



BIBLIOTECA
CENTRO DE ECOLOGIA

**CAMBIOS MORFOLOGICOS EN EL
PROCESO EVOLUTIVO DE LA FAMILIA
CACTACEAE: PAPEL DEL PROCESO DE
DESARROLLO (heterocronía)**

T E S I S

QUE PARA OBTENER EL GRADO DE

DOCTOR EN ECOLOGIA

PRESENTA:

ALICE ALTESOR

000226255

MEXICO, D.F.

MAYO 1995



Universidad Nacional
Autónoma de México



UNAM – Dirección General de Bibliotecas
Tesis Digitales
Restricciones de uso

DERECHOS RESERVADOS ©
PROHIBIDA SU REPRODUCCIÓN TOTAL O PARCIAL

Todo el material contenido en esta tesis esta protegido por la Ley Federal del Derecho de Autor (LFDA) de los Estados Unidos Mexicanos (México).

El uso de imágenes, fragmentos de videos, y demás material que sea objeto de protección de los derechos de autor, será exclusivamente para fines educativos e informativos y deberá citar la fuente donde la obtuvo mencionando el autor o autores. Cualquier uso distinto como el lucro, reproducción, edición o modificación, será perseguido y sancionado por el respectivo titular de los Derechos de Autor.

ÍNDICE

Agradecimientos

Resumen

Abstract

I. Introducción

II. Changes in the photosynthetic metabolism during the early ontogeny of four cactus species.

III. Allometric neoteny and the evolution of succulence in cacti.

IV. Phyllotaxis and the evolution of stem succulence in cacti

V. Conclusiones

Agradecimientos

Debo agradecer a mi querida amiga Consuelo Bonfil, sin ella la presentación de esta tesis tal vez nunca se hubiera concretado. Su colaboración representó meses de entrevistas, cartas, mucha paciencia y una mayor dosis de humor para trasmitirme vía e-mail el estado de los trámites.

Al orientador de este trabajo, tutor, maestro, amigo y responsable de las ideas más locas, al Dr. Exequiel Ezcurra, le agradezco su apoyo y estímulo. A Carlos Silva, coautor de dos de los trabajos que integran esta tesis, quien trabajó con dedicación y creatividad. Al comité revisor integrado por Miguel Franco, Daniel Piñero, Alma Orozco, Ligia Collado, Jorge Meave y Héctor Hernández, les agradezco sus valiosos comentarios y correcciones.

Durante el desarrollo de este trabajo recibí el apoyo de muchos profesores y amigos. Quiero mencionar especialmente a Pedro Miramontes, que reúne ambos atributos. A Esthela Sandoval por sus enseñanzas y el apoyo brindado desde el laboratorio de anatomía del Jardín Botánico. A los miembros del laboratorio de Comunidades, quienes me ayudaron en el trabajo de campo, la obtención de las fotografías, el manejo de programas de cómputo, la discusión y confrontación de ideas y brindándome su compañerismo y amistad: Santiago Arizaga, Ernesto Vega, Alfonso Valiente, Fernando Ramírez de Arellano, Arturo Flores, Leopoldo Valiente, Eduardo Peters, Nina Hinke, Oscar Briones. A mi queridísima Cristina Mourelle, quien estuvo siempre para darme una mano. A Liz Izquierdo, otra amiga entrañable. A Carlos Palleiro, gran amigo y artista plástico que colaboró en el armado de las figuras del tercer trabajo.

A mis compañeros de generación América, Alberto, Horacio, Alejandro y Humberto. Al Dr. Emanuel Rincón y los miembros de su laboratorio, quienes me facilitaron el uso de su equipo fotográfico. Agradezco a todo el personal del Centro de Ecología, especialmente a Chelito, Elena, Carolina, Alicia y Gloria. Finalmente agradezco al Consejo Nacional de Ciencia y Tecnología (Conacyt) por haberme otorgado una beca y a PADEP por su apoyo económico.

RESUMEN

En el proceso evolutivo de las cactáceas ocurrieron tres eventos interrelacionados: (a) la reducción del tamaño de las hojas, hasta su pérdida total; (b) la formación de tejido suculento en la corteza y médula del tallo, y (c) el cambio del metabolismo fotosintético de C₃ a CAM.

Los tres artículos que conforman esta tesis comprenden estudios de procesos en la evolución de la succulencia, a nivel fisiológico, anatómico y morfológico. Se estudiaron comparativamente cinco especies de cactáceas que presentan características morfológicas contrastantes y pertenecen a las tres subfamilias: Pereskioideae, Opuntioideae y Cereoideae.

En el primer trabajo titulado "*Changes in the photosynthetic metabolism during the early ontogeny of four cactus species*" se cuantificó y analizó la fisiología fotosintética durante las primeras semanas de vida. Se detectó una "memoria" ontogenética del carácter ancestral (proceso conocido como recapitulación), ya que todas las especies analizadas presentaron metabolismo C₃ durante las primeras semanas y, excepto *Pereskia aculeata*, las especies derivadas cambiaron al patrón CAM, típico de las cactáceas, entre la décima y la vigésima semana.

En el segundo trabajo, "*Allometric neoteny and the evolution of succulence in cacti*", se hace un análisis comparativo de las características del leño durante el desarrollo en cinco especies. En las especies suculentas se observó que las características de los elementos de vaso (longitud y puntuaciones) se mantenían en el leño adulto iguales al leño juvenil. La comparación alométrica del porcentaje de xilema siguiendo una secuencia temporal de tamaños permitió detectar un retardo en el ritmo de desarrollo del leño en las especies suculentas. La heterocronía se identificó como neotenia alométrica, cuya consecuencia evolutiva es el mantenimiento de las características juveniles en el leño adulto. Sin embargo, la falta de crecimiento secundario genera nuevos problemas geométricos y mecánicos para los tallos, así como nuevas restricciones morfológicas al crecimiento.

Finalmente, en el tercer artículo "*Phyllotaxis and the evolution of stem morphology in cacti*" se expone un modelo matemático de la filotaxia que sugiere que la transformación morfogenética pudo haberse realizado mediante la variación adaptativa de dos parámetros morfológicos que son controlados durante el crecimiento del ápice: el ángulo generador de la filotaxia y la compactación de los entrenudos. Los resultados sugieren que cambios graduales y relativamente pequeños de estos dos parámetros pueden generar nuevas conformaciones de los haces vasculares en los tallos, y podrían estar asociados al desarrollo evolutivo de las costillas del leño como estructura fundamental de soporte.

En resumen, esta tesis concluye que tres mecanismos sencillos (la recapitulación, la heterocronía, y la regulación meristemática de la morfogénesis) y de variación gradual, son posiblemente los elementos más importantes sobre los que operó la selección natural durante el desarrollo de la succulencia del tallo en la familia Cactaceae.

ABSTRACT

Three interrelated events occurred in the evolution of the Cactaceae: (a) the development of stem succulence; (b) the reduction or loss of leaves, and (c) the switching from a C_3 to a CAM photosynthetic pathway.

The three papers presented in this thesis include studies of the evolution of stem succulence in cacti at the physiological, anatomical, and morphological levels. All three papers are based on comparative analyses of five cactus species belonging to the different subfamilies (Pereskioideae, Opuntioideae and Cereoideae) and showing contrasting life-forms.

The first paper, "Changes in the photosynthetic metabolism during the early ontogeny of four cactus species", measured and analyzed the photosynthetic physiology during the first weeks of life. All seedlings showed a C_3 metabolism in their early stages, even in species they are obligate CAM when adult. With the exception of the *Pereskia aculeata*, a leaf-bearing cactus, all other species switched to a typical CAM pattern between 10 and 20 weeks after germination. This result suggests that most cacti tend to maintain the ancestral (C_3) photosynthetic metabolism in the early ontogenetic stage (recapitulation).

In the second paper, "Allometric neoteny and the evolution of succulence in cacti", we made a comparative study of xylem development. In the succulent species it was found that vessel element length did not change between juvenile and adult wood, that wall-pitting in adult plants was similar to that of seedlings, and that the woody tissue in adult cacti was organized in vascular bundles, as in the primary tissue of all dicotyledonous seedlings. Leaf-bearing cacti, in contrast, changed in both vessel element length and wall-pitting when secondary wood was produced, and the secondary woody tissue of adult plants was organized in a continuous cambial cylinder as in most dicotyledons. An allometric analysis suggested that a retardation in the developmental rate of woody tissues (allometric neoteny) is the main anatomical mechanism operating in the evolutionary development of succulence in cacti.

Finally the third paper, "Phyllotaxis and the evolution of stem morphology in cacti", presents a mathematical model using only two parameters: (a) areole density in the stem (a measure of internode length or stem compaction), and (b) the divergence angle between successive areoles with respect to central axis of the stem (a measure of the phyllotactic arrangement of leaf-primordia). Based on existing morphologic and genetic evidence for other taxa, we assumed that both parameters can vary within one cactus species, and hence may be subject to natural selection. Thus, small changes in the phyllotactic parameters may generate new internal arrangements of the vascular bundles and are possibly associated with the evolutionary transformation of contact parastichies into true ribs.

Summing-up our conclusions, three simple mechanisms (recapitulation, heterochrony and meristematic regulation of morphogenesis), which are subject to gradual variation within a species, are possibly the most important elements on which natural selection operated during the development of stem succulence in the Cactaceae.

I. Introducción

INTRODUCCION

Una de las características de la familia Cactaceae que ha suscitado el interés no sólo de biólogos y botánicos, sino de un sinnúmero de aficionados, es la sorprendente diversidad de formas y tamaños que se distribuye a lo largo de todo el Continente Americano.

De acuerdo a la clasificación de Buxbaum (1958) podemos dividir a las cactáceas en tres grandes subfamilias: Pereskioideae, Opuntioideae y Cereoideae. La subfamilia Pereskioideae reúne las especies con características ancestrales, tales como presencia de hojas, hábito arbóreo o arbustivo y tallo leñoso. La característica compartida con el resto de la familia es la presencia de areolas o cojines de espinas, que son realmente yemas axilares transformadas, sustentadas en el tallo primario por una hoja tectriz.

Las especies de la subfamilia Opuntioideae presentan ya sea características semejantes a las Pereskioideas (género *Pereskiopsis*), o tallos suculentos cilíndricos, claviformes o laminares con hojas muy reducidas y efímeras. Las areolas presentan espinas, glóquidas y pelos. Pueden ser arborescentes, arbustivas, subarbustivas, o rastreras.

Finalmente, la subfamilia Cereoideae reúne el mayor número y diversidad de especies. Sus miembros presentan el máximo desarrollo en la succulencia del tallo, el limbo de las hojas desaparece y las areolas se presentan sin glóquidas. La arquitectura de los tallos varía desde tallos no ramificados en algunas especies, hasta profusamente ramificados en otras. La forma exterior del tallo puede ser globosa, oblonga, cilíndrica o en cladodios; con las areolas ubicadas sobre tubérculos o a lo largo de costillas. El tamaño oscila entre escasos centímetros hasta más de quince metros de altura (Bravo-Hollis, 1978).

