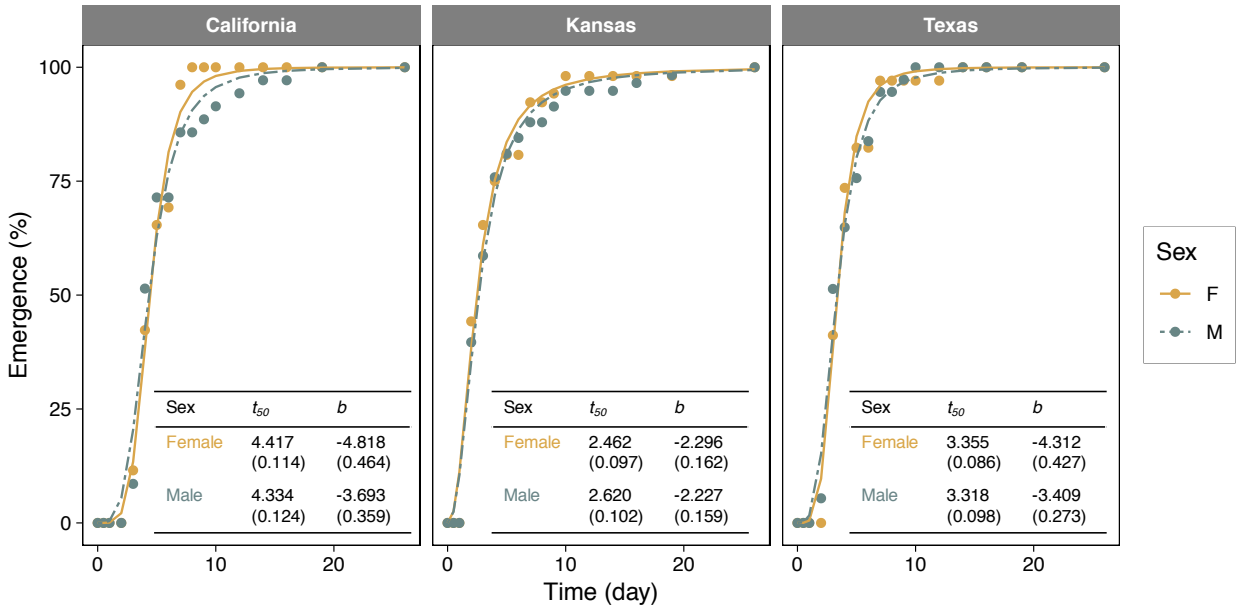
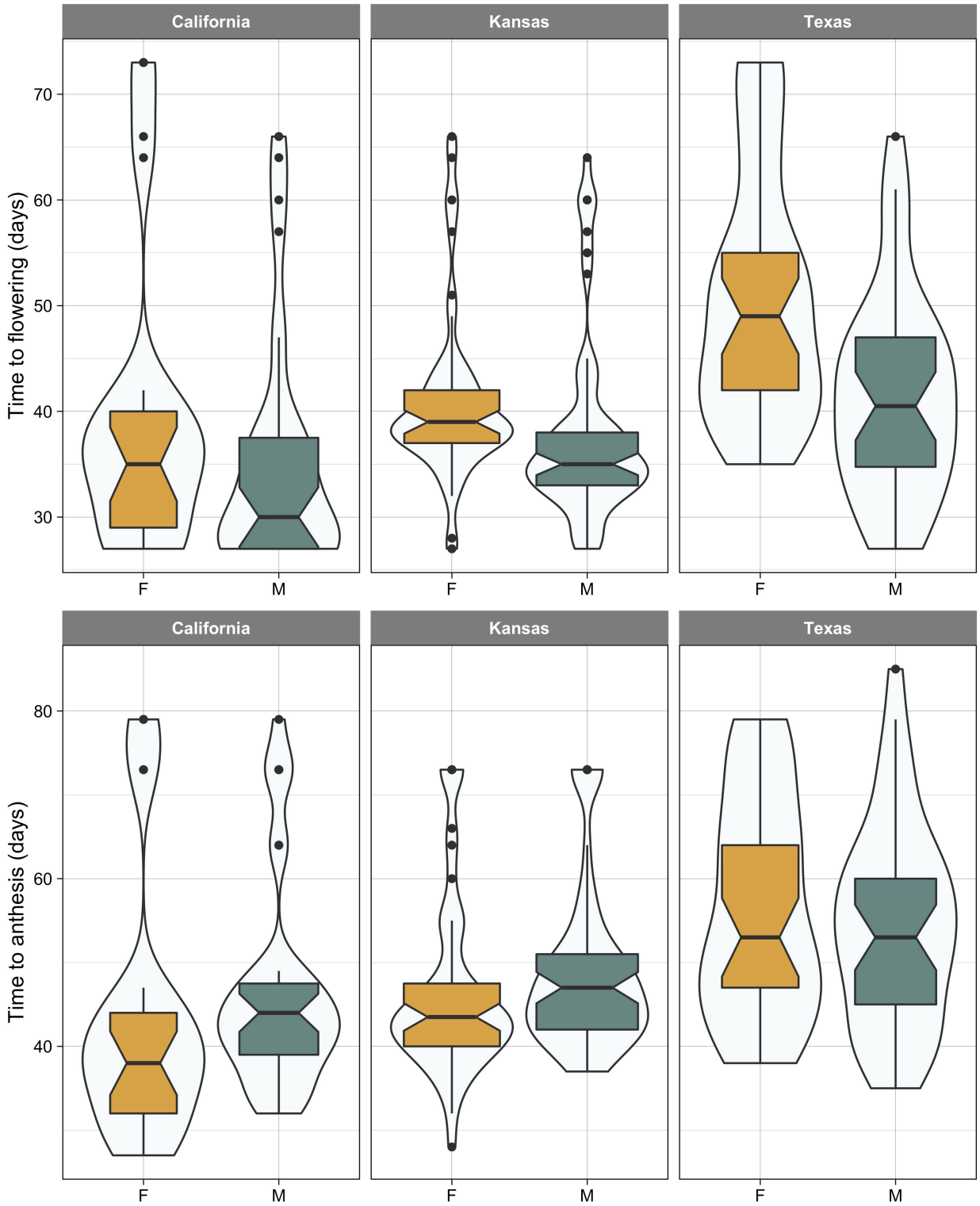

