Driving the evolution of C4 photosynthesis in order

Yong-yao Zhao[1], Yao xin Zhang[123], Xin qi Yu[1], Xin-guang Zhu[12]

1.Center of Excellence for Molecular Plant Sciences, Chinese Academy of Sciences
2.State Key Laboratory for Plant Molecular Genetics
3.University of Chinese Academy of Sciences

C4 photosynthesis is an efficient photosynthetic type (High water use efficiency, High nitrogen use efficiency, High light use efficiency). Although occurring in only 3% plant species, C4 photosynthesis contributes 23% of terrestrial gross primary productivity. C4 photosynthesis has been considered evolved from C3 photosynthesis, although a mass of theories and hypotheses have been made, how does it drive C4 evolution remain unclear. Here, Flaveria genus, the leading model used for C4 evolutionary study, was used to explain the evolutionary driver for C4 photosynthesis through eco-physiological, biochemical and anatomical analysis. Precipitation was negatively associated with C4 metabolic enzyme activity and C4 flux during C4 evolution. Results showed an increasing temperature and precipitation in the earlier evolutionary stage, meanwhile vein density increasing. Decreased precipitation and risen temperature drove the emergence of C2 photosynthesis and the transition from C2 to partial C4 cycle. There was a positive relationship between CO2 compensation point, stomata density and precipitation, indicating decreasing precipitation enhance photorespiration through changing stomata during C4 evolution. This study proposed that different step during the evolution of C4 photosynthesis required different drivers and enhanced water source should be needed in the initial step.

Introduction

Photosynthesis undergone evolution, resulting in different photosynthetic type. C4 photosynthesis is one of these types of photosynthesis, which evolved from the C3 photosynthesis. Compared to its C3 ancestor, the plant with C4 photosynthesis has higher water use efficiency, nitrogen use efficiency and light use efficiency (Vogan and Sage 2011; Taylor et al. 2010). Although occurring in only 3% plant species, C4 photosynthesis contributes 23% of terrestrial gross primary productivity(Still et al. 2003; Sage et al. 2012). The evolution of C4 photosynthesis go through multiple stages (Sage et al. 2012; Sage 2004; Schluter and Weber 2016; Brautigam and Gowik 2016). First stage is preconditioning, when plants have C3 photosynthesis (Sage et al. 2012). At this stage, plants acquired new cis-elements (Akyildiz et al. 2007); gene duplication occurs (Akyildiz et al. 2007; Monson 2003); vein density increases (Marshall et al. 2007; Mckown and dengler 2007) and the number of plastids in the bundle sheath increases(Sage et al. 2013). The second stage is the formation of proto-kranz, when plants still perform C3 photosynthesis (Sage et al. 2013), the number of plastids continue to increase (Stata et al. 2019; Sage et al. 2012) and the chloroplast and mitochondria localize to the centripetal wall of bundle sheath cells, which can be thought to be the initial step of C2 photosynthesis (Hattersley 1989; Sage et al. 2012). The third stage is the formation of C2 photosynthesis (Type I species) (Schulze et al. 2013; Sage et al. 2012; Edwards and KU 1987). C2 photosynthesis is characterize as the transfer of glycine decarboxylase (GDC) and photosynthetic apparatus from mesophyll cell to bundle sheath cell(Schulze et al. 2013; Stata et al. 2019; Sage et al. 2013). The

released CO2 from photorespiration by GDC can moderately concentrate CO2 in the bundle sheath (Keerberg et al. 2014; Sage et al. 2012). The fourth stage is the enhancement of C2 photosynthesis and the induction of C4 cycle (Type II species) (Schluter and Weber 2016; Sage et al. 2018). The induction of C4 cycle is proposed as a mechanism to resolve the imbalance of ammonia between mesophyll cell and bundle sheath cell (Mallmann et al. 2014). The fifth stage is the formation of full C4 cycle (C4-like) (Stata et al. 2019). For this stage, mesophyll remains Rubisco(Taniguchi et al. 2021; Monson et al. 1987; Stata et al. 2019; Sage et al. 2018). The final stage is the optimization of C4 cycle (C4 species) (Sage et al. 2012), and mesophyll cell no longer contains Rubisco (Stata et al. 2019).

The evolution of C4 photosynthesis was considered to be driven by high photorespiration that is caused by the low atmosphere CO2 concentration, drought, salt and heat (Sage et al. 2012; Brautigam and Gowik 2016; Sage 2004; Edwards and Smith 2010; Bromham and Bennett 2014). The low atmosphere CO_2 concentration, drought and salt could decrease the ratio of intercellular CO_2 to O_2 concentration, thereby enhancing photorespiration (Sage et al. 2012). Increasing temperature enhance photorespiration by reducing CO2 relative to O2 in the chloroplast stroma(Ogren 1984) and decrease the specificity of Rubisco for CO2 relative to O2(Ogren 1984; Sage et al. 2012). Previous studies have showed that the decrease in atmosphere CO2 concentration and the appearance of dry, salty and hot environment are correlated with C4 evolution(Christin et al. 2008; Edwards and Smith 2010; Edwards and Still 2008; Bromham and Bennett 2014), which suggests they are the drivers for C4 evolution. However, the above researches may not be comprehensive enough. Given the multiple steps and the complexity during C4 evolution, it is possible that different phase during C4 evolution is under different driving force, rather than the same driving force persistently along the C4 evolution, which has been few studied. Each step of C4 evolution should obtains fitness for plants (Heckmann et al. 2013; Sage et al. 2012). The increase in vein density is an important characteristics in initial stage during C4 evolution(Sage et al. 2012; Sedelnikova et al. 2018; Kumpers et al. 2017; Sage et al. 2014) (Mckown and dengler 2007; Marshall et al. 2007; Williams et al. 2013), which should be to prevent loss of water in the mesophyll and maintain stomatal open, since evaporation is enhanced at this time(Brautigam and Gowik 2016; Sage et al. 2018) (Sage et al. 2012). Therefore, available water content in this stage might be increased(Brautigam and Gowik 2016). This was inconsistent with the notions that the origin of C4 photosynthesis promoted by drier condition(Sage et al. 2018; Edwards and Smith 2010).Since C2 photosynthesis and C4 photosynthesis could largely reduce photorespiration(Sage 2004; Schluter and Weber 2016; Sage et al. 2012), the formation of C2 photosynthesis and the transition from C2 to partial C4 might be driven by the conditions which could enhance photorespiration(Schluter and Weber 2016; Sage et al. 2018; Sage et al. 2012), which remains fewer studies.

Flaveria genus is the model system to study C4 evolution(Sage et al. 2012; Sage 2017; Schluter and Weber 2016; Mallmann et al. 2014; Sage et al. 2013; Vogan and Sage 2011), and the establishment of current C4 evolution pyramid model is largely based on the studies in the Flaveria genus (Sage et al. 2013; Vogan and Sage 2011; Sage et al. 2012). Flaveria genus contain numerous species that represent evolutionary step towards C4 photosynthesis, which includes multiple intermediate stage during the evolution of C4 photosynthesis(Mckown and dengler 2007; Sage et al. 2012; Schulze et al. 2013; Ku et al. 1991; Heckmann et al. 2013). Flaveria is the most young genus among the known C4 linage, which suggested that it might be going through the

evolution(Schulze et al. 2013). Flaveria species has the close relationship, which make it ideal to perform the comparison study (Christin et al. 2011; Sage et al. 2012). The ecology of the habitat for the intermediate species between C3 and C4 species and C4 plants could reflect the environmental conditions drive the evolution of C4 photosynthesis(Sage et al. 2012; Sage et al. 2018). Therefore, the ecology of Flaveria genus enable us to finely study the driving force of C4 evolution. In this study, we collected the ecological data of the C4 evolutionary model genus Flaveria, associated with the biochemical, anatomical and physiological data, finally trying to interpret the drivers and mechanism of C4 evolution. This study attempted to answer:1) how does the conditions promote C4 evolution? 2) how does plant obtain fitness during C4 evolution?

Result

1, Most of Flaveria are distributed inland, and a few are distributed along the coast

The locations of Flaveria species were mapped to the America by downloading the longitude and latitude of each Flaveria samples from Global Biodiversity Information Facility (GBIF) (Fig 1A,B). We found most of Flaveria species are located inland (Fig 1A,B). A few species (bro, flo, lin) are distributed along the coast (Fig 1B), which was consistent with the previous reports(Powell 1978). With evolution, intermediates gradually spread to location of higher latitude (Fig 1B). The C4 species widespread and distribute all over the world (Fig 1A).

2, The temperature and precipitation of Flaveria habitat

The temperature and precipitation of the habitat for each Flaveria species were obtained from the database worldclim(Fick and Hijmans 2017). The results from physiological, molecular experiments and phylogenetic tree indicated the direction of C4 evolution could be well described by species's order of phylogenetic tree towards C4 photosynthesis(Sage et al. 2012; Sage et al. 2018; Schluter and Weber 2016). Along the evolutionary way towards C_4 photosynthesis (Ordinal species towards C4 photosynthesis in the phylogenetic tree), annual mean temperature, mean temperature of warmest quarter, mean temperature of wettest quarter and max temperature of warmest month significantly increased in the F. rob (C3) and F. son (Type I) (Fig 2A, Supplementary fig 2, Supplementary fig 3, Supplementary file), compared with F. Pri. However, it significantly decreased in the F.ang, compared with F.son (Type I) (Fig 2a,b, supplementary file). For the mean temperature of wettest quarter, mean temperature of warmest quarter, max temperature of warmest month, compared with F. son, the habitat of species with C2 photosynthesis (F. ang, F. chl, F. pub, F. opp, F. ano, F. ram) have the lower mean temperature (Fig 2A, supplementary fig 2D). The habitat of species F. flo and F. lin have the higher mean temperature compared to other intermediate species (Fig 2a,b, table 1). Max temperature of warmest month for F. son is extremely high (Fig 2d).

For the annual precipitation, precipitation of wettest quarter, precipitation of warmest quarter and precipitation of wettest month, along the direction of C_4 evolution, the precipitations significantly increased in the F. rob (C3), compared with front species(Fig 2B, Supplementary fig 2, supplementary fig 3). However, it significantly decreased in F. son (Type I), and largely decrease in the F. ang (Type I) (Fig 2A, Supplementary fig 2, Supplementary fig 3, Supplementary file). The precipitation of warmest quarter is greatly higher than precipitation of coldest quarter, and similar to precipitation of wettest quarter (Fig 2B, Supplementary fig 1). Expect for F. flo (Type II) and F. lin (Type II), the precipitation of the species with C2 photosynthesis decrease persistently

along the direction of C4 evolution (Fig 2d,e,f, Supplementary fig 2b). In the latter of C4 evolution, the precipitation was a bit of increase, and the variation was large (Fig 2d,e,f, Supplementary fig 2b).

