

SYSTEMATICS OF PHOTOSYNTHETIC CARBON FIXATION PATHWAYS IN EUPHORBIA*

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Summary

Using the technique of carbon isotopic ratio determinations, 20 selected species of *Euphorbia* (s. lat.) have been investigated to determine their metabolic status. Previous work is reviewed, and as found by previous investigators, enriched ^{13}C values – indicating Crassulacean acid metabolism or C_4 photosynthesis – have been detected only in subgenera *Euphorbia* and *Chamaesyce*, respectively. All taxa investigated in subg. *Chamaesyce* except two species of subsect. *Acutae* show the Kranz syndrome and C_4 photosynthesis; the probable phylogenetic origin of the subgenus is therefore pinpointed within this subsection. Apparently taxa with Crassulacean acid metabolism and C_4 photosynthesis have arisen independently within the genus.

Within the past decade it has become apparent that a large number of plant species, especially in tropical to warm-temperate regions, have a distinctive kind of photosynthesis. Instead of elaborating phosphoglyceric acid as the “initial product” of the well-known Calvin-Benson cycle, such plants fix carbon initially into 4-carbon organic acids (oxaloacetic and then malic or aspartic acids); plants of this kind have been designated by Hatch and Slack (1970) as C_4 plants, in contrast to the more common C_3 plants. It has now been repeatedly demonstrated that C_4 metabolism is correlated with the presence of specialized chlorenchymatous leaf bundle sheaths, in which the chloroplasts show more or less highly modified granar and wall structure: these anatomical specializations have been referred to as the “Kranz syndrome”, and the species in which these features occur as “Kranz species” (Tregunna et al., 1970; Smith and Brown, 1973).

The Euphorbiaceae have been listed by a number of investigators as having Kranz species, in addition to species with Crassulacean acid metabolism (CAM). Within the Euphorbiaceae, Kranz leaf anatomy has been detected only within the single genus *Euphorbia* (sens. lat.) (Welkie and Caldwell, 1970); it now appears virtually certain that all other genera in the family are non-Kranz. A review of the literature indicates that the Kranz syndrome has been reported only in a single group of *Euphorbia*, the subgenus *Chamaesyce* (Moss et al., 1969). However, since previous studies of leaf anatomy and photosynthetic metabolism have not sampled a sufficient number of the *Euphorbia* species of critical taxonomic or phylogenetic position, we have investigated a number of additional species, mainly utilizing the method of determining $^{13}\text{C}/^{12}\text{C}$ ratios outlined by

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Smith and Brown (1973). As pointed out by Tregunna et al. (1970), a number of different criteria may be used in determinations of Kranz status; of these, three have been commonly used in investigations of *Euphorbia*: anatomical preparations of leaves; CO₂ compensation points; and ¹³C/¹²C ratios. In Table I we have listed our results, together with the determinations which have appeared in the previous literature.

Since *Euphorbia* is one of the very few angiosperm genera with both Kranz and CAM species, determination of metabolic adaptation cannot necessarily be made utilizing the ¹³C/¹²C ratio method alone. For example, Bender (1971, 1973) has reported ¹³C enrichment ($\delta^{13}\text{C}/\text{‰}$ of -15.3) in *Euphorbia tirucalli*, a stem-succulent in subgenus *Euphorbia*. However, as indicated by Gaucher (1898), Kranz anatomy does not occur in any of the succulent taxa of subg. *Euphorbia*; in particular, the non-Kranz anatomy of *E. tirucalli* has been described by Trumpke (1914). As shown by McWilliams (1970), the succulents in *Euphorbia* and related genera of the tribe Euphorbieae such as *Monadenium*, *Synadenium*, and *Pedilanthus*, have Crassulacean acid metabolism rather than C₄ photosynthesis.