California
Kansas
Texas
Emergence (%)
Time (day)
Sex
F
M
Sex $t_{50}$ b
Female 4.417 (0.114) -4.818 (0.464)
Male 4.334 (0.124) -3.693 (0.359)
Sex $t_{50}$ b
Female 2.462 (0.097) -2.296 (0.162)
Male 2.620 (0.102) -2.227 (0.159)
Sex $t_{50}$ b
Female 3.355 (0.086) -4.312 (0.427)
Male 3.318 (0.098) -3.409 (0.273)

**Figure 2.**




536

537 **Figure 3.**

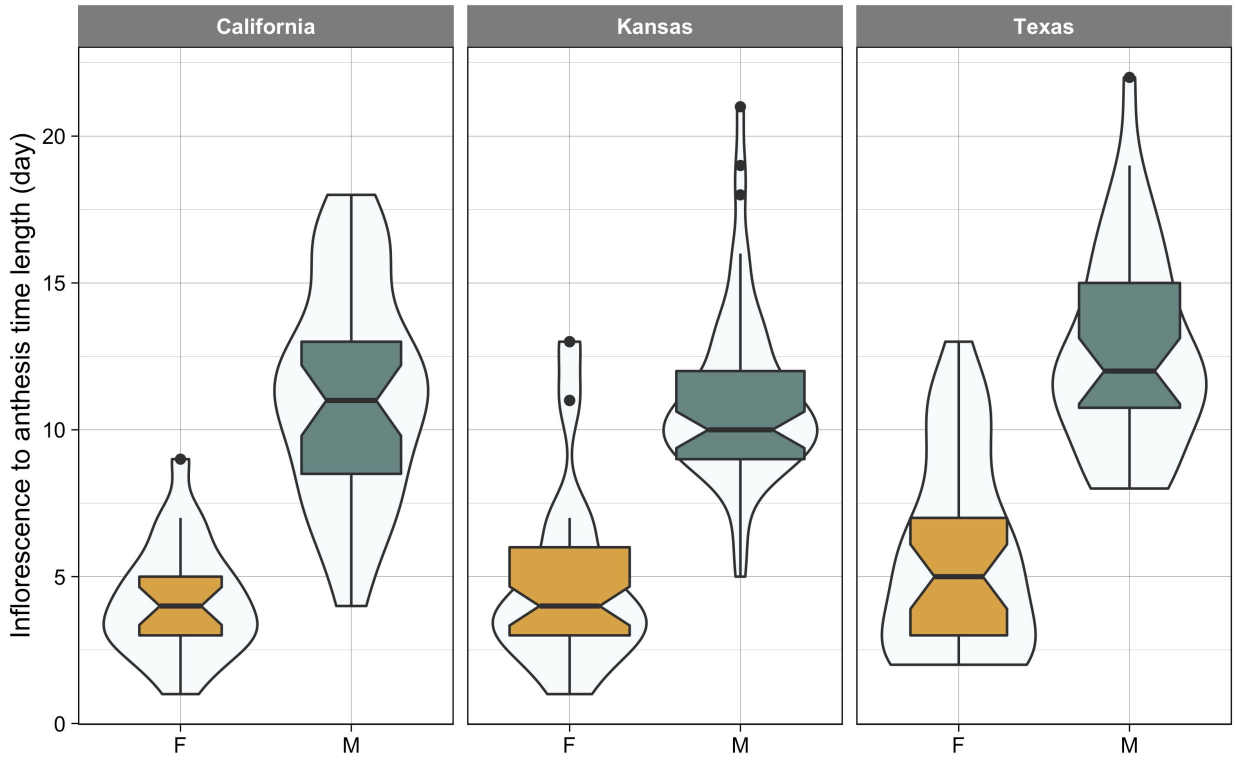
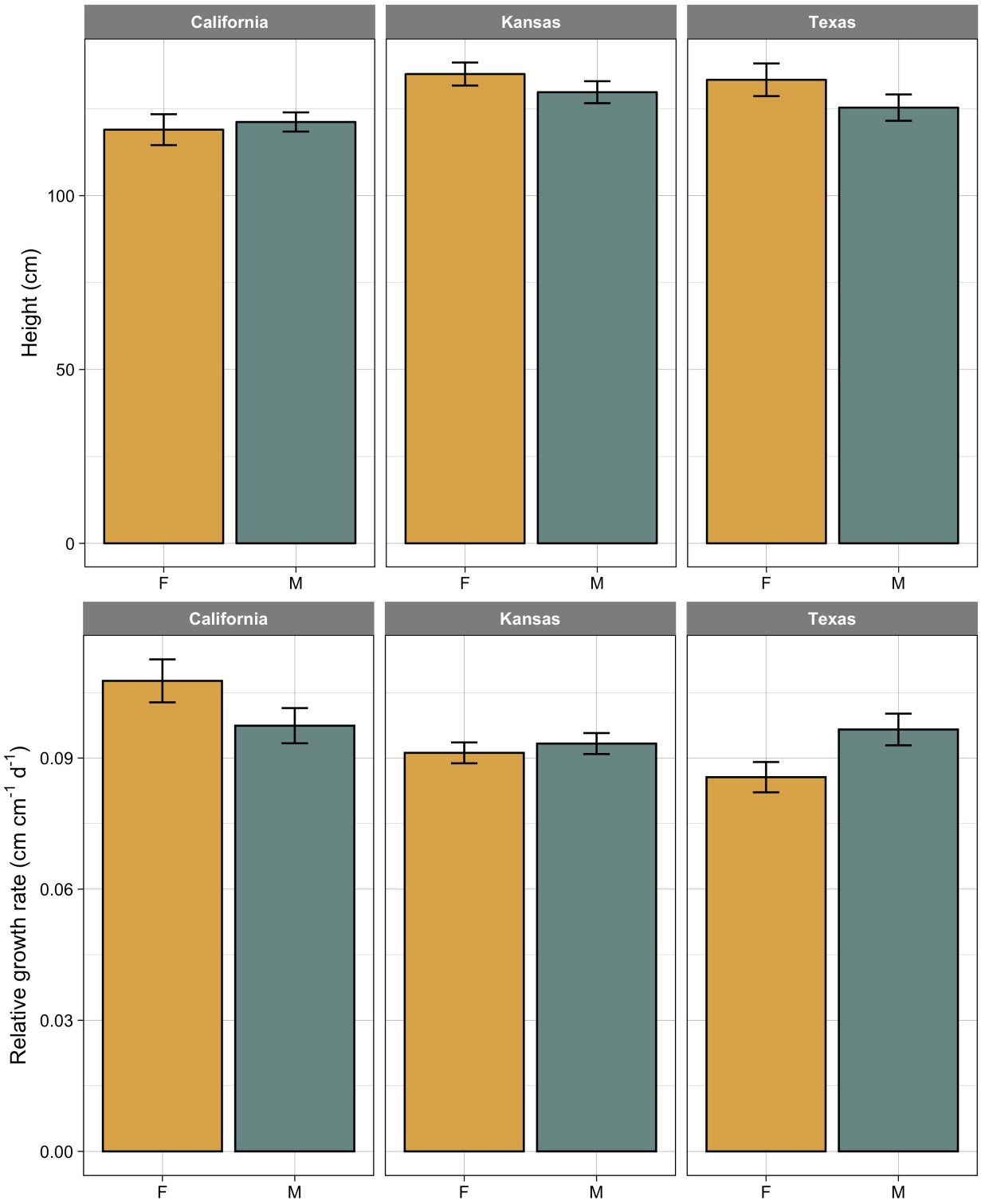