Aún quedan sin responder cuestiones fundamentales sobre la evolución de esta familia, sobre su filogenia, y sobre los eventos geográficos y geológicos responsables de su origen. El ancestro hipotético propuesto por Gibson & Nobel (1986) es una planta leñosa perenne, con hojas deciduas acomodadas de acuerdo a un patrón helicoidal. Las areolas habrían estado presentes en las yemas axilares produciendo nuevas hojas y espinas. El metabolismo fotosintético ancestral habría sido de tipo C_3 , y se propone que el xilema secundario habría estado altamente especializado con elementos de vaso con placas de perforación simple. El cambium vascular secundario habría formado un cilindro completo de xilema secundario hacia dentro y floema hacia fuera. Este perfil ancestral se basa en las características presentes en las especies

vivientes menos especializadas, particularmente en las especies del género *Pereskia* (Subfamilia Pereskioideae) y las especies foliadas de la subfamilia Opuntioideae (*Pereskiopsis* spp.).

En el proceso evolutivo de las cactáceas ocurrieron tres eventos interrelacionados: (a) la reducción del tamaño de las hojas, hasta su pérdida total; (b) la formación de tejido suculento en la corteza y médula del tallo, y (c) el cambio del metabolismo fotosintético de C_3 a CAM. La adquisición de estas características les permitió abrir nuevas fronteras adaptativas, distribuyéndose en las zonas áridas de todo el continente americano. Estas peculiaridades evolutivas, aunadas a la riqueza morfológica, el origen monofilético, y el carácter endémico de la familia, la ubican como un grupo modelo para el estudio de mecanismos evolutivos.

En este trabajo buscamos entender y modelar algunos de los enigmas vigentes en la evolución de la familia. Nos preguntamos cuáles son los mecanismos evolutivos que explican el desarrollo de la suculencia y con ello la gran diversidad morfológica observada. Nuestra hipótesis de trabajo es que pequeños cambios durante la ontogenia pueden magnificarse en el proceso de crecimiento y resultar en grandes saltos evolutivos en términos morfológicos.

Allmon & Ross (1990) y Seilacher (1970 en Allmon & Ross,1990), coinciden en especificar dos tipos de factores determinantes que controlan la evolución morfológica: intrínsecos y extrínsecos. Entre los primeros señalan las restricciones filogenéticas (bióticos intrínsecos) y las restricciones morfogenéticas (abióticos intrínsecos) tales como propiedades físicas de los materiales que afectan el desarrollo morfológico de los organismos y las relaciones alométricas. Los factores extrínsecos comprenden las interacciones con otras especies (bióticos), mientras que las características del sustrato (abióticos) se clasifican como restricciones funcionales.

En esta tesis se exploran particularmente aquellos factores clasificados como bióticos intrínsecos, tales como la fisiología fotosintética, el desarrollo de la succulencia en el tallo, y el arreglo de las areolas y de los haces leñosos en el mismo tallo. También se evalúan aspectos correspondientes a las relaciones alométricas, y a las restricciones físicas y geométricas que afectan el desarrollo morfológico de los organismos.

La pregunta fundamental es: ¿qué mecanismos evolutivos operaron durante el desarrollo de la succulencia de las cactáceas?

De acuerdo a nuestra hipótesis de trabajo, se estudió la heterocronía como paradigma fundamental para entender el papel del desarrollo en la

evolución morfológica de las cactáceas. El concepto moderno de heterocronía propuesto y discutido por Gould (1977) se define como la evolución a través de cambios en los ritmos de crecimiento o desarrollo. Estos cambios se resumen en dos procesos (la aceleración y la retardación) cuyos resultados se traducen en los fenómenos de peramorfosis (recapitulación) y pedomorfosis. Estos resultados no se corresponden uno a uno con los procesos causales, de modo que la recapitulación, o sea la aparición de un carácter del estado adulto del ancestro durante la ontogenia del descendiente, puede obtenerse ya sea por una retardación de la maduración con respecto al desarrollo somático, o por aceleración de la forma con respecto al desarrollo. Asimismo, el resultado contrario; es decir, la pedomorfosis, la cual consiste en la aparición de un carácter juvenil del ancestro en el adulto del descendiente, también puede explicarse por una retardación en las tasas de desarrollo (neotenia) o por una aceleración en la maduración (progenesis).

La heterocronía establece un puente de unión entre la teoría evolutiva y ecológica abarcando dos aspectos fundamentales en la evolución morfológica: las restricciones estructurales y de desarrollo, y la selección ambiental (Gould, 1988). El concepto de heterocronía ha sido más estudiado en sistemas animales que en el desarrollo de plantas (Lord & Hill, 1987; MacNamara,

1986; Gould, 1977). Si bien la embriogénesis en las plantas es más simple que en animales, ya que el número de tejidos y tipos celulares es menor y no hay movimiento celular (Lord & Hill, 1987), existen algunos problemas relacionados con el tipo de crecimiento y con la gran plasticidad fenotípica de las plantas (Guerrant, 1988). El crecimiento indeterminado y la construcción modular conlleva problemas como la presencia de convergencia y paralelismo en la evolución morfológica (Lord & Hill, 1987). Sin embargo, se han publicado una serie de trabajos que analizan procesos de heterocronía en vegetales y sugieren su extensión e importancia en la evolución de las plantas superiores (Takhtajan, 1972; Carlquist, 1962; Guerrant, 1988; Lord & Hill, 1987).

Necesariamente una explicación del ritmo, de los mecanismos y de la dirección del cambio morfológico involucra el estudio de diversos factores y distintos niveles de aproximación. En particular el problema de la evolución de la succulencia tiene expresión a tres niveles: (a) el fisiológico (el cambio de C_3 a CAM); (b) el anatómico (desarrollo de grandes masas de tejido parenquimatoso); y (c) el morfológico (el sostén mecánico de tallos blandos, parenquimatosos y pobres en leño). Para cada uno de estos niveles se plantean preguntas y metodologías específicas.

En la primera etapa del estudio se abordó el nivel fisiológico, el cual se presenta en un trabajo titulado "*Changes in the photosynthetic metabolism during the early ontogeny of four cactus species*". El objetivo de esta etapa fue analizar y cuantificar los cambios en la fisiología fotosintética durante la ontogenia de cuatro especies de cactáceas con morfologías contrastantes. La ruta metabólica CAM (Metabolismo ácido de las crasuláceas), característica de las cactáceas suculentas, implica un desfase de la carboxilación en el tiempo. Los estomas se abren durante la noche y el CO₂ se fija en forma de ácidos orgánicos de cuatro carbonos que durante el día son metabolizados a través de la ruta C₃. Las células vacuoladas de los tejidos parenquimatosos permiten la acumulación de los ácidos orgánicos. La consecuencia más notable de este mecanismo fotosintético es la alta eficiencia en el uso del agua, y su principal costo adaptativo es la disminución en la capacidad fotosintética y por ende el retardo en la tasa de crecimiento.

La hipótesis de trabajo fue que las características ancestrales, en este caso la ruta metabólica C₃ (Gil, 1986) podría expresarse en las etapas tempranas de la ontogenia de las especies derivadas. De ser así, este fenómeno tendría implicaciones no sólo a nivel morfológico sino en la fisiología del crecimiento, y en consecuencia tendría también implicaciones adaptativas.

El segundo nivel de trabajo, correspondiente al estudio anatómico del desarrollo de la succulencia en el tallo, se expone en el Capítulo II. Se hace un análisis comparativo de las características del leño durante el desarrollo en cinco especies de cactáceas con morfologías contrastantes. La pregunta planteada es si la dramática divergencia morfológica observada entre las cactáceas leñosas y las especies suculentas responde a un proceso de heterocronía. Los efectos heterocrónicos pueden manifestarse en una amplia variedad de niveles de la organización estructural, desde el nivel celular, hasta el sistema vascular o la planta como un todo integrado (Guerrant, 1988). De ahí la importancia de concebir los estudios desde una perspectiva jerárquica, a través de una combinación de aproximaciones a distintos niveles (Raff & Wray, 1989). Carlquist (1962) fue el primero en hablar de pedomorfosis en los elementos de vaso del xilema para explicar el desarrollo de la succulencia en el tallo de cactáceas. En este trabajo nos propusimos modelar el mecanismo evolutivo que arroja este resultado, trabajando no sólo con las clásicas curvas edad-longitud de los elementos de vaso, sino con el sistema vascular como un todo a través de modelos de alometría. A través del seguimiento comparativo de la alometría durante las ontogenias del ancestro y descendiente, se puede conocer el tipo de heterocronía, así como su dirección y magnitud (Alberch et al. 1979).

Finalmente, en el Capítulo III se aborda el aspecto morfológico determinado por el arreglo de las areolas sobre el tallo y su estrecha relación con el curso e interconexión del sistema vascular internamente. Dado que las plantas son organismos modulares compuestos por unidades estructurales básicas, fijamos la atención en la expresión más simple de estas unidades constituida por el segmento de tallo que incluye un entrenudo, un nudo, una hoja y una yema axilar (White, 1979). La arquitectura de la planta está modulada por tres variables morfológicas: la longitud del entrenudo, la filotaxia o arreglo de las hojas sobre el tallo, y el patrón de ramificación.

En este trabajo se explora a través de un modelo matemático los dos primeros parámetros: la longitud de los entrenudos y el patrón filotáxico determinado por el arreglo de las areolas en torno al tallo.

Los individuos adultos de las cactáceas suculentas retienen el carácter juvenil en la disposición interna de los haces vasculares y por lo tanto la conexión a lo largo del tallo entre las areolas vecinas. En consecuencia el arreglo filotáxico de las areolas sobre el tallo está estrechamente ligado al curso de los haces vasculares internamente (Tomlinson & Wheat, 1979). La hipótesis que se desprende es que la selección a favor de un cierto patrón

filotáxico implica también una selección para el tipo de distribución de los haces vasculares, o sea determina la conformación biomecánica de la planta.

El principal objetivo de esta etapa es demostrar que la combinación de una selección favorable a la retención de las características juveniles en el tallo y pequeños cambios en los parámetros filotáxicos (longitud de los entrenudos y ángulo de divergencia entre areolas consecutivas) podrían explicar la marcada variación morfológica observada en las cactáceas.

REFERENCIAS

- Alberch P., Gould S., Oster G., Wake D. 1979 . Size and shape in ontogeny and filogeny. *Paleobiology*, **5**:296-317.
- Allmon, W.D.y Ross, R.M. 1990. Specifying causal factors in evolution: The paleontological contribution. In: Ross & Allmon (eds). *Causes of Evolution*: The University of Chicago Press, Chicago.
- Bravo-Hollis, H. 1978. Las Cactáceas de México. Vol. I. Universidad Nacional Autónoma de México, México, D.F..
- Buxbaum, F. 1958. The phylogenetic division of the subfamily Cereoideae, Cactaceae. *Madroño* **14**: 177-206.
- Carlquist, S. 1962. A theory of pedomorphosis in dicotyledonous woods. *Phytomorphology* **12**: 29-45.
- Gil, F. 1986. Origin of CAM as an alternative photosynthetic carbon fixation pathway. *Photosynthetica* **20**: 494-507.