Along the phylogenetic tree towards C4 photosynthesis, the initial increase in precipitation and temperature (F. pri to F. rob) coincided with the initial appearance of the higher vein density(Fig 2, supplementary fig 4).

3, The association between precipitation (or temperature) and C4 metabolism related traits during the transition from proto-kranz to C4-like.

For the clade A, we found the relationship between (PEPC, PPDK and MDH activity) and precipitation is described as one in which (PEPC, PPDK and MDH activity) decreases with precipitation in the form of a negative power function, which can be described as a linear relationship between log enzyme activity and log precipitation (Fig 3 A,B,C). The relationship between C4 flux and precipitation can be described as the linear function (Fig 3D). For the clade B, the relationship between log enzyme activity and log precipitation also can be described as the linear function (Fig 3 I,J,K). But the linear relation between C4 flux and precipitation in clade B is weaker(Fig 3L). The linear relation between log enzyme activity and log temperature is weaker than that between Log enzyme activity and Log precipitation, whether in clade A or clade B (Fig 3E,F,M,N,G,H,O,P).

4, The change of temperature and precipitation between C3 and C2, C2 and initial C4, initial C4 and developed C4.

Previous study has reported that C3-C4 species can represent the transition between C3 and C4 photosynthesis(Heckmann et al. 2013), and *F. ram* has the enhanced C4 cycle flux, increased C4 cycle related enzyme activity and rised gene expression (Adachi et al. 2023; Mallmann et al. 2014; Vogan and Sage 2011). According to the phylogenetic tree, *F. ang* was closer to *F.ram* as compared with *F.son*, therefore, among all Flaveria species, *F.son* most likely to represent the initial appearance of C2 photosynthesis in Flaveria; *F. ram* and *F. ano* most likely to represent the initial appearance of C4 photosynthesis in clade A and clade B, respectively; *F. Pal* and *F. Bro* most likely to represent the initial appearance of the fully functional C4 cycle for clade A and clade B. The comparison of *F. rob* and *F. son* suggested that the first decreased precipitation along the evolutionary way towards C4 photosynthesis concurrently occurred with the increased mean temperature (Fig 4A,F). Precipitation of *F.ano* and *F.ram* is lower than that of ang, and Temperature of ram and ano is higher than that of *F. ang*(Fig 4B,G,C,H). Precipitation of pal is lower than that of *F. ram*, and precipitation of *F. bro* is lower than that of *F. flo* (Fig 4D,I,E,J). However, temperature of *F.Pal* and *F. Bro* showed the inconsistent change, *F. Pal* is same as *F. ram*, and *F. bro* is lower than *F. flo* (Fig 4D,I,E,J). Interestingly, compared with their front species, we observed that the decreased precipitation for the species habitat (F. son, F.ram, F.flo, F.pal and F.bro) concurrently appeared with the decreased stomatal density, decreased inhibition of O2 and CO2 compensation point, decreased maximum stomatal conductance (Supplementary Fig 4E,F,G,H). Although precipitation of *F. flo* and *F. lin* were increased compared to other species with C2 photosynthesis (Fig 3b), they also have the decreased stomatal density, maximum stomatal conductance and increased stomatal size, which might attribute to their salty habitat.

5, The association between precipitation, stomata and CO_2 compensation point.

Previous work found there is a difference in stomata in Flaveria genus(Zhao et al. 2022). Since stomata have long been thought to be associated with water content, we checked the relationship between stomata and precipitation in Flaveria genus. We found there was a positive and linear relationship between the stomata, CO2 compensation point and precipitation during the transition between proto-kranz and C4-like in Flaveria clade A and clade B(Fig 5A,B,C,D,E,F). The linear relationship between stomata, CO2 compensation point and precipitation in Flaveria clade B was weaker than that in clade A (Fig 5A,B,C,F,G,H). The relation between temperature and stomata (or temperature and CO2 compensation point) was not apparent as the relation between these two parameters and precipitation(Fig 5D,I,E,J).

Discussion

Evolutionary conditions along the path towards C4 photosynthesis.

The heat and drought have long been thought to be associated with the evolution of C4 photosynthesis(Sage et al. 2018; Taylor et al. 2012). Discovering the environmental conditions for the habitats of C4 and C3-C4 species could contribute to explain the drivers for C4 evolution(Sage et al. 2018; Sage et al. 2012; Edwards and Smith 2010). In this study, we examined the data for precipitation and temperature of the diverse Flaveria species. The precipitation and temperature of different time-scale was shown (Fig 2a, b, supplementary fig 1, 2, 4). We noticed that the trajectory trend in the wettest quarter mostly coincides with the warmest quarter(Fig 2, supplementary fig 2) and mean temperature of wettest quarter was overall higher than mean temperature of driest quarter (Supplementary fig 2D, Supplementary fig 1E), suggesting that the habitat of Flaveria is raining and hot during the same period. Sufficient water content is necessary to maintain high photosynthetic activity for plants, and the description from previous work indicated that Flaveria species perform higher photosynthesis during summer (Powell 1978). Therefore, this period should approximately be the summer in Mexico, and Flaveria have higher photosynthetic activity during this time as compared with the rest of time (Powell 1978) (Fig2a,b,d,e, supplementary fig 1). In the perspective of long-term, higher photosynthetic activity indicating higher photorespiratory flux in C3 plants (Previous work has proved that there is no significant difference in the Sc/o between different Flaveria species(Kubien et al. 2008), and Ci between the C3 and type I species has no significant difference(Sage et al. 2013)), thus C3 and initial intermediate Flaveria species in this period would have the potential of high photorespiratory flux which has long been thought to be the driver force for the origin of C4 plants(Sage et al. 2012; Sage et al. 2018). Due to high photosynthetic activity and high photorespiratory flux, this period for Flaveria is valuable to explain the driving of C4 evolution (Fig 2a,b,c,d,e,f).

The evolutionary selective pressure on photosynthesis should act on the period that photosynthesis function during a year(Sage et al. 2018), therefore the driest and coldest season should not be considered (Supplementary fig 1a,b,c,d,e,f). Since Flaveria genus is the leading model for C4 evolutionary study, and the habitat ecology for C4 species and the intermediate species that branch the lineage of C3 and C4 species could reflect the environmental conditions drive the evolution of C4 photosynthesis, three type of rainy and warm periods suggested that the conditions promote C4 evolution step by step and in order. Specifically, the initial stage during C4 evolution need rising precipitation and temperature, and later stage need decreased

221 precipitation (Fig 2a,b,c,d,e,f, supplementary fig 2,a,b). Heat previously is considered to be the
222 factor that promote the origin of C4 photosynthesis(Sage et al. 2018), however, our study
223 proposed that, in addition to increasing temperature, precipitation increasing is a vital factor to
224 launch the evolution of C4 photosynthesis (Fig 2a,b,c,d,e,f). Previous study proposed it is possible
225 that heat occurred with drought simultaneously to enhance photorespiration and then promote
226 the evolution of C4 photosynthesis(Sage et al. 2012), our study indicated that this process indeed
227 exist (Fig 2a,b,c,d,e,f), and specifically, this process is the appearance of C2 photosynthesis (son)
228 and partial C4 (F. ram, F. ano). Although the precipitation of the Type II species F. flo and F. lin was
229 high (Fig 2), they inhabit contain saline region (Fig 1, table 1), which even under high
230 precipitation may experience strong osmotic stress. The habitat of C4 species was various,
231 indicating that adaptation extent of C4 plants will be wider (Fig1, table1).
232
233 The role of water content and temperature
234 Some studies have proposed that drought is driver force for C4 evolution(Sage 2004; Zhou et al.
235 2018; Sage et al. 2018; Schluter and Weber 2016; Edwards and Smith 2010). Some studies
236 suggested that heat is the crucial environment to drive the C4 evolution(Sage et al. 2018; Sage et
237 al. 2012). In this study, our result proposed that drought play a more important role than heat
238 during C4 evolution. During the transition between C3 and C4 photosynthesis, precipitation
239 decline step by step, however, there is no clear trend for temperature (Fig 2). Whether the clade
240 A or Clade B, there showed a negative association between core C4 enzyme activity and
241 precipitation (Fig 3), and there is a strong negative relationship between precipitation and core
242 C4 enzyme activity in clade A (Fig3). However, the relationship isn't apparent for temperature (Fig
243 3). These suggested that, in terms of the whole evolutionary process, drought might be the main
244 driver for the transition from C3 to C4 photosynthesis rather than heat.
245 Initial step is key for C4 evolution(Sage et al. 2012). For this step, the increased vein density and
246 increased number of chloroplasts in bundle sheath is the important characters during the C4
247 evolution(Sage et al. 2012). The increased vein density has been deducted to be used to maintain
248 enough water content in the leaf and avoid the reduce of stomata aperture, since the
249 evaporation might be increased in this stage(Sage et al. 2012; Sage et al. 2018). In agreement
250 with this statement, our data showed that, when was at the stage of vein density increasing,
251 there was no change in stomatal development and temperature was indeed increased (Fig 2A,
252 supplementary fig 4). The increased temperature could increase vapor pression between leaf and
253 air (supplementary fig 5B), hence enhance the evaporation(Sage et al. 2018); while no change in
254 the stomatal development indicate that water shortage in leaf might not happen, since water
255 shortage could alter stomatal development(Franks et al. 2009; Taylor et al. 2012). Our data
256 showed that precipitation was increased in this stage, which matched with the increased vein
257 density, together ensure the enough water content in the leaf (Fig 2B). Due to the sufficient
258 supply of water, stomatal aperture can be maintained. Maintaining stomatal open could not only
259 maintain intercellular CO2 concentration, but also could help alleviate the increase of leaf
260 temperature through transpiration, which benefits for maintaining efficient function of
261 photosynthesis. The increase in vein density inevitably decreases proportion of mesophyll in a
262 leaf, decreasing the photosynthetic capacity of the leaf. In theory, this should create a strong
263 evolutionary pressure to select for the plants with increased photosynthetic capacity of BSC to
264 offset the loss of photosynthetic capacity. Indeed, the relative number and size of chloroplast of