Although phylogenetic relationships within the large genus *Euphorbia* are still rather poorly understood, morphological details and the basic chromosome number ($x = 10$) suggest an origin of the succulents in subg. *Euphorbia* from non-succulent woody representatives of subg. *Esula* (Webster, 1967). In contrast, the relationships of subg. *Chamaesyce* appear to lie with subg. *Agaloma*. Wheeler (1941) stated that *Chamaesyce* might be allied to either subg. *Euphorbia* or subg. *Agaloma*; however, recent cytological studies of Urbatsch et al. (1975) indicate a basic chromosome number of $x = 7$ in both *Agaloma* and *Chamaesyce* and support the views of Dressler (1957) and Webster (1967) that the origin of the *Chamaesyce* group is to be sought within subg. *Agaloma*.

Because of the likelihood that the Kranz species of subg. *Chamaesyce* have been derived from non-Kranz ancestors within subg. *Agaloma*, we have made a special attempt to investigate species which were indicated by Boissier (1862) as transitional or boundary-line in his classical monograph of *Euphorbia*. Species placed in subsect. *Pleiadeniae* of *Chamaesyce* by Boissier have been given particular scrutiny.

The results of our studies prove to be of considerable interest in assessing speculations about the phylogenetic origin and taxonomic circumscription of *Chamaesyce*. *Euphorbia macropus* (incl. *E. biformis*), although placed in subsect. *Pleiadeniae* of *Chamaesyce* by Boissier, has more recently been referred to subg. *Agaloma* by McVaugh (1961). Both *E. macropus* and the related *E. macropodoides* prove to be non-Kranz by the ¹³C/¹²C ratio test, thereby substantiating McVaugh's opinion. Another neotropical species, *E. peperomioides*, which was noted by Boissier as having the habit of *Chamaesyce*, is also non-Kranz, and should be referred to subg. *Agaloma*. The problematical *E. innocua* of the Texas coast, which was referred to subg. *Agaloma* as a possible progenitor of the *Chamaesyce* group by Webster (1967), likewise proves to be non-Kranz.

Within subg. *Chamaesyce*, the expected Kranz values indicating ¹³C enrichment were found by us for most of the species tested. However, the species in subsect. *Acutae* have provided a surprise: although *E. lata* is indeed a Kranz species with $\delta^{13}\text{C}/\text{‰}$ values of -11.8 and -13.0 (in two determinations), the two species *E. acuta* and *E. angusta* are non-Kranz, with $\delta^{13}\text{C}/\text{‰}$ values of -25.5 and -24.9. Boissier, Wheeler, and Correll and

Johnston (1970) all treat these three species as closely related, and no one has suggested that they do not belong together. The cytological studies of Urbatsch et al. (1975) show that in all three species $n = 14$ or 28. Johnston (personal communication) has pointed out that the stipules are more reduced in *E. acuta* and *E. angusta* than in *E. lata*; nevertheless, the three species seem clearly related to one another and not particularly close to any other species of subg. *Chamaesyce*. It is interesting that Welkie and Caldwell (1970) correctly listed *E. acuta* and *E. lata* as having non-Kranz and Kranz anatomy respectively, but they did not comment on the implications of this difference between the two closely related species.

As pointed out by Gaucher (1898), the chlorenchymatous leaf bundle sheaths of subg. *Chamaesyce* provide one of the best diagnostic features for the group. The unanticipated results of this study therefore somewhat undermine part of the arguments of Croizat and Degener (1936), Burch (1966, 1968), and Webster (1967) for recognition of *Chamaesyce* as a genus distinct from *Euphorbia*. Conservative taxonomists doubtlessly would object to defining *Chamaesyce* in such a manner as to split subsect. *Acutae* and thus place *E. acuta* and *E. angusta* in a different genus from *E. lata*.

On the other hand, other characters (abortion of main axis, opposite and inaequilateral leaves, prominent stipules) still remain to characterize *Chamaesyce* as a whole; and indeed, except within subsect. *Acutae* the chlorenchymatous leaf sheath is diagnostic. Many other instances are known in the angiosperms (e.g., *Aster/Erigeron*, *Pyrus/Malus*, *Astragalus/Oxytropis*) where genera are separated despite transitional species. Therefore, recognition of *Chamaesyce* as a distinct genus can still be defended on the basis of data presently available.