California
Kansas
Texas
Inflorescence to anthesis time length (day)
0
5
10
15
20
F
M
F
M
F
M

**Figure 4.**




547

548 **Figure 5.**

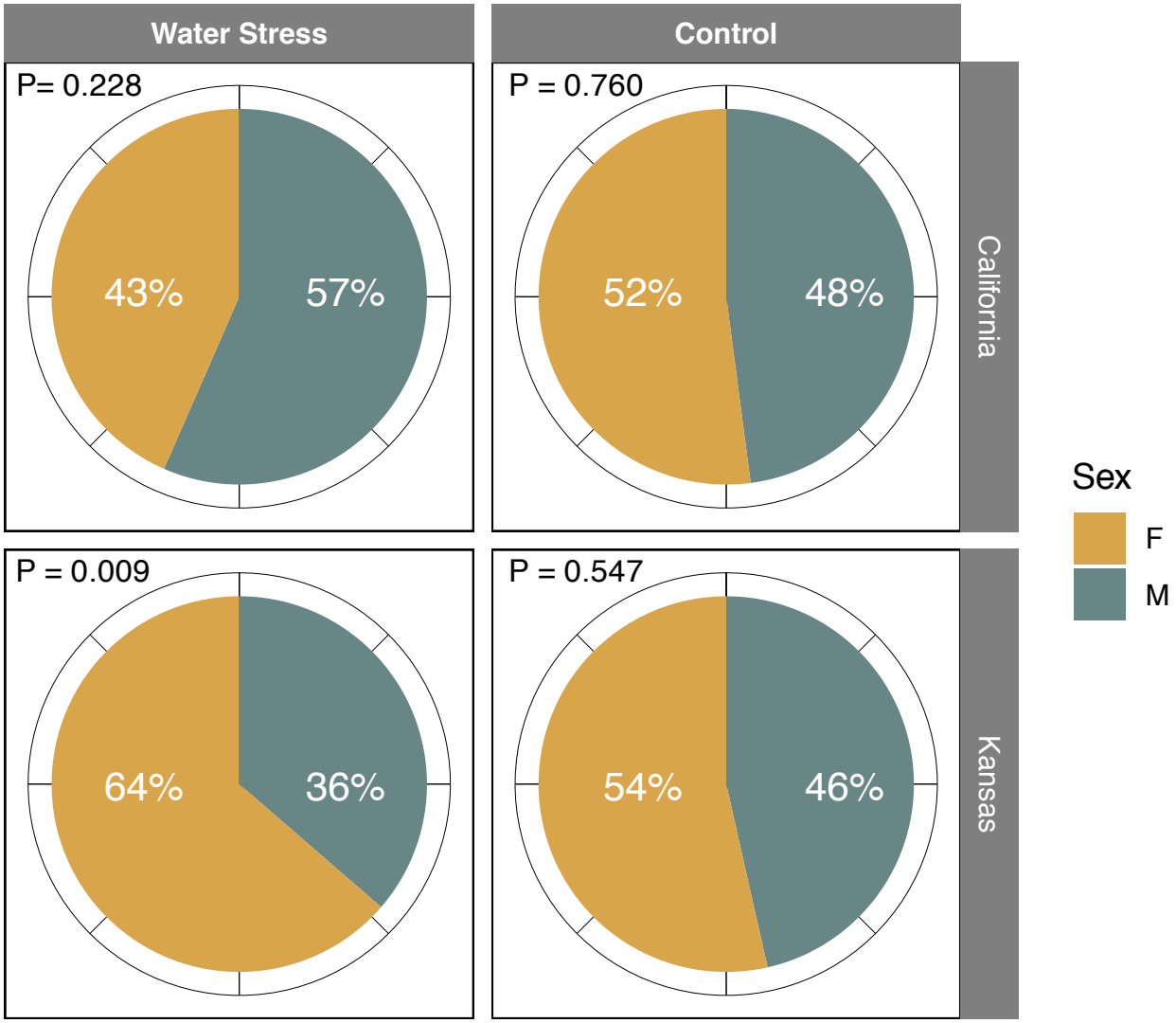

Water Stress
Control
P= 0.228
43%
57%
P = 0.760
52%
48%
California
P = 0.009