265 vascular bundle sheath in F. rob increase(Sage et al. 2013). Besides, the light intensity of F. rob
266 habitat is higher than that of F. pri (supplementary fig 5A), which further enhances the
267 photosynthesis of bundle sheath. The size of vascular bundle sheath in F. rob decrease compared
268 with F. pri(Sage et al. 2013; Mckown and dengler 2007), the size of mesophyll cell decrease
269 compared with F. pri(Mckown and dengler 2007), and thickness of leaf become thin(Mckown and
270 dengler 2007), these all enhancing the intensity of the light that cast vascular bundle sheath,
271 rising the probability of the selection for bundle sheath with higher photosynthetic capacity.
272
273 Different evolutionary step promoted by different driver force
274 A mass of researches focused on the driver force of C4 evolution. For example, decreasing
275 atmosphere CO2 concentration has been thought to be associated to the driving force of C4
276 evolution(Christin et al. 2008; Concentrations and Paleogene). Drought, salt and heat is also
277 thought to be the conditions benefit for C4 evolution(Sage et al. 2018; Sage 2004; Bromham and
278 Bennett 2014) (Edwards and Still 2008; Edwards and Smith 2010; Pearcy and Ehleringer 2006;
279 Sage et al. 2012). However, most of these studies suggest that C4 evolution promoted by single
280 ecological condition in the entire evolutionary process. Since C4 photosynthesis is the complex
281 traits, we proposed that single and same ecological driver is difficult to achieve the C4
282 photosynthesis. Since Flaveria species is an excellent representative for the different step during
283 C4 evolution, in this work, from the results of the study on Flaveria genus, which supported that
284 the different step of the C4 evolution is driven by the different driver mechanism.
285 After the initial step of C4 evolution, the promotion of C2 photosynthesis is considered to be a
286 crucial step during C4 evolution (Sage et al. 2012; Schluter and Weber 2016). Even though, there
287 is a viewpoint that the way to C4 evolution could be turned into the way to C2 evolution
288 (Brautigam and Gowik 2016). Our data indicated increasing temperature and decreasing
289 precipitation simultaneously promote the emergence of C2 photosynthesis (Fig 3). The increase
290 in temperature and decrease in precipitation could largely enhance photorespiration, promoting
291 the selection of the transfer of GDC from mesophyll to bundle sheath (photorespiration CO2
292 pump), which could improve the fitness of plants. Previous work has proposed that drought
293 enhance photorespiration through close stomata(Sage et al. 2012). In this study, we provided the
294 evidence that stomata play a role in the process that decreased precipitation enhanced
295 photorespiration, supported by the relation between stomatal density and CO2 compensation
296 point (Fig 5). The photorespiratory CO2 pump could largely weaken the photorespiration(Sage et
297 al. 2018; Sage et al. 2012; Schluter and Weber 2016; Ku et al. 1991), which indicated by the
298 largely decrease in CO2 compensation point and O2 inhibition(Sage et al. 2013; Ku et al. 1991).
299 The gradual enhancement of C2 photosynthesis mainly associate to the decreasing precipitation
300 (Fig 2a,b,c,d,e,f, Fig 3). The gradual enhancement of C2 photosynthesis has long been considered
301 to cause the imbalance of nitrogen between mesophyll cell and bundle sheath cell, which induce
302 the partial C4 cycle(Mallmann et al. 2014). In case of the progress from the C2 photosynthesis to
303 partial C4 photosynthesis, precipitation decreases and temperature increases, just like the former
304 step (Fig 4 B,C,G,H). After the induce of partial C4 photosynthesis, the formation of fully
305 functional C4 photosynthesis was drived by inconsistent conditions for Clade A and Clade B.
306 Precipitation was significantly decreased both in clade A and clade B, but there is no difference in
307 temperature in clade A(Fig 4), even though, temperature was decreased in clade B(Fig 4). These
308 results suggested that precipitation was the driving force rather than temperature at this

transitional stage. Lacking of photosynthetic capacity and growing under high temperature, plants suffering the carbon limitation in the early phase during C4 evolution, suggesting the main driving force for C4 evolution at this phase is carbon limitation. After the preconditioning for C2 photosynthesis, plants suffering water limitation and water limitation lead to deceasing of carbon acquirement, which gradually established C2 photosynthesis and further partial C4 photosynthesis. Therefore, the main driving reason for C4 evolution at these phases is the water limitation.

Methods

1, Obtain the ecological data of Flaveria species.

First, the geographical distribution of Flaveria was acquired from Global Biodiversity Information Facility (GBIF) database using the rgibf software package. Using ArcMAP software, the elevation data of the respective locations and 19 Bioclimatic factors, including Mean Diurnal Range, annual mean Temperature, Isothermality, Temperature Seasonality, Max Temperature of Warmest Month, Temperature Annual Range, Mean Temperature of Wettest Quarter, Mean Temperature of Warmest Quarter, Annual Precipitation, Precipitation of Wettest Month, Precipitation of Warmest Quarter, Precipitation of Wettest Quarter, Precipitation of Coldest Quarter, Min Temperature of Coldest Month, Precipitation of Driest Quarter, Mean Temperature of Coldest Quarter, Precipitation of Driest Month, Precipitation Seasonality (Coefficient of Variation), Precipitation of Coldest Quarter, were acquired from the Worldclim database based on the geographical distribution information of the Flaveria. Specifically, downloading the data from the worldclim at first. Secondly, preparing the file for the information of sampling location. Thirdly, importing the layers file of climate factor. Fourthly, importing the information of sampling location. Fifth, generating the new layers. Lastly, exporting the file that including sample and the data of geographical factors. All bioclimatic variables are averages from 1970 to2000. The elevation data came from SRTM elevation data. All data from the worldclim database have a maximum spatial resolution of 30 arc-seconds. The water vaper pression and solar radiation were also extracted from the World clim database(Fick and Hijmans 2017) based on the geographical distribution information of Flaveria.

2,Obtain the geology, anatomical and physiological data of Flaveria species.

The anatomical and physiological data for Flaveria was acquired from previous research. The data of veinal data was from (Mckown and dengler 2007). The data of CO2 compensation point and O2 inhibition is from (Ku et al. 1991). The data related to stomata is from (Zhao et al. 2022). The C4 metabolic enzyme activity data is from (Adachi et al. 2023) (supplementary table S7). The data in the graph was digitized by Origin 2018. The growth habitat is aquired from(Powell 1978; Kocacinar et al. 2008).

3, Map the Flaveria species.

We mapped the location of Flaveria species using the software google earth Pro according the latitude and longtitude which is extracted from Global Biodiversity Information Facility (GBIF).

4, Statistical analysis, regression analysis and Graph drawing.

The graph was plotted and the regression was performed by using graphpad prism 8. The statistic test was performed by graphpad prism 8 and WPS office. The statistic test for the difference in precipitation or temperature between all Flaveria species was performed by

one-way ANOVA and Tukey's multiple test (supplementary file). The rest of other statistic test was performed through T-test. The precipitation of the wettest quarter and the C4 metabolic enzyme activity took the logarithm with 2 as the base, and then they were performed linear regression by graphpad prism 8. The precipitation of the wettest quarter and the C4 metabolic enzyme activity was directly performed linear regression using graphpad prism 8. The equations for the regression were showed in supplementary file. The type of the statistic test used in each analysis was stated in the supplementary file.

Table 1 The habitat of Flaveria species

Species (photosynthetic type)	Growth habitat	Geographic location
Flaveria pringlei (C3)	Rather arid and gypseous habitats, scattered localities, waste places, cultivated fields, roadsides	Southern Mexico
Flaveria cronquistii (C3)	Rocky limestone (possibly gypseous) areas	Southern Mexico
Flaveria robusta (C3)	Gypsum and slate, sandy clay, or open rocky slopes, weedy at roadsides	Western Mexico
Flaveria sonorensis (Type I)	Close to warm mineral springs	North-western Mexico
Flaveria angustifolia (Type I)	Sandy or loamy soils in open fields	Southern Mexico
Flaveria pubescens (Type I)	Scattered localities, gypsum soils	Central Mexico
Flaveria oppositifolia (Type I)	high gypsum plains or other areas with dilute gypsum, saline, or rarely loamy soil, usually in moist areas or in disturbed habitats at roadside	Central Mexico
Flaveria linearis (Type II)	Near the coast and on offshore islands	Western Florida, USA
Flaveria floridana (Type II)	Restricted to saline, white sand near the beaches of brackish marshes	Western Florida, USA
Flaveria ramosissima (Type II)	Sandy-clay bottoms, along dry washes, fields and roadsides, possibly in gypseous soils,	Southern Mexico
Flaveria anomala (Type II)	High gypsum plains, often in disturbed sites	Central Mexico
Flaveria palmeri (C4-like)	Gypsum flats and knolls, or clay-loam soils probably with dilute gypsum, bolsons near lagunas, distributed areas near roads and fields	Northern Mexico
Flaveria brownie (C4-like)	Saline, sandy, and marshy areas of the coastal flats and islands of the lower Texas Gulf Coast	North-eastern Mexico
Flaveria vaginata (C4-like)	Arid and gypseous soils, scrub vegetation, cultivated fields	South-central Mexico

Flaveria bidentis (C4)	A weed in moist places on waste ground, near rivers, streams, and barrancas, in fields, pastures, disturbed ground, near streets and sidewalks in towns, claygravel, or sand	Africa, Caribbean, South America, southern USA
Flaveria trinervia (C4)	a variety of habitats, usually saline, gypseous, and disturbed, usually near ephemeral or permanent water sources	Africa, Caribbean, Central/South America, Mexico, USA
Flaveria chloraefolia (Type I)	Permanent or ephemeral saline marshy soils, springs, streams, roadsides	North Mexico

The information of habitat of Flaveria species was from (Powell 1978; Kocacinar et al. 2008)

Figure legend

Fig 1 Distribution of the Flaveria species

A, The location of Flaveria species was mapped to the world map. The main habitat of Flaveria is Mexico which was displayed. B, The location of Flaveria species except C4 species was mapped to the world map. C,D, The latitude and longitude of Flaveria species. The latitude and longitude was acquired from the GBIF. Different colour indicate different photosynthetic type.

Fig 2 The environment conditions of the habitat for Flaveria species showed rhythmic change

A, mean temperature of warmest quarter of Flaveria species. B, precipitation of wettest quarter of Flaveria species. C,mean temperature of wettest quarter for F. rob and F. pri. D, precipitation of wettest quarter for F.rob and F.pri. E, the change in vein density in the C3 and intermediate species. Different colour indicates different photosynthetic types. Orange indicates C3, grey indicates intermediate species, green indicates C4-like, blue indicates C4. Black lines indicate median.

Fig 3 The association between precipitation (temperature) and C4 enzyme activity or C4 flux

The precipitation indicating precipitation of the wettest quarter. The temperature indicating mean temperature of the wettest quarter. (A-H, clade A). A, the relation between Log PEPC activity per chl and Log precipitation. B, the relation between Log PPDK activity per chl and Log precipitation. C, the relation between Log MDH activity per chl and Log precipitation.D, the relation between C4 flux and precipitation. E, the relation between Log PEPC activity per chl and Log temperature. F, the relation between Log PPDK activity per chl and Log temperature. G, the relation between Log MDH activity per chl and Log temperature. H, the relation between C4 flux and log temperature. (I-P, clade B). I, the relation between Log PEPC activity per chl and log precipitation. J, the relation between Log PPDK activity per chl and log precipitation. The change trend of precipitation of wettest quarter. K, the relation between Log MDH activity per chl and log precipitation. L, the relation between C4 flux and precipitation. M, the relation between log PEPC activity per chl and log temperature. N, the relation between log PPDK activity per chl and log temperature. O, the relation between log MDH activity per chl and log temperature. P, the relation between C4 flux and temperature. (a), mean temperature of wettest quarter (b), O2 inhibition (c), CO2 compensation point (d), vein density (e), distance between vein (f), Maximum stomatal conductance (gsmax) (g) and stomatal density (SD) (h) from C3 to C4 photosynthesis in

Flaveria genus. Different colour indicates different photosynthetic types. Orange indicates C3, purple indicates Type I, yellow indicates Type II, green indicates C4-like, blue indicates C4.