- Gibson, A.C. y Nobel, P. 1986. *The Cactus Primer*, Harvard University Press, Cambridge.
- Gould, S.J. 1977. *Ontogeny and Phylogeny*. Cambridge: Bellknap Press of Harvard University Press.
- Gould, S. 1988. The uses of heterochrony. In McKinney M., (ed.) *Heterochrony in evolution*: Plenum Press, N.Y., 1-13.
- Guerrant, E.O. 1988. Heterochrony in plants: the intersection of evolution, ecology and ontogeny. In: McKinney M. (ed.) *Heterochrony in Evolution*: Plenum Press, New York, 111-133.
- Lord E.M. y Hill J.P. 1987. Evidence for heterochrony in the evolution of plant form. In Raff, R., Raff, E. (eds.) *Development as an evolutionary process*: MBL Lectures in Biology 8: Alan R. Liss, U.S.A., 47-70.
- MacNamara, K. J. 1986. A guide to the nomenclature of heterochrony. *Journal of Paleontology*. 60:4-13.
- Raff, R. y Wray G. 1989. Heterochrony: developmental mechanisms and evolutionary results. *Journal of Evolutionary Biology*, 409-434.
- Takhtajan, A. 1972. Patterns of ontogenetic alterations in the evolution of higher plants. *Phytomorphology*, 22:164-171.
- Tomlinson, P.B. y Wheat, D.W. 1979. Bijugate phyllotaxis in Rhizophoreae. *Botanical Journal of the Linnean Society* 78: 317-321.
- White, J. 1979. *The plant as a metapopulation*. *Annual Review of Ecology and Systematic*. 10:109-145.

II. Changes in the photosynthetic metabolism during the early ontogeny of four cactus species.



BIBLIOTECA
CENTRO DE ECOLOGIA

Changes in the photosynthetic metabolism during the early ontogeny of four cactus species

Alice Altesor, Exequiel Ezcurra and Carlos Silva

Centro de Ecología, Universidad Nacional Autónoma de México. Apartado Postal 70-275, CP 04510, México, DF

Abstract

1. Changes in the photosynthetic physiology during the early ontogeny of four species of cacti (*Pereskia aculeata*, *Opuntia pilifera*, *Neobuxbaumia tetetzo* and *Ferocactus recurvus*) were studied. Each species presents a contrasting morphology, and represents a different group within the main taxonomic subdivisions of the family. We measured daily variations in the acid concentration of seedling tissues at different ages for each of the four species up to 40 weeks after germination.

2. In the first weeks, the daily pattern of acid concentration did not show a night-time peak in any of the four species. This indicates that seedlings have a C_3 metabolism during their early ontogeny, even in species that are typically CAM when adult.

3. With the exception of the *Pereskia aculeata*, a leaf-bearing cactus, all other species switched to a typical CAM pattern between 10 and 20 weeks after germination.

4. The results suggest that most cacti tend to maintain the ancestral (C_3) photosynthetic metabolism in the early ontogenetic stages.

Keywords: Crassulacean acid metabolism (CAM), ontogeny, phylogeny, Cactaceae, cactus seedling, C_3 metabolism.

Résumé

1. On a étudié les modifications de la physiologie de la photosynthèse de quatre espèces de cactées (*Pereskia aculeata*, *Opuntia pilifera*, *Neobuxbaumia tetetzo* et *Ferocactus recurvus*) au cours des premiers stades de l'ontogénèse. Chaque espèce présente une morphologie contrastée et représente un groupe différent parmi les principales subdivisions taxonomiques de la famille. Nous avons mesuré les variations journalières de la concentration en acide dans les tissus des plantules à des âges différents, pour chacune des quatre espèces, jusqu'à 40 semaines après la germination.

2. Au cours des premières semaines, le patron journalier de la concentration en acide ne présente de pic nocturne chez aucune des quatre espèces. Cela indique que les plantules ont un métabolisme C_3 en début d'ontogénèse, même chez des espèces qui sont typiquement CAM à l'âge adulte.

3. A l'exception de *Pereskia aculeata*, un cactus portant des feuilles, toutes les espèces passent à un patron typiquement CAM dans les 10 à 20 semaines suivant la germination.

4. Les résultats suggèrent que la plupart des cactées tendent à maintenir un métabolisme photosynthétique ancestral (C_3) au cours des premiers stades de l'ontogénèse.

INTRODUCTION

The study of ontogeny and developmental biology has reappeared with new vigour during the last ten years. Haeckel's ideas, which occupied a very important

place during the second half of the 19th Century, were readopted, enriched and upgraded. GOULD'S (1977) work became a milestone in this conceptual upgrading, because of its thorough theoretical and methodological revision of the research on ontogeny and on the causes of its parallelism with phylogeny. Possibly for historical causes, most of the published ontogenetic studies deal with animals and very few discuss the problem in plants.

The goal of this work is to analyze and quantify the changes in photosynthetic physiology during the ontogeny of four cactus species. The selected species were *Pereskia aculeata* Miller, *Opuntia pilifera* Weber, *Ferocactus recurvus* var. *greenwoodii* Miller and *Neobuxbaumia tetetzo* Backeberg. They represent the three subfamilies within the Cactaceae: Pereskioideae, Opuntioideae and Cereoideae, according to BUXBAUM'S classification (1958). Their physiological, anatomical and architectural features possibly exemplify the most important evolutionary steps in the family's phylogeny. *Pereskia aculeata* shows the primitive features assigned to the ancestral condition: it is a tree with fully developed leaves and woody stems. Its metabolic route of CO₂ fixation is C₃ type (Calvin and Benson cycle, GIBSON & NOBEL, 1986). The other three species present a full Crassulacean Acid Metabolism (CAM) in their adult stage.

An interesting aspect of some CAM plants is their ability to shift the CO₂ fixation mode in the same individual, depending on the water availability and the age of the leaves (HARTSOCK & NOBEL, 1976; NOBEL & HARTSOCK, 1987; BORLAND & GRIFFITHS, 1990). Different modes of expression of the acid metabolism in plants have been described. The first one is known as "CAM-idling" and was described in *Opuntia basilaris* by SZAREK *et al.* (1973). This plant during periods of drought closes its stomata completely and stops nocturnal CO₂ fixation, but continues to show fluctuations in organic acid concentration as a result of the reassimilation of CO₂ generated by respiration.

Some succulent plants may show fluctuating concentrations of organic acids even though the CO₂ fixation takes place during the day (TING, 1985). The nocturnal acid increase occurs as a result of the reassimilation of the CO₂ produced by respiration (WINTER *et al.*, 1986; PATEL & TING, 1987). This variant named "CAM-cycling" seems to be intermediate, in evolutionary terms, between the full C₃ metabolism and the "full CAM" (MONSON, 1989). RAYDER and TING (1981) found that adult plants of *Pereskia aculeata* behave as C₃ when grown under adequate moisture, but tend to accumulate organic acids at night when grown under water-stressed conditions. MONSON (1989) suggested this species as an example of facultative CAM-cycling. NOBEL and HARTSOCK (1986*b*) analyzed the CO₂ uptake patterns of three species of the subfamily Pereskioideae (including *P. aculeata*, used in our study) and found that in all three the net CO₂ uptake was by the leaves and during day-time.

In this study we analyze the ontogenetic variations in the photosynthetic metabolism of each of the four species described above during the first weeks after germination. Our hypothesis was that the ancestral characters, in this case the C₃ pathway (GIL, 1986), could still be expressed during the first stages of the life cycle. By following the photosynthetic metabolism during the ontogeny of each one of these species, it was our aim to observe if an ancestral character, in this case the C₃ pathway, was expressed during the juvenile stages of the descendants.

METHODS

Seeds of the four species were sown in agar gel. Germination was recorded daily in order to know the seedling age. Eight weeks after germination, the seedlings were transplanted from the agar gel into pots with soil and sand. The cohorts were always kept under controlled light, temperature and humidity conditions. Light conditions were maintained at low levels, as it is known that some of these species establish under the shade of "nurse plants" that eliminate a substantial amount of the incoming solar radiation (VALIENTE-BANUET & EZCURRA, 1991). The photosynthetically active radiation (PAR), measured with a Li-Cor quantimeter, averaged $70 \mu\text{mol m}^{-2} \text{s}^{-1}$. The total radiation was determined with a Li-Cor pyranometer, and averaged 12.4 W/m^2 . Mean air temperatures were 25°C during light periods and 20°C during dark periods, with an absolute range between 27°C and 18°C . The light and dark periods had both a duration of 12 h. The plants were watered three times per week to maintain soil moisture.

Seedlings were sampled one, three, six, ten, fourteen, twenty and forty weeks after germination. For each individual species, sampling was continued at the described intervals until a full CAM metabolism was observed. Sampling was done during a 24-hour period, with two sample replicates. Every three hours, from 0:00 to 21:00, one gramme of seedling tissue was taken per sample and was immediately frozen in dry ice (OSMOND *et al.*, 1989). Once a complete daily series was obtained, the individual samples were first macerated, then diluted in 5 ml of distilled water and finally boiled in order to insure vacuole breakage and release of organic acids (HARTSOCK & NOBEL, 1976). The samples were then titrated with NaOH 0.002N, and the total concentration of acid was estimated. The results were expressed both as acid concentration per unit fresh weight (meq/g) and as acid concentration per unit photosynthetic area (meq/mm²). In the case of cotyledons and leaves, the photosynthetic area was estimated by means of a Delta T Area meter. The photosynthetic area of succulent seedling stems was estimated through geometric formulae, assuming that the seedlings approach in shape a three-dimensional ellipse.

For each of the species we made two analyses of variance. In the first one the independent variable was the acid concentration per unit of fresh weight of the seedlings, while in the second one we used acid concentration per unit photosynthetic surface. In both analyses the predictors were two categoric variables or factors: seedling age and hour of the day.

The accumulation of organic acids in CAM plants is a periodic phenomenon: it peaks daily around dawn (after accumulating CO₂ during the night in the form of malate), and shows a minimum value towards sunset (during the day the stomata are closed and malate is metabolized by the plant). Thus, organic acid concentration must roughly follow a sinusoidal function of the type

$$x = a + b \sin(h) \quad (1)$$

where x stands for the predicted concentration of organic acids in the plant, a is the mean concentration of acids, b is the amplitude of the periodic variation in acid concentration and h is the hour of the day expressed in radians, such that $h = 2\pi t/24$, where t stands for the local solar time (in hours).