Of greater general interest than these taxonomic problems are the phylogenetic implications. We feel that the $^{13}\text{C}/^{12}\text{C}$ ratio tests have pinpointed the place of origin of *Chamaesyce* more definitely than could have been expected before. The close relationship between Kranz and non-Kranz species in the *E. acuta-E. lata* group is reminiscent of the situation in *Atriplex* elucidated by Bjorkman et al. (1971), and offers a similar possibility of testing the evolution of photosynthetic mechanisms by experimental interspecific crosses.

Finally, a negative result of some importance is that although C_4 and CAM species both occur in *Euphorbia*, the species involved are not at all closely related. The CAM species all appear to belong to subg. *Euphorbia*, and to have originated in the African tropics or subtropics; whereas the C_4 species of *Chamaesyce* evidently arose in subtropical/warm temperate areas in North America. Since then the two groups have spread to become sympatric in arid regions of Africa and India, where they now coexist by means of photosynthetic strategies which differ from those of C_3 plants but which have completely independent phylogenetic origins.

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TABLE I. Distribution of photosynthetic syndromes in *Euphorbia*

Taxon*	$\delta^{13}\text{C}/\text{‰}^{**}$	status***	references ^o
Subgenus ESULA			
Sect. BALSAMIS			
<i>E. balsamifera</i>		Na	12
<i>E. calyculata</i> (Hinton 13953) ^{oo}	-24.6	Nr	
<i>E. dendroides</i>		Na	14
Sect. LATHYRIS			
<i>E. lathyris</i>		Na	2, 14
Sect. MYRSINITEAE			
<i>E. biglandulosa</i>		Na	12
<i>E. broteri</i>		Na	5
Sect. ESULA			
<i>E. cyparissias</i>		Na	14
Sect. TITHYMALUS			
<i>E. helioscopia</i>		Na	14
<i>E. portulacoides</i> (Garcia 206)	-23.5	Nr	
Subgenus EUPHORBIA			
Sect. TREISIA			
<i>E. bubalina</i> (B 66.415)	-13.2	Cr	
Sect. EUPHORBIA			
<i>E. caducifolia</i>		Cm	11
<i>E. grandidens</i>		Cm	9
<i>E. ingens</i>		Nm	10
<i>E. nivulia</i> (67.525)	-15.7	Cr	
<i>E. submammillaris</i>		Cm	7
<i>E. trigona</i> (B 72.119)	-19.4	Cr	
<i>E. xylophylloides</i>		Cm	7
Sect. ELAEOPHORBIA			
<i>E. drupifera</i> (B 67.301)	-14.1	Cr	
Sect. GONIOSTEMA			
<i>E. didierioides</i> (B 70.130)	-26.3	Nr	
<i>E. milii</i> (s. lat.)		Nm	7, 9
Sect. APHYLLIS			
<i>E. tirucalli</i>		Cr	1
Subgenus POINSETTIA			
<i>E. cyathophora</i> (Wilbur and Webster 2539)	-25.8	Nr	
<i>E. dentata</i>		Nac	14, 8
<i>E. eriantha</i>		Na	14
<i>E. heterophylla</i>		Nac	14, 8
<i>E. pulcherrima</i> (Hallberg 1009)	-25.5	Nr	1