Fig 4 Precipitation and temperature during the transition from proto-kranz to C4-like

A, precipitation of the wettest quarter during the transition from proto-kranz(F.rob) to C2(F.son). B, precipitation of the wettest quarter during the transition from C2 (F.son) to partial C4 (F.ano) in clade B. C, Precipitation of the wettest quarter during the transition from proto-kranz(F.son) to partial C4 in clade A. D, Precipitation of the wettest quarter during the transition from partial C4 to fully functional C4 photosynthesis. E, Precipitation of the wettest quarter during the transition from partial C4 to fully functional C4 photosynthesis. F, Mean temperature of the wettest quarter during the transition from proto-kranz (F.rob) to C2(F.son). G, Mean temperature of the wettest quarter during the transition from C2 (F.son) to partial C4 (F.ano) in clade B. H, Mean temperature of the wettest quarter during the transition from C2(F.son) to partial C4 (F.ram) in clade A. I, Mean temperature of the wettest quarter during the transition from partial C4 to fully functional C4 photosynthesis (F.pal) in clade A. J, Mean temperature of the wettest quarter during the transition from partial C4 (F.ano) to fully functional C4 photosynthesis (F.bro) in clade B. The C4 evolution was launched by increased temperature and increased precipitation. The latter of C4 evolution was promote by increased temperature and decreased precipitation.

Fig 5 The association between stomatal density, CO2 compensation point, precipitation and temperature

(A-E, clade A) A, The relation between stomatal density and precipitation. B, the relation between CO2 compensation point and precipitation. C, The relation between stomatal density and CO2 compensation point. D, The relation between stomatal density and temperature. E, the relation between CO2 compensation point and temperature. (F-J, clade B) F, The relation between stomatal density and precipitation. G, the relation between CO2 compensation point and precipitation. H, The relation between stomatal density and CO2 compensation point. I, the relation between stomatal density and temperature. J, the relation between CO2 compensation point and temperature.

Fig 6, Schematic diagram for the driver process of C4 evolution

The schematic diagram for the conditions driving the evolutionary process for C4 photosynthesis. The raindrop number denotes the precipitation. The thermometer denotes the temperature. The arrows denote the direction of C4 evolution. The cycle composed by white and black denotes the main driving element that promote the corresponding stage of C4 evolution. The driving reason in C3 and proto-kranz was mainly carbon limitation, and the driving reason in the latter stage was mainly water limitation. More and more black indicating the carbon limitation was increasing stepwise, and more and more white indicating the water limitation was increasing stepwise.

Supplementary fig 1, Temperature and precipitation of habitat for Flaveria species in driest and coldest period.

A, precipitation of driest quarter. B, precipitation of coldest quarter. C, precipitation of driest month. D, Mean temperature of driest quarter. E, Mean temperature of coldest quarter. F, Min temperature of coldest month. Orange indicates C3, purple indicates Type I, yellow indicates Type II, green indicates C4-like, blue indicates C4. Black lines indicate median.

Supplementary fig 2, Temperature and precipitation of habitat for Flaveria species in wettest and warmest period. Orange indicates C3, purple indicates Type I, yellow indicates Type II, green

indicates C4-like, blue indicates C4. Black lines indicate median.

Supplementary fig 3, annual temperature and precipitation of habitat for Flaveria species

a, Annual mean temperature. b, Annual precipitation. Orange indicates C3, purple indicates Type I, yellow indicates Type II, green indicates C4-like, blue indicates C4. Black lines indicate median.

Supplementary fig 4, The change trend for the evolution towards C4 photosynthesis.

A, Precipitation of wettest quarter. B, Mean temperature of wettest quarter. C, Vein density. D, Distance between vein. E, O2 inhibition. F, CO2 compensation point. G, Gs_{max}. H, stomatal density (SD).

Supplementary fig 5, The difference in solar radiation and water vaper pression between F. pri and F. rob.

A, The difference in solar radiation between F.pri and F.rob. B, The difference in water vaper pression between F.pri and F.rob.

Table 1 The habitat for Flaveria species

Supplementary file The statistics test and regression analysis

Adachi S, Stata M, Martin DG, Cheng S, Liu H, Zhu X-G, Sage RF (2023) The Evolution of C4 Photosynthesis in Flaveria (Asteraceae): Insights from the Flaveria linearis Complex. Plant physiology 191 (1):233-251. doi:10.1093/plphys/kiac467

Akyildiz M, Gowik U, Engelmann S, Koczor M, Streubel M, Westhoff P (2007) Evolution and function of a cis-regulatory module for mesophyll-specific gene expression in the C_4 dicot Flaveria trinervia. Plant Cell 19 (11):3391-3402. doi:10.1105/tpc.107.053322

Brautigam A, Gowik U (2016) Photorespiration connects C3 and C4 photosynthesis. J Exp Bot 67 (10):2953-2962. doi:10.1093/jxb/erw056

Bromham L, Bennett TH (2014) Salt tolerance evolves more frequently in C4 grass lineages. Journal of Evolutionary Biology 27 (3):653-659. doi:10.1111/jeb.12320

Christin PA, Besnard G, Samaritani E, Duvall MR, Hodkinson TR, Savolainen V, Salamin N (2008) Oligocene CO2 decline promoted C4 photosynthesis in grasses. Curr Biol 18 (1):37-43. doi:10.1016/j.cub.2007.11.058

Christin PA, Osborne CP, Sage RF, Arakaki M, Edwards EJ (2011) C(4) eudicots are not younger than C(4) monocots. J Exp Bot 62 (9):3171-3181. doi:10.1093/jxb/err041

Concentrations MDiACD, Paleogene Dt <science.1110063.pdf>.

Edwards EJ, Smith SA (2010) Phylogenetic analyses reveal the shady history of C4 grasses. Proc Natl Acad Sci U S A 107 (6):2532-2537. doi:10.1073/pnas.0909672107

Edwards EJ, Still CJ (2008) Climate, phylogeny and the ecological distribution of C4 grasses. Ecol Lett 11 (3):266-276. doi:10.1111/j.1461-0248.2007.01144.x

Edwards Ge, KU Msb (1987) Biochemistry of C3-C4 Intermediates. The Biochemistry of Plants, 10

Fick SE, Hijmans RJ (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37 (12):4302-4315. doi:10.1002/joc.5086

Franks PJ, Drake PL, Beerling DJ (2009) Plasticity in maximum stomatal conductance constrained by negative correlation between stomatal size and density: an analysis using Eucalyptus globulus. Plant,

cell & environment 32 (12):1737-1748. doi:10.1111/j.1365-3040.2009.002031.x

Hattersley RHBaPW (1989) Leaf Anatomy of C_3-C_4 Species as Related to Evolution of C_4 Photosynthesis.pdf. Plant physiology 91:1543-1550

Heckmann D, Schulze S, Denton A, Gowik U, Westhoff P, Weber AP, Lercher MJ (2013) Predicting C4 photosynthesis evolution: modular, individually adaptive steps on a Mount Fuji fitness landscape. Cell 153 (7):1579-1588. doi:10.1016/j.cell.2013.04.058

Keerberg O, Parnik T, Ivanova H, Bassuner B, Bauwe H (2014) C_2 photosynthesis generates about 3-fold elevated leaf CO_2 levels in the C_3-C_4 intermediate species Flaveria pubescens. J Exp Bot 65 (13):3649-3656. doi:10.1093/jxb/eru239

Kocacinar F, McKown AD, Sage TL, Sage RF (2008) Photosynthetic pathway influences xylem structure and function in Flaveria (Asteraceae). Plant, cell & environment 31 (10):1363-1376. doi:10.1111/j.1365-3040.2008.01847.x

Ku MS, Wu J, Dai Z, Scott RA, Chu C, Edwards GE (1991) Photosynthetic and photorespiratory characteristics of flaveria species. Plant physiology 96 (2):518-528. doi:10.1104/pp.96.2.518

Kubien DS, Whitney SM, Moore PV, Jesson LK (2008) The biochemistry of Rubisco in Flaveria. Journal of Experimental Botany 59 (7):1767-1777. doi:10.1093/jxb/erm283

Kumpers BM, Burgess SJ, Reyna-Llorens I, Smith-Unna R, Boursnell C, Hibberd JM (2017) Shared characteristics underpinning C4 leaf maturation derived from analysis of multiple C3 and C4 species of Flaveria. J Exp Bot 68 (2):177-189. doi:10.1093/jxb/erw488

Mallmann J, Heckmann D, Brautigam A, Lercher MJ, Weber AP, Westhoff P, Gowik U (2014) The role of photorespiration during the evolution of C_4 photosynthesis in the genus Flaveria. Elife 3:e02478. doi:10.7554/eLife.02478

Marshall DM, Muhaidat R, Brown NJ, Liu Z, Stanley S, Griffiths H, Sage RF, Hibberd JM (2007) Cleome, a genus closely related to Arabidopsis, contains species spanning a developmental progression from C(3) to C(4) photosynthesis. Plant J 51 (5):886-896. doi:10.1111/j.1365-313X.2007.03188.x

Mckown A, dengler N (2007) Key innovations in the evolution of Kranz anatomy and C4 vein pattern in Flaveria (Asteraceae). American Journal of Botany 94:382–399

Monson, Willlam S. Schuster, Ku MSB (1987) Photosynthesis in Flaveria brownil A.M. Powell. Plant physiology 85:1063-1067

Monson RK (2003) Gene duplication, neofunctionalization, and the evolution ofC4 photosynthesis. Int J Plant Sci

Ogren DBJaWL (1984) The CO2_O2 specificity of ribulose 1,5-bisphosphate carboxylase oxygenase_ dependence on ribulosebisphosphate concentration, pH and temperature. planta 161:308-313

Pearcy RW, Ehleringer J (2006) Comparative ecophysiology of C3 and C4 plants. Plant, cell & environment 7 (1):1-13. doi:10.1111/j.1365-3040.1984.tb01194.x

Powell AM (1978) Systematics of Flaveria (Flaveriinae--Asteraceae). Annals of the Missouri Botanical Garden 65:590-636

Sage RF (2004) The evolution of C_4 photosynthesis. New Phytologist 161:341-270. doi:10.1046/j.1469-8137.2004.00974.x

Sage RF (2017) A portrait of the C4 photosynthetic family on the 50th anniversary of its discovery: species number, evolutionary lineages, and Hall of Fame. J Exp Bot 68 (2):4039-4056. doi:10.1093/jxb/erx005