Taking the sine of h as the independent variable, eq. (1) becomes a straight line which can be analyzed by linear regression in order to determine the parameters a and b , and the correlation coefficient (r). This last number can be interpreted as a measure of the periodicity in the concentration of acids for each age and species. The r values obtained were plotted in order to make comparisons between species for each age.

RESULTS

The results of both analyses for *Pereskia aculeata* showed the absence of a periodic pattern in daily acid fluctuation. As the seedling age increased, the trend was to maintain a constant acid concentration during the day (fig. 1). *Opuntia pilifera*, *Neobuxbaumia tetetzo* and *Ferocactus recurvus* showed at first the same pattern, with erratic fluctuations in acid concentration during the initial weeks of life. Later on, though, they developed a sinusoidal trend that became more evident

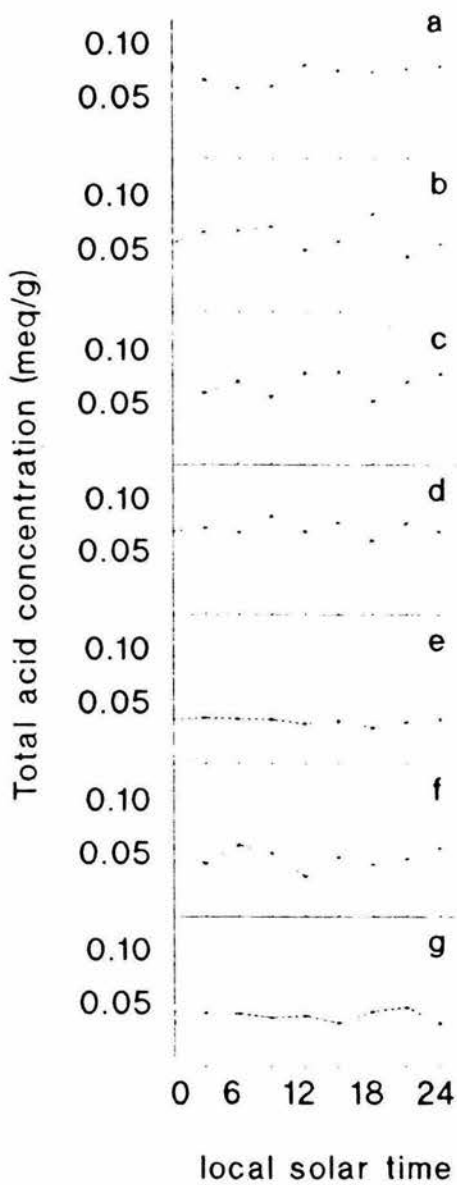


Fig. 1

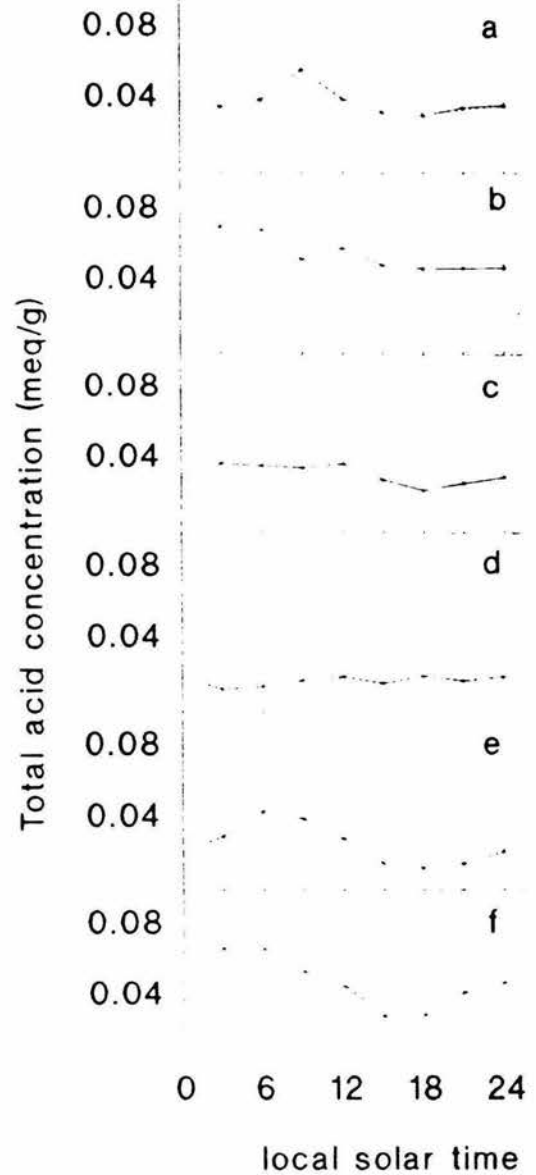


Fig. 2

Fig. 1. - Total acid concentration per unit fresh weight at different hours of the day and for different ages in *Pereskia aculeata* seedlings. The curves from a to g represent 1, 3, 6, 10, 14, 20 and 40 weeks of life, respectively. The analysis of variance for acid concentration per unit fresh weight and for acid concentration per unit photosynthesis area indicated that variation for both statistical factors (hour and age) was not significant. The dotted lines indicate standard errors.

Fig. 2. - Total acid concentration per unit fresh weight at different hours of the day and for different ages in *Opuntia pilifera*. The curves from a to f represent 1, 3, 6, 10, 14, and 20 weeks of life, respectively. The analysis of variance for acid concentration per unit fresh weight and for acid concentration per unit photosynthetic area indicated a significant variation for both factors (hour and age) and their interaction ($P < 0.001$). The dotted lines indicate standard errors.

as the experiment progressed, with a high acid concentration at dawn that decreased during the light hours. In the three species, however, the typical CAM sinusoidal

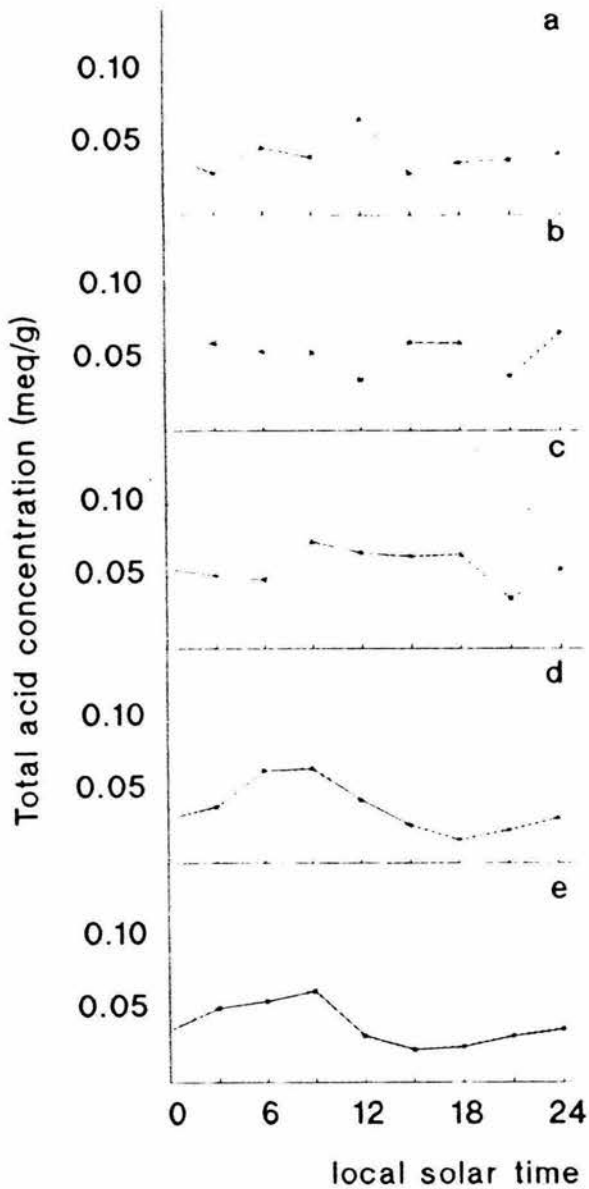


Fig. 3

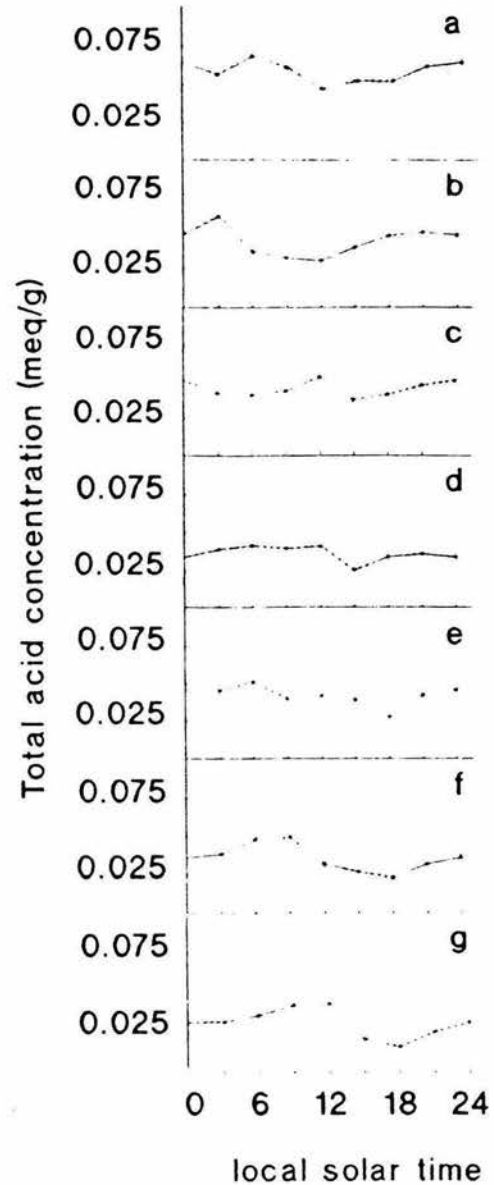


Fig. 4

FIG. 3. - Total acid concentration per unit fresh weight at different hours of the day and for different ages in *Ferocactus recurvus*. The curves from a to e represent 1, 3, 6, 10, and 14 weeks of life, respectively. The analysis of variance for acid concentration per unit fresh weight and for acid concentration per unit photosynthetic area indicated a significant variation for both factors (hour and age) and their interaction ($P < 0.001$). The dotted lines indicate standard errors.

FIG. 4. - Total acid concentration per unit fresh weight at different hours of the day and for different ages in *Neobuxbaumia tetetzo*. The curves from a to g represent 1, 3, 6, 10, 14, 20 and 40 weeks of life, respectively. The analysis of variance for acid concentration per unit fresh weight and for acid concentration per unit photosynthetic area indicated a significant variation for both factors (hour and age) and their interaction ($P < 0.001$). The dotted lines indicate standard errors.

pattern appeared at different ages. In *Opuntia pilifera* and *Ferocactus recurvus* it was observed between ten and fourteen weeks of age, while in *Neobuxbaumia tetetzo* the

nocturnal acid accumulation was expressed significantly from the twentieth week onwards (fig. 2, 3 and 4). It is interesting to note that *Opuntia pilifera* showed a feeble trend to accumulate acids at night during the first three weeks of life, while the seedlings still had cotyledons. Once the cotyledons were lost, the nocturnal accumulation disappeared (fig. 2*d*), to be regained later when the typical CAM pattern developed in the 14th week.