64%
36%
P = 0.547
54%
46%
Kansas
Sex
F
M

**Figure 6.**

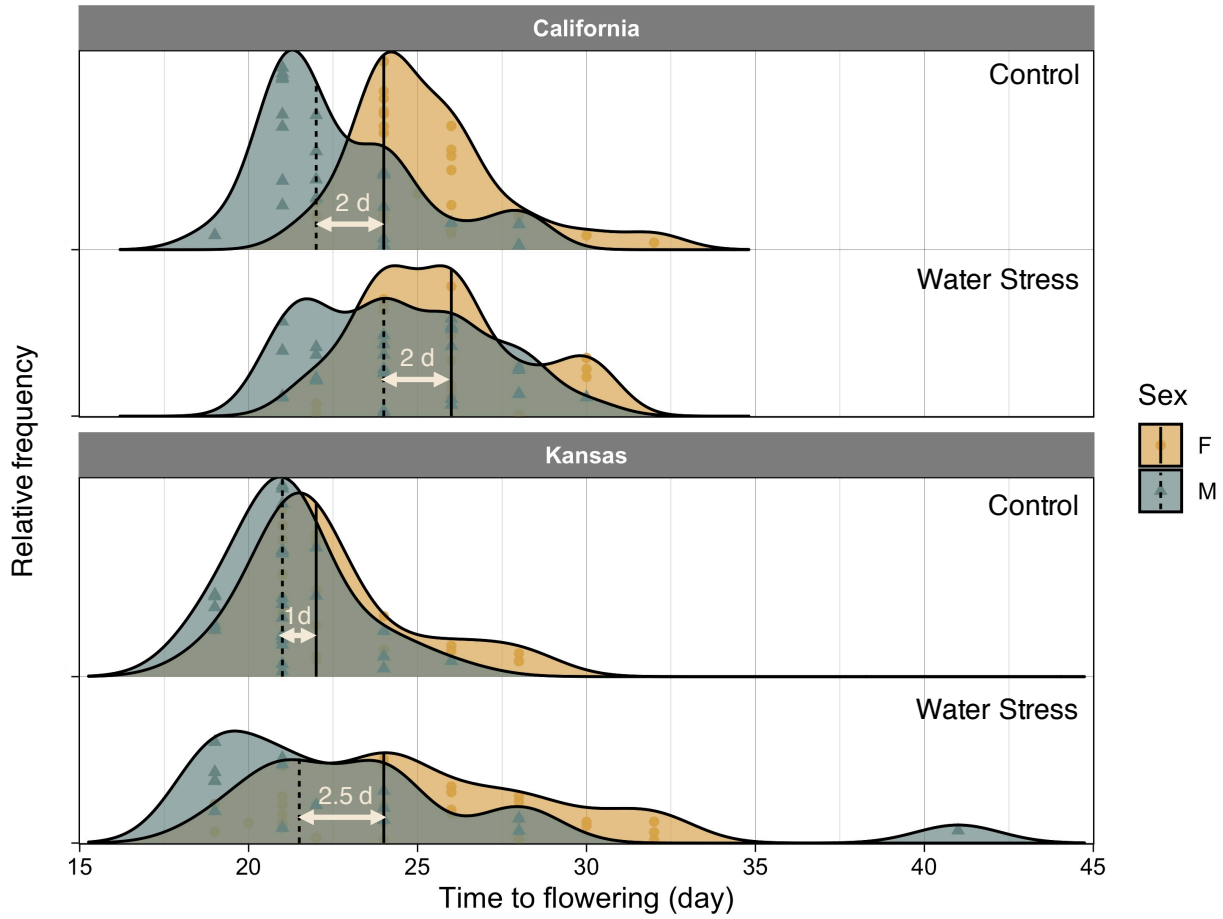

California
Control
2 d
Water Stress
2 d
Kansas
Control
1 d
Water Stress
2.5 d
Relative frequency
15
20
25
30
35
40
45
Time to flowering (day)
Sex
F
M