Sage RF, Khoshravesh R, Sage TL (2014) From proto-Kranz to C4 Kranz: building the bridge to C4 photosynthesis. J Exp Bot 65 (13):3341-3356. doi:10.1093/jxb/eru180

Sage RF, Monson RK, Ehleringer JR, Adachi S, Pearcy RW (2018) Some like it hot: the physiological ecology of C4 plant evolution. Oecologia 187 (4):941-966. doi:10.1007/s00442-018-4191-6

Sage RF, Sage TL, Kocacinar F (2012) Photorespiration and the evolution of C_4 photosynthesis. Annu Rev Plant Biol 63:19-47. doi:10.1146/annurev-arplant-042811-105511

Sage TL, Busch FA, Johnson DC, Friesen PC, Stinson CR, Stata M, Sultmanis S, Rahman BA, Rawsthorne S, Sage RF (2013) Initial events during the evolution of C4 photosynthesis in C3 species of Flaveria. Plant physiology 163 (3):1266-1276. doi:10.1104/pp.113.221119

Schluter U, Weber AP (2016) The Road to C4 Photosynthesis: Evolution of a Complex Trait via Intermediary States. Plant Cell Physiol 57 (5):881-889. doi:10.1093/pcp/pcw009

Schulze S, Mallmann J, Burscheidt J, Koczor M, Streubel M, Bauwe H, Gowik U, Westhoff P (2013) Evolution of C4 photosynthesis in the genus flaveria: establishment of a photorespiratory CO2 pump. Plant Cell 25 (7):2522-2535. doi:10.1105/tpc.113.114520

Sedelnikova OV, Hughes TE, Langdale JA (2018) Understanding the Genetic Basis of C_4 Kranz Anatomy with a View to Engineering C_3 Crops. Annu Rev Genet 52:249-270. doi:10.1146/annurev-genet-120417-031217

Stata M, Sage TL, Sage RF (2019) Mind the gap: the evolutionary engagement of the C4 metabolic cycle in support of net carbon assimilation. Curr Opin Plant Biol 49:27-34. doi:10.1016/j.pbi.2019.04.008

Still CJ, Berry JA, Collatz GJ, DeFries RS (2003) Global distribution of C3and C4vegetation: Carbon cycle implications. Global Biogeochemical Cycles 17 (1):6-1-6-14. doi:10.1029/2001gb001807

Taniguchi YY, Gowik U, Kinoshita Y, Kishizaki R, Ono N, Yokota A, Westhoff P, Munekage YN (2021) Dynamic changes of genome sizes and gradual gain of cell-specific distribution of C4 enzymes during C4 evolution in genus Flaveria. Plant Genome:e20095. doi:10.1002/tpg2.20095

Taylor SH, Franks PJ, Hulme SP, Spriggs E, Christin PA, Edwards EJ, Woodward FI, Osborne CP (2012) Photosynthetic pathway and ecological adaptation explain stomatal trait diversity amongst grasses. New Phytol 193 (2):387-396. doi:10.1111/j.1469-8137.2011.03935.x

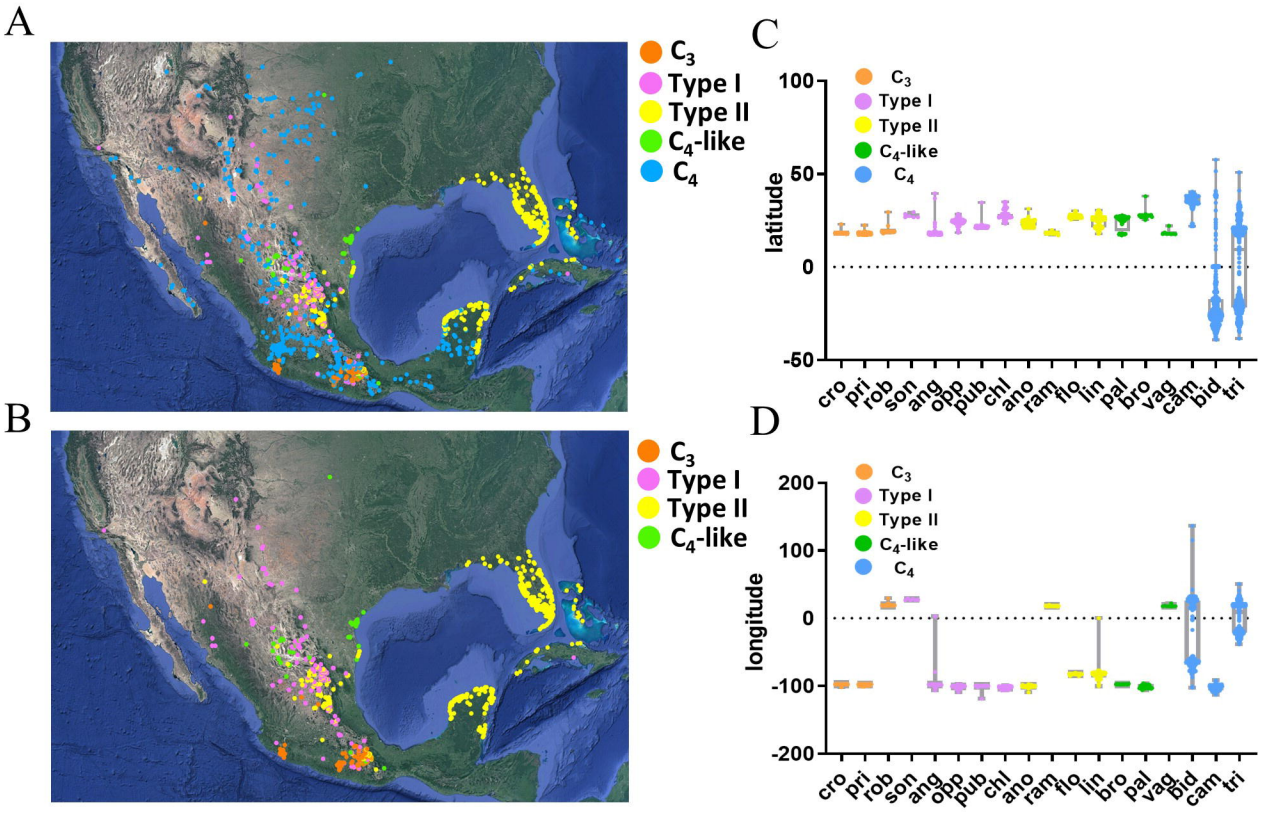
Taylor SH, Hulme SP, Rees M, Ripley BS, Woodward FI, Osborne CP (2010) Ecophysiological traits in C_3 and C_4 grasses: a phylogenetically controlled screening experiment. New Phytol 185 (3):780-791. doi:10.1111/j.1469-8137.2009.03102.x

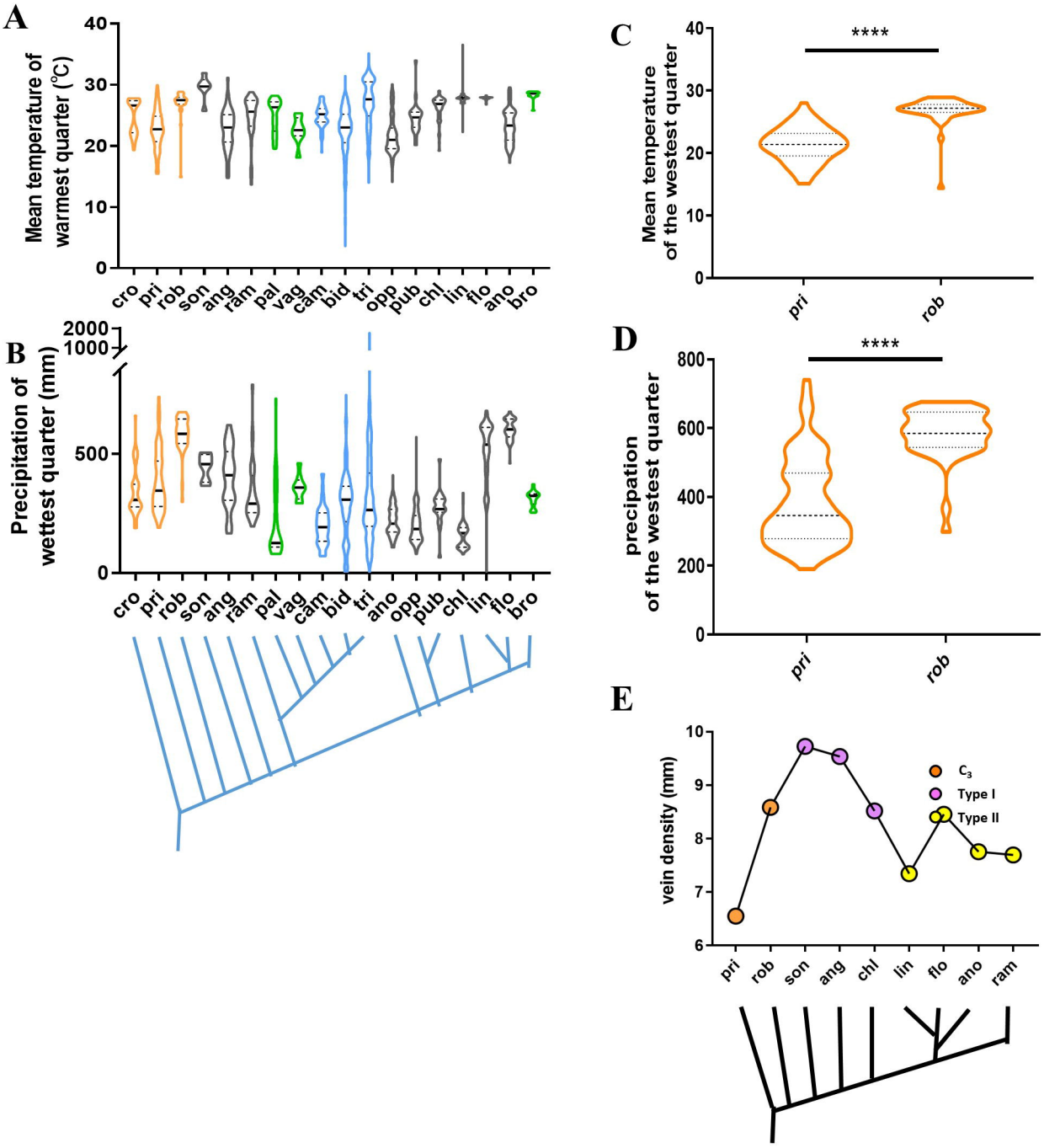
Vogan PJ, Sage RF (2011) Water-use efficiency and nitrogen-use efficiency of C_3-C_4 intermediate species of Flaveria Juss. (Asteraceae). Plant, cell & environment 34 (9):1415-1430. doi:10.1111/j.1365-3040.2011.02340.x