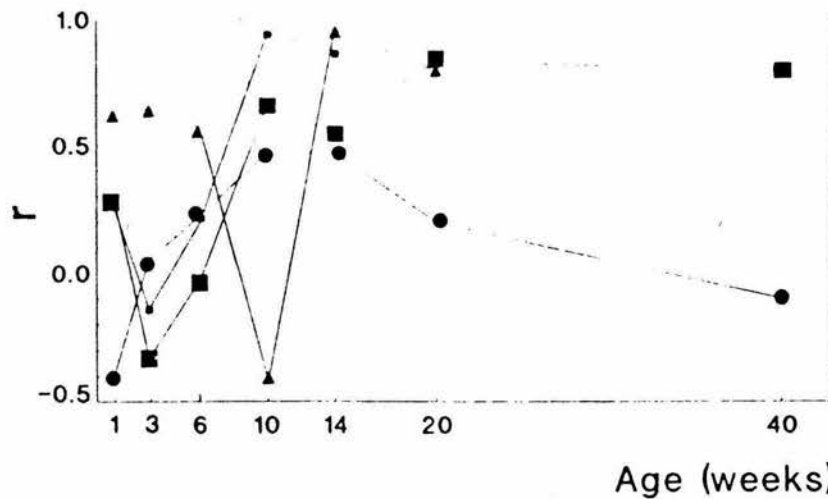


FIG. 5. – Index of periodicity (r values) plotted against seedling age. The symbols indicate different species and the broken line indicates the $P=0.001$ significance level for r with 14 degrees of freedom (● *Pereskia aculeata*, ▲ *Opuntia pilifera*, ■ *Neobuxbaumia tetetzo*, ◆ *Ferocactus recurvus*).

In the case of *Pereskia aculeata*, the r values as a measure of acid periodicity were never significant during the experiment (fig. 5). In the other three species the r values showed significant ($P<0.001$) correlations between acid concentration and the sine function [$\sin(h)$] starting between the 10th and the 20th week.

DISCUSSION

An ontogenetic “memory” revealed by the presence of a C_3 metabolism during the early development of otherwise typical CAM species has important implications at the systematic, ecological and morpho-physiological levels. In this study we shall restrict our discussion to the last two levels.

From the point of view of plant-environment relationships, to understand the metabolism during the initial weeks of life – a very critical time for survival in cactus seedlings – may allow us to interpret adaptively some observed patterns of plant establishment. Establishment patterns have been extensively studied in many species belonging to the subfamilies Opuntioideae and Cereoideae, and have been shown to bear a great importance in the dynamics and the structure of arid communities. As a general rule, cactus seedlings tend to establish under the canopy of “nurse” plants that provide shade and protection from radiation damage (McAULIFFE, 1988; VALIENTE-BANUET, 1991). Shrubs also allow nitrogen retention and accumulation in the soil (GARCIA MOYA & MCKELL, 1969), they give protection against wind erosion (MULLER, 1953) and in some cases they can pump water from deeper soil-profile horizons (CALDWELL & RICHARDS, 1989). In other cases they may even serve

as refuges against predators (McAULIFFE, 1984). The shade of the nurse shrubs has a positive effect on seedling survival, as it increases the probability of their successful establishment (VALIENTE-BANUET & EZCURRA, 1991). These advantages, however, imply a cost in the seedling growth rate, as nurse shading decreases PAR.

Cacti in general are characterized by a low surface-to-volume ratio and by the nocturnal opening of stomata. These traits tend to reduce water loss (JORDAN & NOBEL, 1981; BARCIKOWSKI & NOBEL, 1984) but they also decrease the CO₂ absorption surface (which in turn decreases the growth rate) and the convective heat exchange surface (which in turn increases the risk of temperature damage, NOBEL, 1978). Recent studies have shown that excessive radiation and the associated high temperatures are the main mortality factor for cactus seedlings (FRANCO & NOBEL, 1989; VALIENTE-BANUET & EZCURRA, 1991). Thus, a C₃ metabolism in the initial weeks of life of cactus seedlings could have important consequences from an adaptive point of view.

On the one hand, the faster growth rate that characterizes C₃ plants may possibly compensate the lower PAR levels under the nurse plant, allowing the seedlings to root and escape sooner from the critical establishment period and thus to increase their probabilities of survival. On the other hand, a C₃ metabolism implies the diurnal opening of stomata and the possibility of thermoregulation through transpiration, decreasing the chances of temperature damage. This, obviously, implies a higher water expense, but seedling germination in the field usually occurs after heavy rains when water is relatively abundant. After the first weeks of seedling growth, pubescence and thorns are developed that affect the convection coefficient and produce self-shading, helping to generate passive means of temperature control. Thus, an early C₃ metabolism may help to promote fast growth and efficient thermoregulation at the expense of a higher water use, at a time in which water is relatively abundant and in which rooting and cooling are critical factors. Presumably, water availability could change the timing of the shift period.

At the morpho-physiological level, the results have important implications on plant architecture and allometric relationships. The surface-to-volume ratio for *Opuntia pilifera*, *Neobuxbaumia tetetzo*, and *Ferocactus recurvus* decreased in 40%, 39% and 48% respectively, during the first 100 days of life (fig. 6). In the case of *Pereskia aculeata*, however, the ratio increased by around 63% during the first 100 days of life (fig. 6). Thus, the switch from C₃ to CAM metabolism occurs in plants in which the allometric relationships during ontogeny tend to decrease the surface-to-volume ratio. It is interesting to note that as the surface-to-volume ratio decreases, the capacity of the plant to accumulate substantial amounts of nocturnal CO₂ in the form of malate also increases.

As mentioned, *Pereskia aculeata* shows ancestral physiological and anatomical features showing well-developed leaves and ligneous stems without cortical succulence. The species in this genus grow mostly in dry or subhumid tropical forests, and do not prosper in arid environments. As far as we know, a nurse-plant-mediated establishment pattern has not been reported for the genus. *Pereskia* species have been proposed as representatives of the CAM-cycling pathway. According to the results of this paper, however, during the first life stages *Pereskia aculeata* behaves as a typical C₃ with no nocturnal acid accumulation; CAM-cycling probably manifests itself at older ages and possibly in a facultative way. Some studies have reported this shift capacity in species of the genus *Pereskia* (RAYDER & TING, 1981).

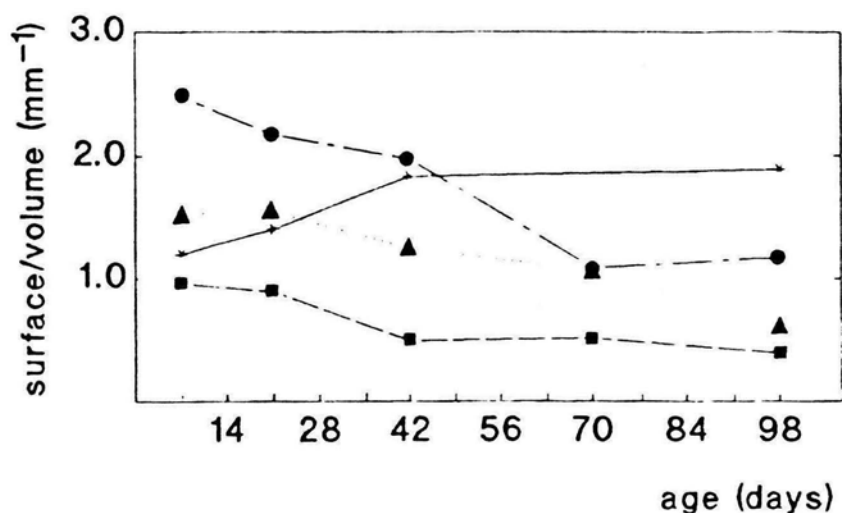


Fig. 6. - Surface-to-volume ratio for the first 100 days after germination in *Pereskia aculeata* (—★—), *Opuntia pilifera* (—■—), *Neobuxbaumia tetetzo* (—▲—) and *Ferocactus recurvus* (—●—).

An important aspect to be pointed out is that the PAR level used in this study was low ($70 \mu\text{m s}^{-1} \text{m}^{-2}$), and corresponds to 70% of the saturation level for adult plants of *Pereskia aculeata* (NOBEL & HARTSOCK, 1986a) and approximately to 15% of the saturation levels of adult *Opuntia ficus-indica* and *Ferocactus acanthodes* (NOBEL & HARTSOCK, 1983). The amount of PAR considered in this study scarcely exceeds the compensation point in the case of the above mentioned adult cacti. Nevertheless, it is important to consider that in seedlings the surface-to-volume ratio is higher and the compensation point must be considerably lower. In the case of *Pereskia aculeata*, the amount of PAR used is sevenfold the compensation point for adult plants. It is possible that if PAR were increased the seedling shift from C_3 to CAM metabolism would occur earlier. This remains to be investigated.

In conclusion, this study shows that species of cacti from widely different subfamilies all show a C_3 metabolism immediately after germination. We believe that this may be a widespread characteristic in the whole family. With the exception of *Pereskia aculeata*, CAM metabolism develops in all other species during the first weeks of seedling ontogeny. On the one hand, this fact sheds some interesting elements on the theory of recapitulation, *i.e.* the parallelism between ontogeny and phylogeny, which has not been well studied in plants. Natural selection may operate differently at the seedling stage, and ancestral characters that are adaptive for seedling survival may be preserved during the early stages of the life history of the plant. In the case of cactus seedlings, there are strong reasons to infer that a C_3 metabolism may be adaptive in the early stages of germination and development, when water is seldom a limiting factor.

ACKNOWLEDGMENTS

We would like to thank Alfonso VALIENTE-BANUELI, who read the manuscript and made important suggestions, and Santiago ARIZAGA for his invaluable technical support.

REFERENCES

- BARCIKOWSKI W. & NOBEL P., 1984. - Water relations of cacti during desiccation distribution of water tissues. *Bot. Gaz.*, **145**, 1, 110-115.