**Figure 7.**

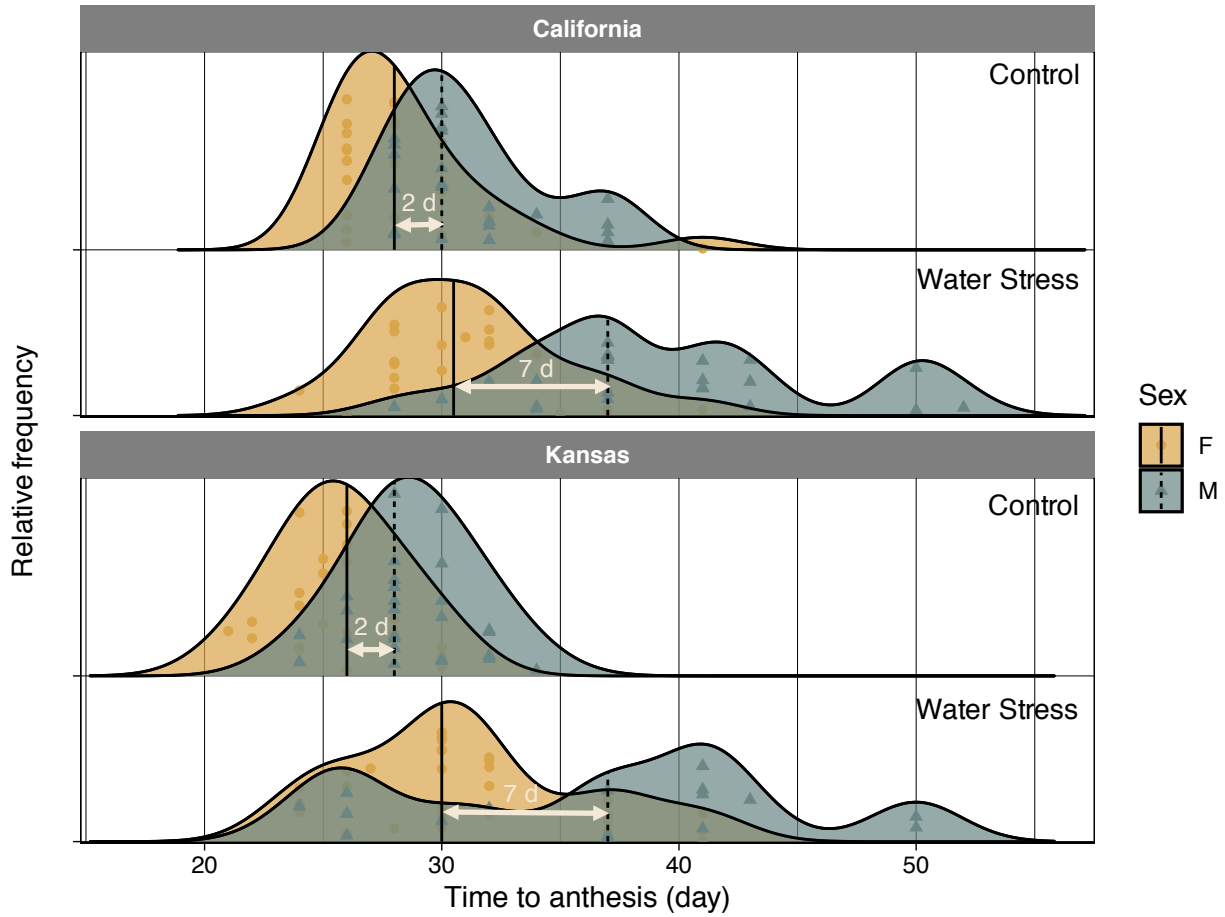

California
Control
2 d
Water Stress
7 d
Kansas
Control
2 d
Water Stress
7 d
Relative frequency
20
30
40
50
Time to anthesis (day)
Sex
F
M

**Figure 8.**