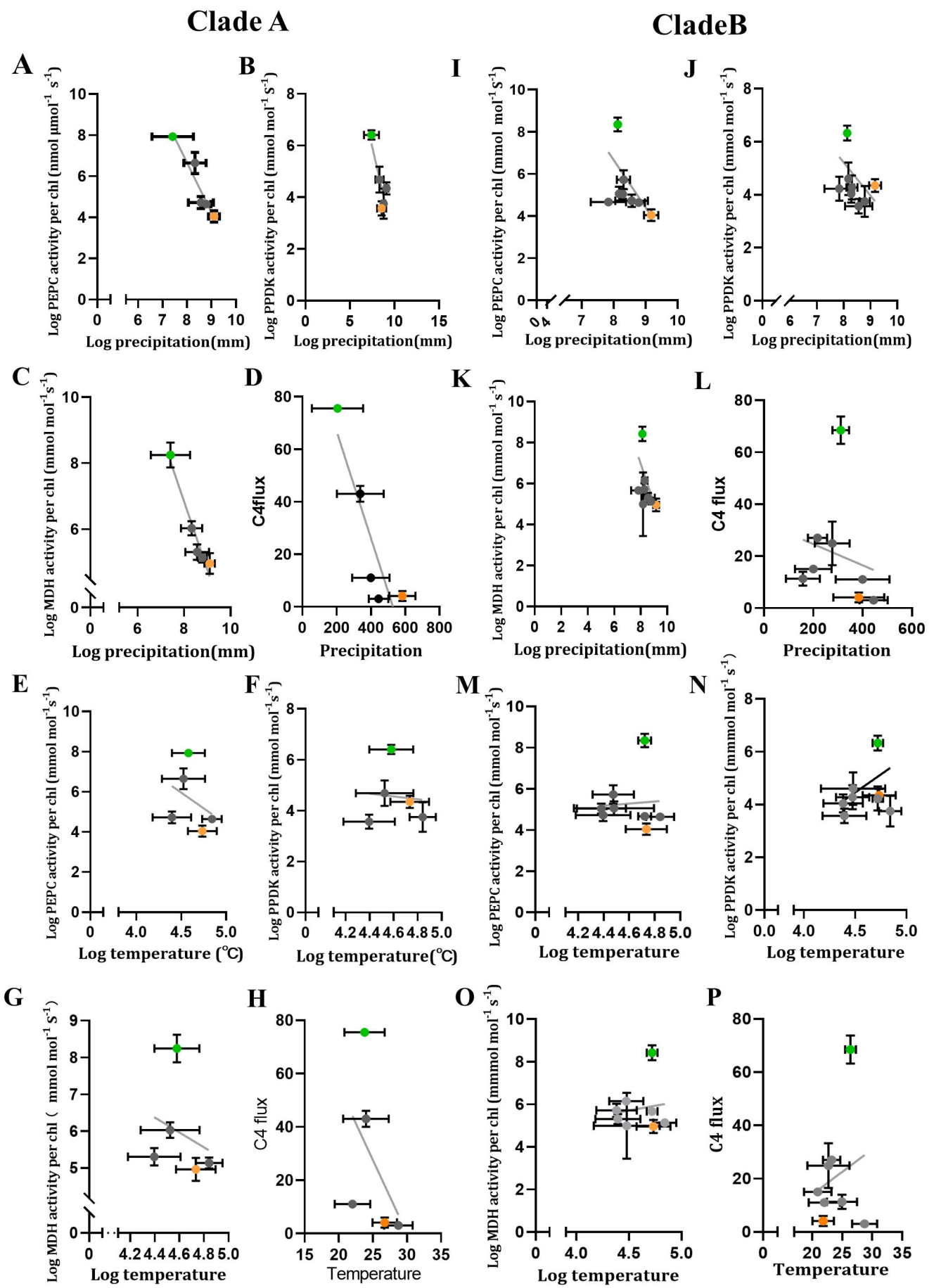
Williams BP, Johnston IG, Covshoff S, Hibberd JM (2013) Phenotypic landscape inference reveals multiple evolutionary paths to C_4 photosynthesis. Elife 2:e00961. doi:10.7554/eLife.00961

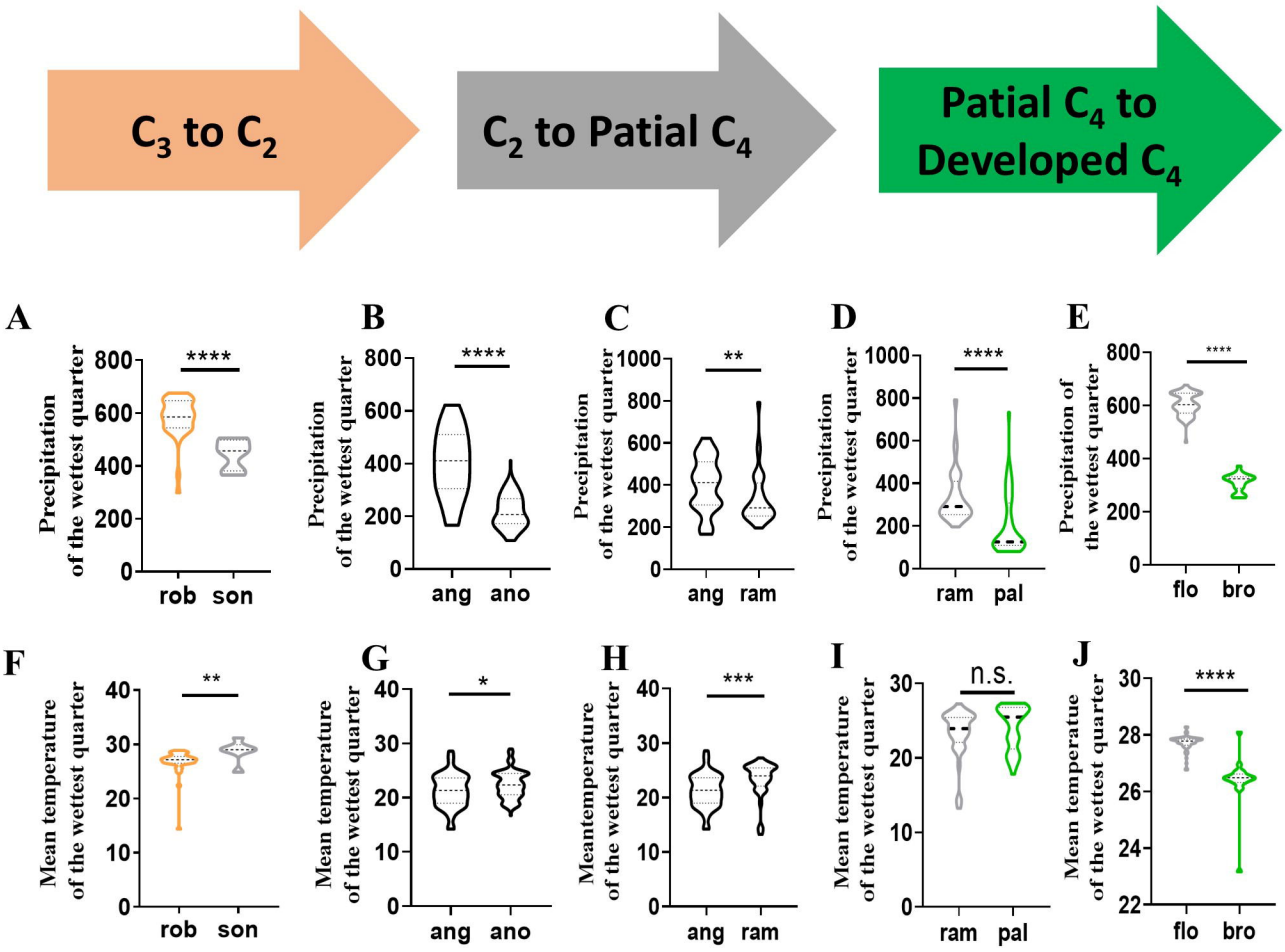
Zhao YY, Lyu MA, Miao F, Chen G, Zhu XG (2022) The evolution of stomatal traits along the trajectory toward C4 photosynthesis. Plant physiology 190 (1):441-458. doi:10.1093/plphys/kiac252

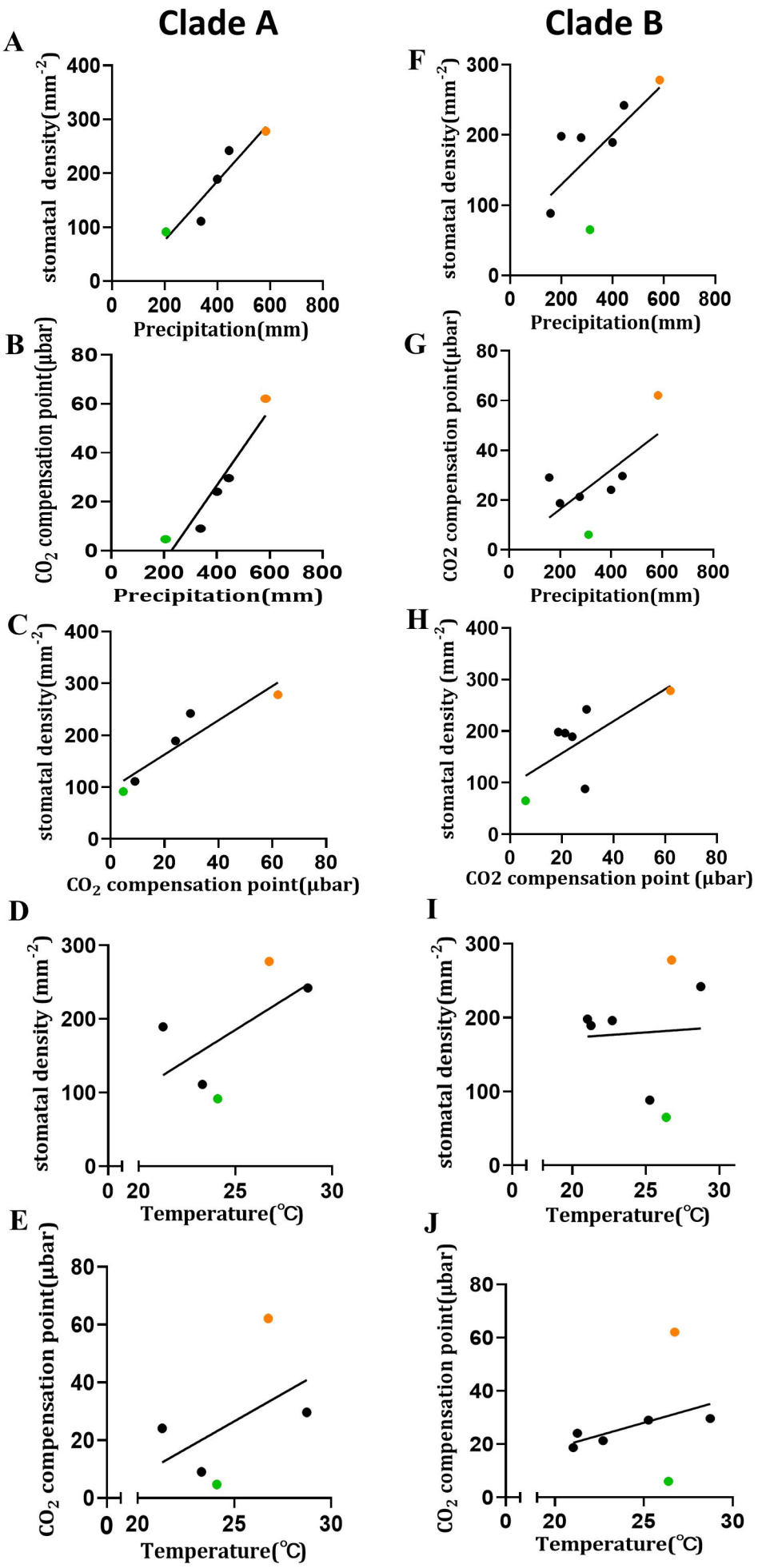
Zhou H, Helliker BR, Huber M, Dicks A, Akcay E (2018) C_4 photosynthesis and climate through the lens of optimality. Proc Natl Acad Sci U S A 115 (47):12057-12062. doi:10.1073/pnas.1718988115