- BORLAND A. M. & GRIFFITHS H., 1990. – The regulation of CAM and respiratory recycling by water supply and light regime in the C₃-CAM intermediate *Sedum telephium*. *Function. Ecol.*, **4**, 33-39.
- BUXBAUM F., 1958. – The phylogenetic division of the subfamily Cereoideae, Cactaceae. *Madroño*, **14**, 177-206.
- CALDWELL M. M. & RICHARDS J. H., 1989. – Hydraulic lift: water efflux from upper roots improves effectiveness of water uptake by deep roots. *Oecologia*, **79**, 1-5.
- FRANCO A. C. & NOBEL P., 1989. – Effect of nurse plants on the microhabitat and growth of cacti. *J. Ecol.*, **77**, 870-886.
- GARCIA-MOYA E. & MCKELL C., 1969. – Contribution of shrubs to the nitrogen economy of a desert-wash plant community. *Ecology*, **51**, 1, 81-88.
- GIBSON A. C. & NOBEL P., 1986. – *The Cactus Primer*. Harvard University Press, Cambridge, MA.
- GIL F., 1986. – Origin of CAM as an alternative photosynthetic carbon fixation pathway. *Photosynthetica*, **20**, 4, 494-507.
- GOULD S. J., 1977. – *Ontogeny and phylogeny*. The Belknap Press of Harvard University Press. EUA.
- HARTSOCK T. & NOBEL P., 1976. – Watering converts a CAM plant to daytime CO₂ uptake. *Nature*, **262**, 574-576.
- JORDAN P. & NOBEL P., 1981. – Seedling establishment of *Ferocactus acanthodes* in relation to drought. *Ecology*, **62**, 4, 901-906.
- McAULIFFE J., 1984. – Prey refugia and the distributions of two Sonoran desert cacti. *Oecologia*, **65**, 82-85.
- McAULIFFE J., 1988. – Markovian dynamics of simple and complex desert plant communities. *Am. Natur.*, **131**, 4, 459-490.
- MONSON R., 1989. – On the evolutionary pathways resulting in C₄ photosynthesis and Crassulacean Acid Metabolism (CAM). *Adv. Ecol. Res.*, **19**, 58-100.
- MULLER C. H., 1953. – The association of desert annuals with shrubs. *Am. J. Bot.*, **40**, 53-60.
- NOBEL P., 1978. – Surface temperatures of cacti-influences of environmental and morphological factors. *Ecology*, **59**, 5, 986-996.
- NOBEL P. & HARTSOCK T., 1983. – Relationships between photosynthetically active radiation, nocturnal acid accumulation and CO₂ uptake for a crassulacean acid metabolism plant *Opuntia ficus-indica*. *Plant Physiol.*, **71**, 71-75.
- NOBEL P. & HARTSOCK T., 1986a. – Temperature, water, and PAR influences on predicted and measured productivity of *Agave deserti* at various elevations. *Oecologia*, **68**, 181-185.
- NOBEL P. & HARTSOCK T., 1986b. – Leaf and stem CO₂ uptake in the three subfamilies of the Cactaceae. *Plant Physiol.*, **80**, 913-917.
- NOBEL P. & HARTSOCK T., 1987. – Drought-induced shifts in daily CO₂ uptake patterns for leafy cacti. *Physiol. Plantarum*, **70**, 114-118.
- OSMOND B., ADAMS W. & SMITH S., 1989. – Crassulacean Acid Metabolism. In: PEARCY R. W., EHRLINGER J., MOONEY H. A. & RUNDEL P., Eds., *Plant Physiological Ecology*, Chapman & Hall, London NY, 255-275.
- PATEL A. & TING I. P., 1987. – The relationship between respiration and CAM-cycling in *Peperomia campotricha*. *Plant Physiol.*, **84**, 640-642.
- RAYDER L. & TING I. P., 1981. – Carbon metabolism in two species of *Pereskia* (Cactaceae). *Plant Physiol.*, **68**, 139-142.
- SZAREK S., JOHNSON H. & TING I. P., 1973. – Drought adaptation in *Opuntia basilaris*. Significance of recycling carbon through crassulacean acid metabolism. *Plant Physiol.*, **52**, 539-541.
- TING I. P., 1985. – Crassulacean acid metabolism. *Ann. Rev. Plant Physiol.*, **36**, 595-622.
- VALIENTE-BANUET A. & EZCURRA E., 1991. – Shade as cause of the association between the cactus *Neobuxbaumia tetetzo* and the nurse plant *Mimosa luisana* in the Tehuacán Valley, Mexico. *J. Ecol.*, **79**, 4, 961-971.
- VALIENTE-BANUET A., BOLONGARO CREVENNA A., BRIONES O., EZCURRA E., ROSAS M., NUÑEZ H., BARNARD G. & VAZQUEZ E., 1991. – Spatial relationships between cacti and nurse shrubs in a semi-arid environment in central Mexico. *J. Vegetation Sci.*, **2**, 15-20.
- WINTER K., SCHROPP-LEIHER G. & CALDWELL M. M., 1986. – Respiratory CO₂ as carbon source for nocturnal acid synthesis at high temperature in three species exhibiting crassulacean acid metabolism. *Plant Physiol.*, **81**, 390-394.

III. Allometric neoteny and the evolution of succulence in cacti.

Allometric neoteny and the evolution of succulence in cacti

A. ALTESOR, C. SILVA AND E. EZCURRA

Centro de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, C.P. 04510, Mexico

Received March 1993, accepted for publication August 1993

ALTESOR, A., SILVA, C. & EZCURRA, E. 1993. **Allometric neoteny and the evolution of succulence in cacti.** With the objective of analysing the role of heterochrony in the evolution of succulence in the cactus family, a comparative study of xylem development in six species with contrasting morphologies was carried out. Two woody leaf-bearing cacti and four succulent cactus species belong to different subdivisions within the family were analysed. In each species and for different ages, vessel-element length was measured, vessel-element lateral wall-pitting described and the percentage of xylem and parenchyma in the stem quantified. In the succulent species it was found that vessel element length did not change between juvenile and adult wood, that wall-pitting in adult plants was similar to that of seedlings, and that the woody tissue in adult plants was organized in vascular bundles as in the primary tissue of seedlings. Leaf-bearing cacti, in contrast, changed in both vessel element length and wall-pitting when secondary wood was produced, and the secondary woody tissue of adult plants was organized in a continuous cambial cylinder as in most dicotyledons. An allometric analysis suggests that a retardation in the developmental rate of woody tissues (allometric neoteny) is the main mechanism in the development of succulence in cacti.

ADDITIONAL KEY WORDS:—allometry – Cactaceae – heterochrony – plant anatomy.

CONTENTS

Introduction	283
Methods	285
Characterization of the vessel elements	285
Quantification of xylem in histological slides of the main shoot	286
Results	287
Characterization of the vessel elements	287
Quantification of xylem in histological slides of the main shoot	287
Conclusions	289
Acknowledgements	292
References	292

INTRODUCTION

The modern concept of heterochrony, discussed in detail by Gould (1977), has been defined as evolution through changes in the rates of growth or development. These changes consist mainly of two processes: acceleration and retardation, which cause peramorphosis (recapitulation) and paedomorphosis (reverse recapitulation) without the existence of an exact one-to-one correspondence between them and the causal processes. Thus paedomorphosis,

the retention of juvenile ancestral characters in the descendent adults, can be either the result of an acceleration (progenesis) or a retardation (neoteny) of development (Gould, 1977); it may even be the consequence of a change in the parameters that control the ontogenetic trajectory (post-displacement), i.e. a delay in the onset of morphologic development (Alberch *et al.*, 1979; McNamara, 1986). The concept of heterochrony explains a wide variety of morphological transformations.

Size, shape and rates of development are closely related phenomena. The study of allometry (i.e. the analysis of the effects of variation in size on variation in shape) allows us to recognize the causes of changes in the rates of development. Alberch *et al.* (1979) formulated and quantified the heterochronic 'clock model' proposed by Gould (1977), with special attention on systems whose functions are characterized by their morphological appearance. By following and comparing allometry during the ontogeny of ancestor and descendant, the type of heterochrony as well as its direction and magnitude can be recognized and quantified. Allometric modelling allows an approximation to problems of morphological evolution from a dynamic perspective.

Heterochrony establishes a point of confluence between evolutionary and ecological theories through the study of the result of 'structural and development constraints' vs. 'environmental selection' upon evolutionary patterns (Gould, 1988). The heterochronic effects can be seen in a wide variety of levels of structural organization, from the cellular level to the vascular system or the individual plant as an integrated unity (Guerrant, 1988). It is therefore important to approach the studies from a hierarchical perspective through a combination of approximations at different levels—genetic, molecular, cellular and morphological (Raff & Wray, 1989). Heterochronic processes in plants are expressed differently from those in animals; the indeterminate growth and the modular construction of the former allow more pronounced phenotypic changes (Lord & Hill, 1987; Guerrant, 1988). Gould (1988) proposed that ultimately all processes of heterochrony can be regarded as adaptations once the proper ecological correlations have been established.

Although literature analysing heterochrony in plants is scarce, a few studies have been published that suggest this approach as an important tool for understanding the evolution of plant form. Takhtajan (1972) established the role of neoteny in the evolution of herbaceous angiosperms and the reduction in size of the male and female gametophyte. Lord & Hill (1987) have suggested that heterochrony is the major mechanism in the evolution of the cleistogamous floral form from the chasmogamous ancestral form in several species of unrelated families. On a microevolutionary scale, Guerrant (1988) studied neoteny as the main mechanism in the origin of a species of *Delphinium*, and Kellogg (1990) analysed ontogenetic changes in the allometry of florets in *Poa*. At the cellular level, Carlquist (1962, 1988) was one of the first authors to discuss the role of pedomorphosis in the evolution of wood anatomy. He used the concept to explain exceptional attributes in the wood of certain plants departing from the common trends (Carlquist, 1962). In particular, he analysed how certain characteristics of the primary xylem—such as length and shape of the vessel elements, lateral-wall pitting, and ray cells—have been protracted into the secondary xylem of some plants. The result is secondary wood with juvenile (i.e. primary) characteristics.

Because of the incredible variation in life-forms in the Cactaceae, it is particularly challenging to analyse the changes in wood anatomy linked to the evolution of size and growth-form in this family of mostly succulent, long-lived plants with highly parenchymatous stems. There are numerous studies on the anatomy and vascular organization in the subfamilies Pereskioideae and Opuntioideae (Bailey, 1960, 1962, 1962c, 1964a) and later works including the subfamily Cactoideae (Gibson, 1973, 1976, 1977, 1978). In these studies the ancestral characteristics of the secondary xylem of *Pereskia* (a genus of woody, only slightly-succulent cacti) have been described. *Pereskia* xylem is essentially a solid woody tissue, interrupted by small non-lignified primary rays in a helicoidal array (Gibson, 1978). It is highly specialized and totally lignified, composed of short vessel elements with simple perforation plates and with small pits in the lateral walls (Bailey, 1964a). On the other hand, Carlquist (1962) noticed that *Carnegiea gigantea*, a giant columnar cactus, does not show the well-established trends of wood evolution in dicotyledons, because the juvenile traits of the ancestor (those of the primary xylem) are retained into the secondary xylem.