Taxon*	$\delta^{13}\text{C}^0/00^{2\text{‰}}$	status***	references ^o
Subgenus AGALOMA			
Sect. ZYGOPHILLIDIUM			
<i>E. henricksonii</i>		Na	13
<i>E. hexagona</i>		Nac	14, 8
Sect. CYTTAROSPERMUM			
<i>E. macropus</i> (Johnston 9041)	-28.9	Nr	
<i>E. macropodoides</i> (Beaman 3807)	-28.3	Nr	
Sect. TITHYMALOPSIS			
<i>E. corollata</i>		Nacr	14, 8, 1
<i>E. innocua</i> (Tharp and Brown 50-56)	-28.1	Nr	
Sect. PETALOMA			
<i>E. bicolor</i>		Na	14
<i>E. marginata</i>		Nac	14, 8
Sect. TRICHEROSTIGMA			
<i>E. misera</i>		Na	14
Sect. NUMMULARIOPSIS			
<i>E. peperomioides</i> (Smith and Klein 13802)	-25.6	Nar	
Subgenus CHAMAESYCE			
Sect. SCLEROPHYLLAE			
Subsect. GYMNADENIAE			
<i>E. forbesii</i>		Ka	3
<i>E. rockii</i>		Ka	3
Subsect. SCLEROPHYLLAE			
<i>E. articulata</i> (Wilbur 8239)	-12.9	Kr	
<i>E. mesembrianthemifolia</i>		Ka	14
Sect. CHAMAESYCE			
Subsect. ACUTAE			
<i>E. acuta</i> (Warnock 46186)	-25.5	Nar	14
<i>E. angusta</i> (Warnock and Krodel 46531)	-24.9	Nr	
<i>E. lata</i> (Johnston 8617)	-13.0	Kar	14
(Henderson 63-696)	-11.8		
Subsect. HYPERICIFOLIAE			
<i>E. hirta</i>		Kac	14, 4
<i>E. hypericifolia</i>		Kc	4
<i>E. nutans</i>		Ka	12
Subsect. CHAMAESYCEAE			
<i>E. carunculata</i> (Warnock and Asher 8109)	-13.3	Kr	
<i>E. drummondii</i>		Ka	2
<i>E. glyptosperma</i>		Kac	14, 8
<i>E. granulata</i>		Ka	12
<i>E. maculata</i>		Kace	14, 8, 6
<i>E. missurica</i>		Kac	14, 8
<i>E. peplis</i>		Ka	5, 12
<i>E. wheeleri</i>		Ka	2
Subsect. PLEIADENIAE			
<i>E. coecorum</i> (Heringer 7749)	-13.8	Kr	
<i>E. viscooides</i> (Pires et al. 9670)	-13.4	Kr	

(Legend to table)

* Circumscriptions and arrangement of the infrageneric taxa of *Euphorbia* follow the classifications of Boissier (1862) and Webster (1967). Species studied only from leaf anatomy have not been listed unless of particular interest; a detailed enumeration of anatomically studied species (especially in subg. *Esula*) is provided by Welkie and Caldwell (1970).

** Determinations according to the carbon isotope ratio method of Smith and Brown (1973).

*** Letter symbols indicate the following: C, CAM; K, Kranz (C₄); N, non-CAM and non-Kranz; a, determined by leaf *anatomy*; c, determined by CO₂ *compensation* point; e, determined by *electron* microscopy; m, determined by various *metabolic* studies, especially diurnal variations in organic acid concentration; r, determined by ¹³C/¹²C *ratio*. Ratio numbers are cited only for species examined in this study.

° Reference numbers indicate publications as follows: (1) Bender (1971); (2) Downton (1971); (3) Herbst (1971, 1972); (4) Hofstra et al. (1972); (5) Gaucher (1898); (6) Laetsch (1971); (7) McWilliams (1970); (8) Moss et al. (1969); (9) Nuernbergk (1961); (10) Schütte et al. (1967); (11) Sen et al. (1971); (12) Vindt (1960); (13) Webster (in Johnston, 1974); (14) Welkie and Caldwell (1970).

°° Voucher herbarium specimens for ¹³C/¹²C ratio determinations are deposited in the Botany Department herbaria at the University of Texas, Austin, and the University of California, Davis; voucher designations by number only (under subg. *Euphorbia*) indicate accession numbers of living plants in the Botany greenhouses at Davis