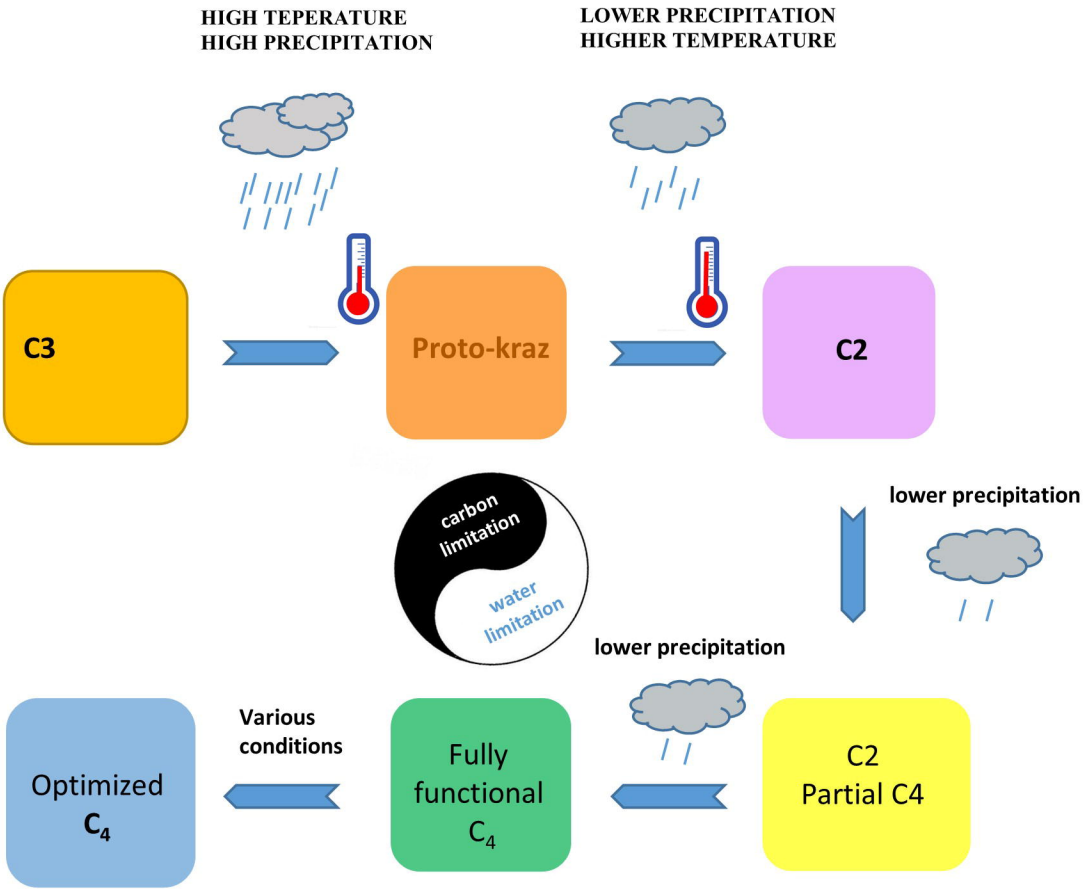

A
B
C
D
C3
Type I
Type II
C4-like
C4
latitude
longitude
100
50
0
-50
200
-100
-200
cro
pri
rob
son
ang
opp
pub
chl
ano
ram
flo
lin
pal
bro
vag
cam
bid
tri

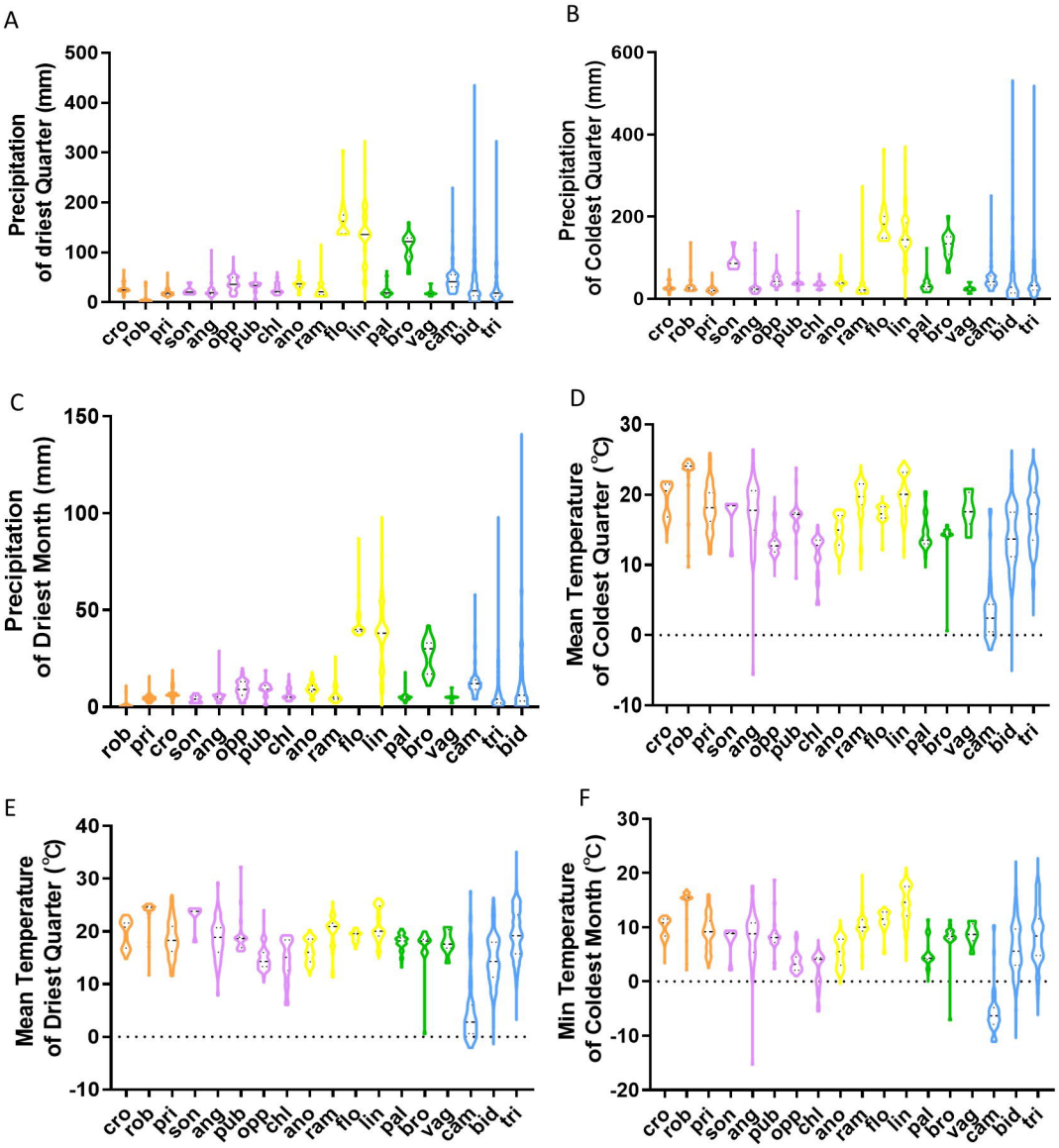

A
Mean temperature of warmest quarter (°C)
40
30
20
10
0
cro pri rob son ang ram pal vag cam bid tri opp pub chl lin flo ano bro
B
Precipitation of wettest quarter (mm)
2000
1000
500
0
cro pri rob son ang ram pal vag cam bid tri ano opp pub chl lin flo bro
C
Mean temperature of the westest quarter
****
40
30
20
10
0
pri
rob
D
precipation of the westest quarter
****
800
600
400
200
0
pri
rob
E
vein density (mm)
10
9
8
7
6
C3
Type I
Type II
pri rob son ang chl lin flo ano ram


Clade A
CladeB
A
B
I
J
C
D
K
L
E
F
M
N
G
H
O
P
Log PEPC activity per chl (nmol μmol-1 s-1)
Log PPDK activity per chl (mmol mol-1 s-1)
Log MDH activity per chl (mmol mol-1 s-1)
C4flux
C4 flux
Log precipitation(mm)
Precipitation
Log temperature (℃)
Log temperature
Temperature