In this work we make a comparative analysis of the vascular systems of five species of cacti belonging to different subfamilies and showing contrasting life-forms. We analyse the extent to which the dramatic morphological dissociation of the adult forms can be the result of a heterochronic process which becomes evident during the process of growth and structural organization. At the cellular level, we analyse age-on-length curves of the vessel elements as paedomorphic evidence. At the level of plant tissues, we study heterochrony taking as an allometric variable the percentage of xylem in transverse sections of the main shoot.

METHODS

We analysed six cactus species: *Pereskia lychnidiflora* De Candolle, *Pereskia aculeata* Miller, *Opuntia pilifera* Weber, *Neobuxbaumia tetetzo* (Coulter) Backeberg, *Ferocactus latispinus* (Glass) Taylor, and *Mammillaria collina* Purpus, representing typical and contrasting morphologies within the family (two woody leaf-bearing cacti, and four fully succulent life-forms: a cladode-stemmed cactus, a giant columnar cactus, a barrel cactus and a globose cactus, respectively). The genus *Pereskia*, comprising woody plants with sparingly succulent stems, has been considered to have ancestral characteristics (Gibson & Nobel, 1986; Bailey, 1962), and it is therefore the point of reference for our comparisons. We took several samples during part of the ontogeny from seedlings grown from seeds, and from juveniles and adults collected in the field.

Characterization of the vessel elements

We obtained series of samples of macerated xylem tissue following the Jeffrey method (Johansen, 1940), from the part nearest to the pith, and continuing radially towards the cambium. The vessel elements were measured in a Carl Zeiss, Axioscop mod. D-7082 microscope, considering the length from one terminal plate of the vessel element to the plate in the opposite side. The ligulae, when present, were not considered. In this study we used *Pereskia lychnidiflora*, a

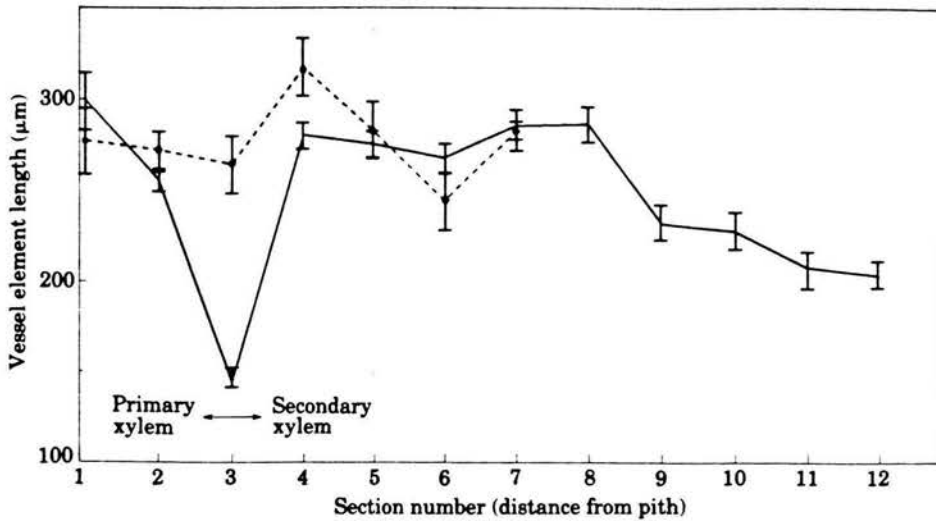


Figure 1. Age-on-length curve of the vessel elements of *Pereskia lychnidiflora* (—) and *Neobuxbaumia tetetzo* (·····). The abscissa shows the ranked order of the anatomical sections (each section is separated from the previous one by 3 mm), and is thus a measure of the distance from the pith of the stem to each section.

woody tree, instead of *Pereskia aculeata*, a woody climber, as sampling across the larger woody tissues of the former is easier. Quantitative data for vessel elements recorded in age-on-length curves (Fig. 1), were based on twenty measurements for each point for *Pereskia lychnidiflora*, and between five to ten points for *Neobuxbaumia tetetzo* (vessel elements are scant in this last species).

Quantification of xylem in histological slides of the main shoot

We obtained transverse sections of the main shoot at different ages and sizes for each species (in this case we used *Pereskia aculeata* for comparison instead of *P. lychnidiflora*, as its narrower stem diameter made the sectioning easier). In every section we measured the percentage of transverse area represented by xylem, with a Carl Zeiss, Axioscop mod. D-7082 microscope, and a computer program for image analysis (Videoplan). The usual techniques for sectioning and staining were used (Sass, 1961). In all cases, we measured the percentage of xylem at the base of the plant.

The percentage of xylem was considered an allometric characteristic that was correlated with the height of the stem (a measure of plant size). The allometric analysis was carried out following the classic allometric bivariate expression $y = kx^b$ (Huxley, 1932), where y is the percentage of xylem in the transverse section at the base of the main shoot and x is the height of the individual. The parameters k and b were obtained by non-linear estimation (Himmelblau, 1972).

Because of the very slow growth of cacti, our allometric study used both seedlings grown in the laboratory and adult plants collected in the field. Thus, our study is partly an ontogenetic allometric analysis, and partly a static allometric analysis *sensu* Cheverud (1982) and Gould (1966). The independent variable (height) is not strictly a comparable measure of age, as the same height may represent different ages for the different species. Nevertheless, for each

TABLE 1. Lateral-wall pitting of the vessel elements in the different cactus species. In all cases, the most frequent type of pitting found in the samples of macerated tissue is indicated

Species	Primary xylem	Secondary xylem
<i>P. lychnidiflora</i>	pseudoscalariform	alternate
<i>O. pilifera</i>	pseudoscalariform	pseudoscalariform and helical
<i>N. tetetzo</i>	pseudoscalariform	pseudoscalariform
<i>F. latispinus</i>	helical	helical
<i>M. collina</i>	helical	helical

species there is a monotonic relationship between height and age, and the independent variable (height) defines a well-established temporal sequence.

RESULTS

Characterization of the vessel elements

The age-on-length curves of the vessel elements of *Pereskia lychnidiflora* and *Neobuxbaumia tetetzo* are shown in Fig. 1. While *Pereskia* showed a pronounced and significant ($P < 0.00001$) change in vessel element length during stem growth, coinciding with the transition from primary to secondary wood, *Neobuxbaumia* showed no significant changes in vessel element length. The other species (*Ferocactus latispinus* and *Opuntia pilifera*) showed a similar pattern to *Neobuxbaumia tetetzo*. Table 1 shows the comparative analysis of the characteristics found in the vessel elements of each species. Again, it can be seen that while *Pereskia* showed a marked change in lateral-wall pitting of the vessel elements from pseudoscalariform to alternate pits, this change was not observed in the wood of the more succulent species.

Quantification of xylem in histological slides of the main shoot

The allometric model gave a significant ($P < 0.00001$) fit to the data ($r^2 = 0.93$, Table 2). Plant size explained a large part (43%) of the variance,

TABLE 2. Analysis of variance of the non-linear fit of the allometric function (percentage xylem vs. plant size) for the five species (in the case of non-linear models the variances are not strictly additive and the probabilities indicated by the tests are only approximate). Although the model fitted most (93% of the observed variance), a significant between-replicates error term was found. For this reason, and using a conservative criterion in the statistical tests, the F ratios were calculated using the largest error term (between-replicates error) in the denominator

Source of variation	Sum of squares	deg. of freedom	Mean square	F	Probability	r^2
Total model	6931.3	9	770.1	30.6	0.00002	0.93
Main effects						
Length	3187.4	1	3187.4	126.5	< 0.00001	0.43
Species	1038.1	4	259.5	10.3	0.00003	0.14
Interactions						
Length \times species	2705.8	4	676.5	26.8	< 0.00001	0.36
Error terms						
Total error	514.8	48	10.7			
Between replicates	302.2	12	25.2	3.5	0.001	
Within replicates	260.6	36	7.2			
Total variation	7446.1	57	130.6			

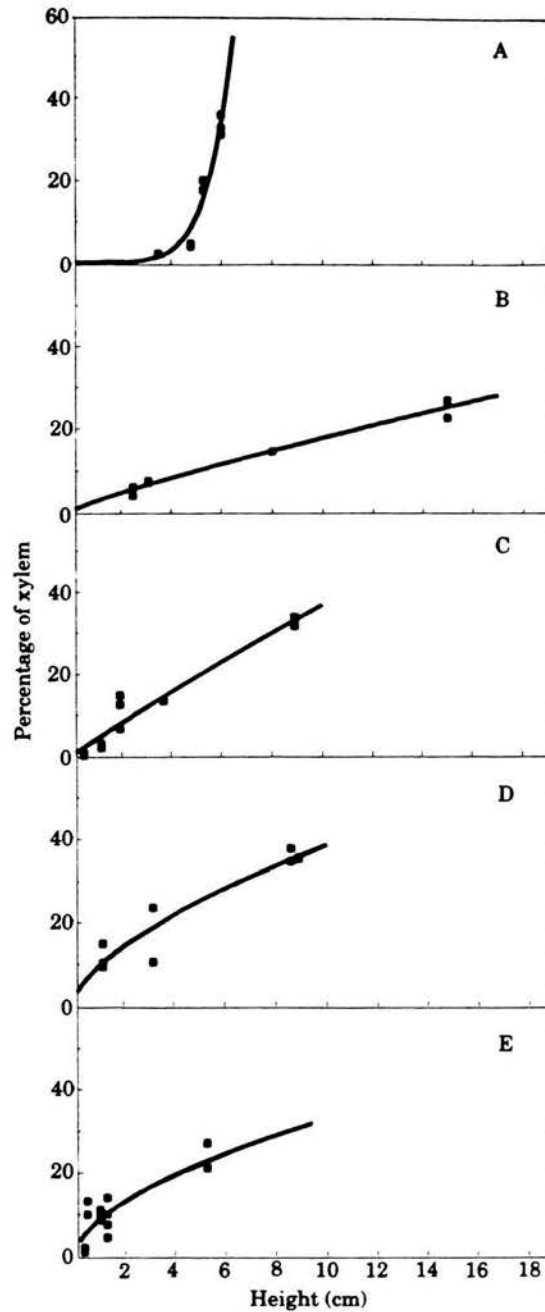


Figure 2. A-E. Allometric growth curves showing the percentage of xylem at the base of the plant vs. length. A, *Pereskia aculeata*. B, *Opuntia pilifera*. C, *Neobuxbaumia tetetzo*. D, *Mammillaria collina*. E, *Ferocactus latispinus*.

indicating that in all species there was a clear tendency to increase the percentage of xylem as the individuals grew. The interaction between plant size and species, however, was also highly significant and explained 36% of the observed variance. This indicates that, although the general trend for all species

TABLE 3. Values of the allometric parameter (b) and standard errors for the different species, obtained by non-linear estimation

Species	b	SE
<i>P. lychnidiflora</i>	6.20	± 0.780
<i>O. pilifera</i>	0.85	± 0.069
<i>N. tetelzo</i>	0.94	± 0.089
<i>F. latispinus</i>	0.57	± 0.084
<i>M. collina</i>	0.62	± 0.094