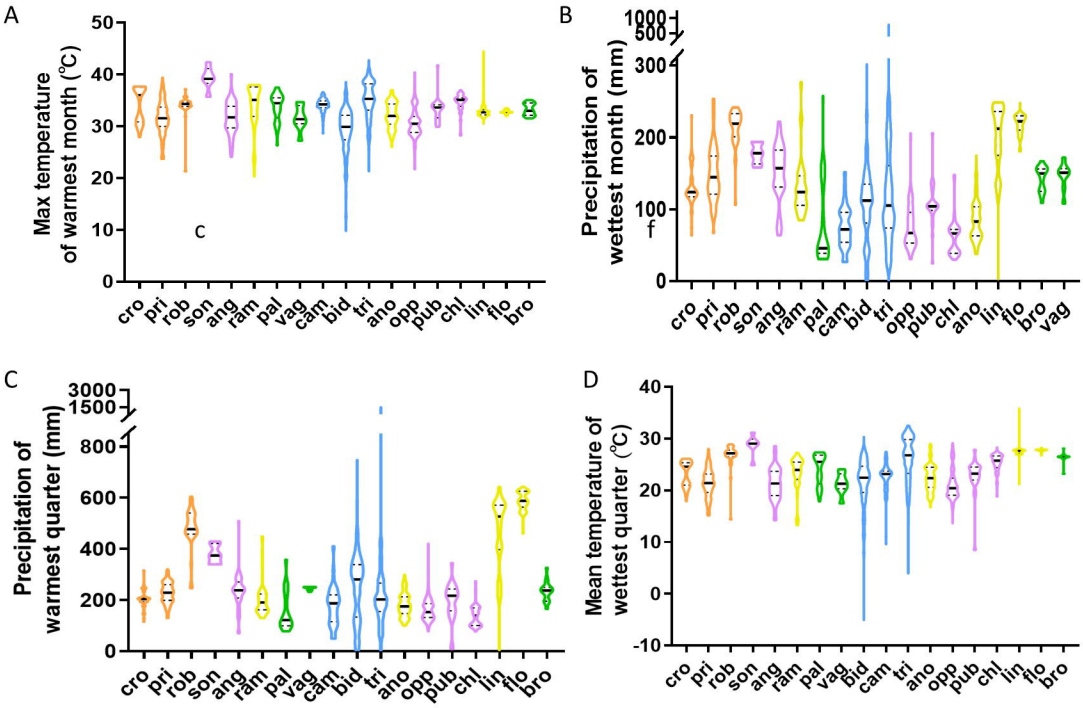

C3 to C2
C2 to Patial C4
Patial C4 to Developed C4
A
Precipitation of the wettest quarter
****
rob
son
B
Precipitation of the wettest quarter
****
ang
ano
C
Precipitation of the wettest quarter
**
ang
ram
D
Precipitation of the wettest quarter
****
ram
pal
E
Precipitation of the wettest quarter
****
flo
bro
F
Mean temperature of the wettest quarter
**
rob
son
G
Mean temperature of the wettest quarter
*
ang
ano
H
Meantemperature of the wettest quarter
***
ang
ram
I
Mean temperature of the wettest quarter
n.s.
ram
pal
J
Mean temperatue of the wettest quarter
****
flo
bro


Clade A
Clade B
A
stomatal density(mm-2)
Precipitation(mm)
F
stomatal density(mm-2)
Precipitation(mm)
B
CO2 compensation point(μbar)
Precipitation(mm)
G
CO2 compensation point(μbar)
Precipitation(mm)
C
stomatal density(mm-2)
CO2 compensation point(μbar)
H
stomatal density (mm-2)
CO2 compensation point (μbar)
D
stomatal density (mm-2)
Temperature(℃)
I
stomatal density(mm-2)
Temperature(℃)
E
CO2 compensation point(μbar)
Temperature(℃)
J
CO2 compensation point(μbar)
Temperature(℃)

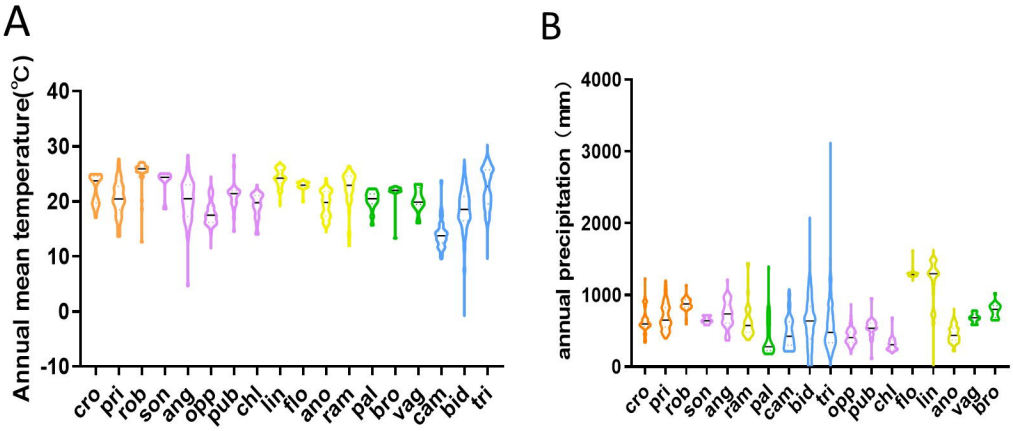

HIGH TEPERATURE
HIGH PRECIPITATION
LOWER PRECIPITATION
HIGHER TEMPERATURE
C3
Proto-kraz
C2
carbon limitation
water limitation
lower precipitation
lower precipitation
Various conditions
Optimized C_4
Fully functional C_4
C2
Partial C4



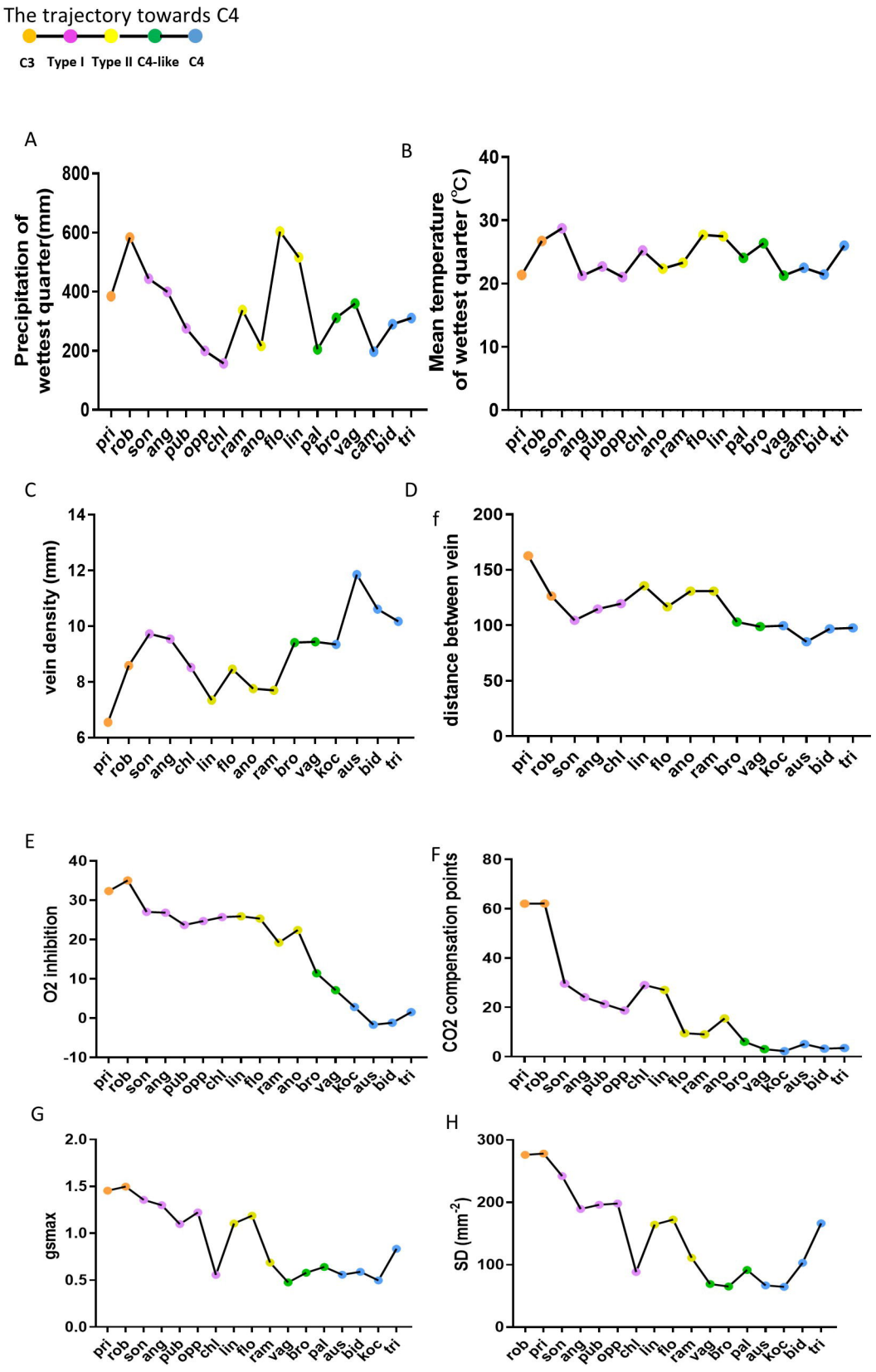
Supplementary Figure 1



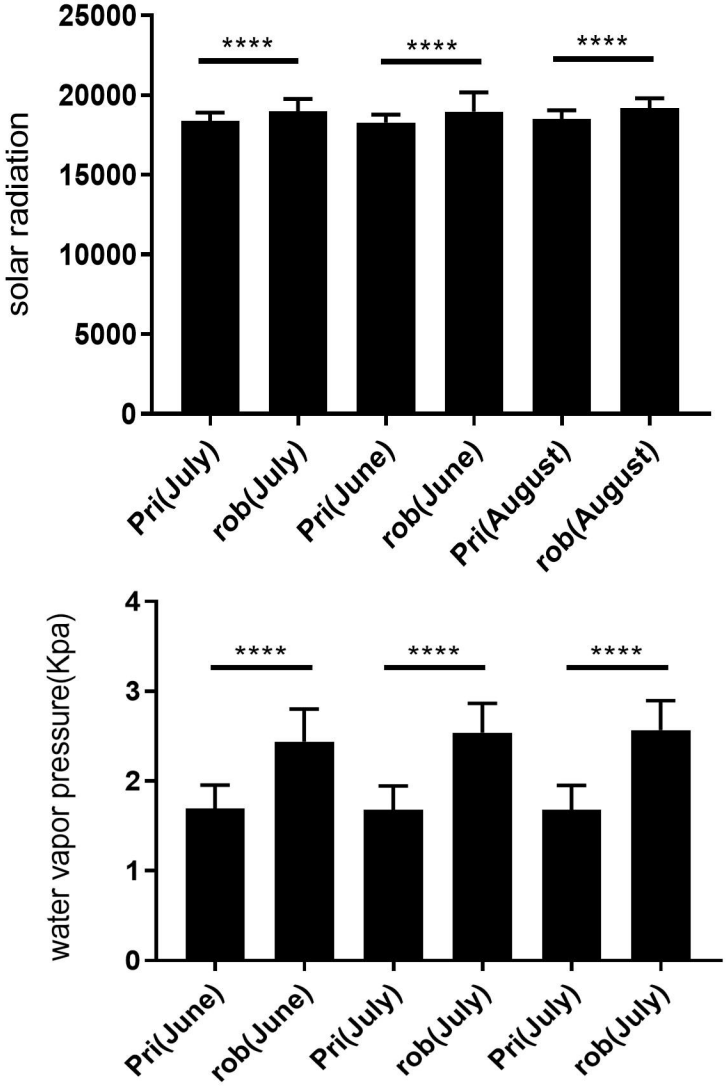
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5