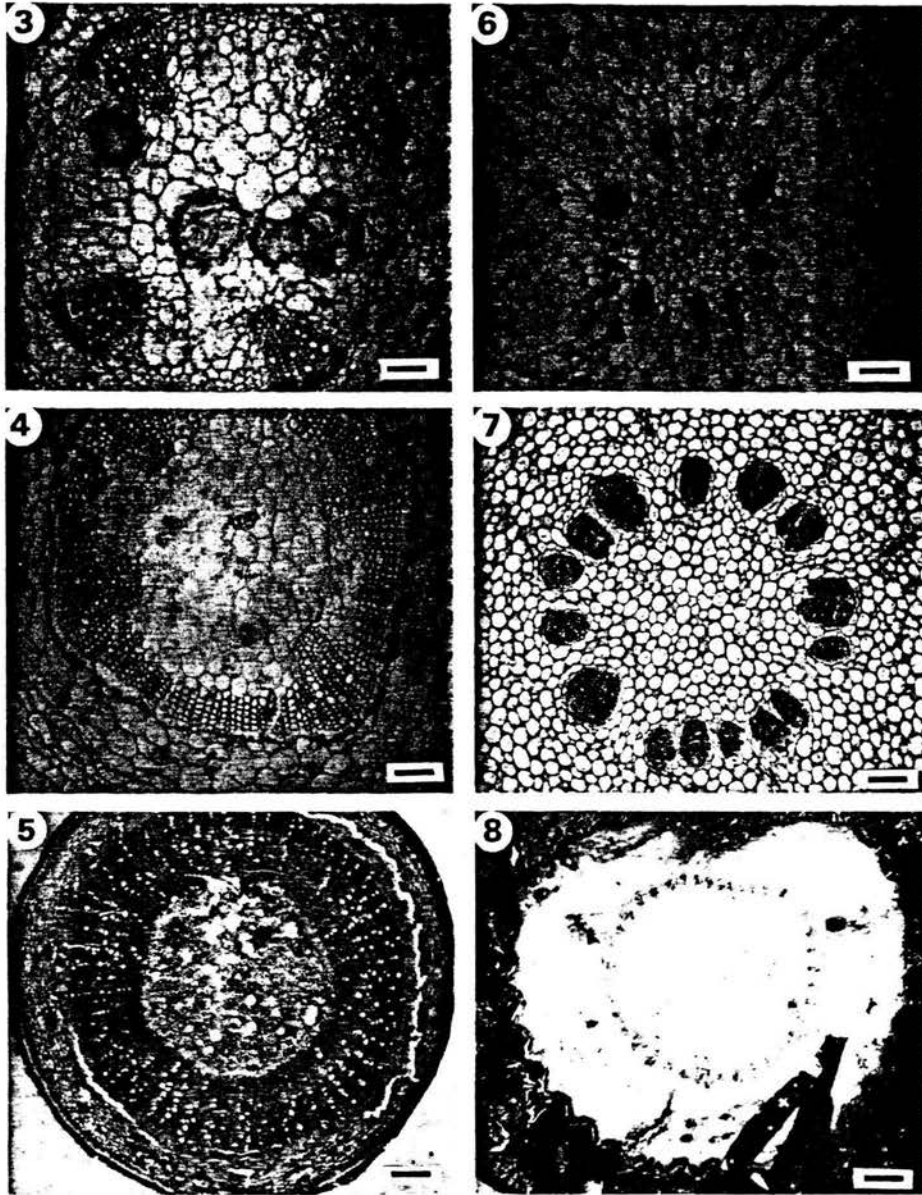
was to increase the proportion of xylem as they grew, some species increased their woody tissues at a significantly higher rate.

In the case of *Pereskia aculeata*, the allometric parameter (b) was significantly higher than unity ($P < 0.001$, Table 3), indicating that the proportion of xylem in the stem increases more rapidly than plant size (i.e. the plant lignifies quicker than it grows). In all other cases, the parameter was lower than unity ($P < 0.01$, with the exception of *N. tetelzo* where the parameter was lower than, but not significantly different from unity), indicating that in highly succulent cacti the proportion of xylem in the stem increases slowly in relation to size. The fitted allometric functions are shown in Fig. 2. Figs 3-9 and 9-12 show the development of xylem anatomy in transverse sections in an ordered time sequence, from seedlings to adults or from the tip of the main stem to the base, for four of the species studied.

CONCLUSIONS

Many physiological, anatomical and morphological features have changed in cacti as the species within the phylum developed succulence. The photosynthetic metabolism changed from C_3 to CAM, leaves were lost, axillary buds became transformed into areoles arranged along ribs or tubercles, and the stems became succulent and parenchymatous, with the woody tissues arranged in discrete vascular bundles. In most dicotyledons, a discontinuous cambium-producing xylem arranged in vascular bundles separated by parenchyma is typical of primary growth. Indeed, the most noticeable characteristic of secondary wood in most dicots is the development of a continuous cambial layer, responsible for the annual increment in stem diameter.

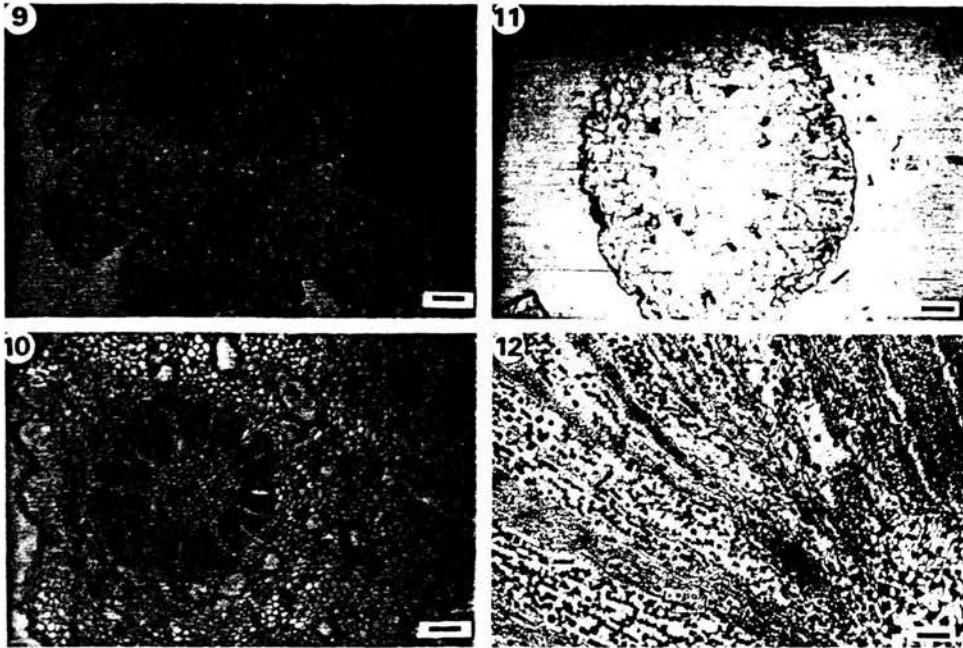
Our results show that, from many points of view, the evolution of succulence in cacti has implied a selection for juvenile characteristics in the woody tissues. Firstly, the change in lateral wall pitting that occurs in most plants during the transition from primary to secondary growth did not occur in the succulent cacti we studied, and was observed only in *Pereskia*, a scarcely succulent genus showing ancestral stem characteristics. Secondly, the marked decrease in vessel element length described by Carlquist (1962) as an important indication of the transition from primary to secondary growth, was observed only in *Pereskia*, and was conspicuously absent in the other species. Lastly, the allometric analysis showed that, while *Pereskia* lignified very quickly, producing the continuous cambial layer that is typical of secondary growth in dicotyledons, the other species lignified very slowly and maintained their xylem arranged in vascular



Figures 3-8. Transversal sections of stems of *Pereskia aculeata* (Figs 3-5), and *Neobuxbaumia tetelzo* (Figs 6-8). Fig. 3. Stem of a six-week-old seedling, showing separate vascular bundles. Fig. 4. Stem of thirty-week-old seedling, note that the cambium is now arranged in a continuous cylinder. Fig. 5. Stem section showing the adult pattern of xylem arrangement. Fig. 6. Apical section of a juvenile plant (c. 10 years old). Fig. 7. Lower section of a juvenile stem. Fig. 8. Breast-height stem section from an adult plant (more than 100 years old and c. 7 m high), note that xylem never fuses into a single cylinder. Scale bars: Figs 3-4 = 0.08 mm, Figs 5-7 = 0.32 mm and Fig. 8 = 24 mm.

bundles, separated by parenchyma and enclosing a large pith. In all cases it is obvious that while the species with the ancestral stem anatomy (*Pereskia*) produced true and typical secondary wood, the more succulent species retained in the adult xylem the characteristics of their primary wood.

Other authors have suggested that paedomorphosis, or juvenilism, may be the



Figures 9–12. Transversal sections of stems of *Mammillaria collina* (Figs 9, 10), and *Opuntia pilifera* (Figs 11, 12). Fig. 9. Apical stem section from a juvenile plant. Fig. 10. Basal stem section from an adult plant. Fig. 11. Stem of a thirty-week-old seedling, showing separate vascular bundles. Fig. 12. Stem section from an adult plant, showing the network of vascular bundles, in both cases the xylem remains in separate bundles. Scale bars: Figs 9–12 = 0.32 mm.

mechanism by which the stem anatomy of succulent cacti evolved. Gibson (1973) made a comparative study of the secondary xylem of cacti in order to find which factors have been important in the evolution of the succulent shoot in xeric environments. He suggested that the presence of vascular tracheids (a trait common in the primary xylem of plants) in mature succulent cacti is a product of juvenilism, i.e. the delay of the onset of maturity (see also Gibson & Nobel, 1986). Carlquist (1962) reported that *Carnegieia gigantea* has an almost horizontal age-on-length curve of vessel elements that did not reflect the transition from primary to secondary xylem.

In *Opuntia pilifera* the network of vascular bundles forms a wood-skeleton (Fig. 12) which only in very old stems becomes a solid cylinder (Gibson, 1978). In *Neobuxbaumia tetetzo*, a columnar growth-form, the bundles form separated rods of wood that run beneath the external ribs of the plant (Fig. 8). In *Ferocactus latispinus*, a barrel cactus, the wooden rods also follow the external arrangement of ribs. Finally, in *Mammillaria collina*, a globose plant, the bundles form an internal reticular network that follows the external phyllotactic arrangement (Fig. 10).

From the low values estimated for the allometric parameter (b) we can conclude that paedomorphosis in succulent cacti corresponds to allometric neoteny (McKinney, 1988; Alberch *et al.*, 1979; Gould, 1977). In other words, paedomorphosis in the wood anatomy of cacti is the result of a retardation in the rate of development. Selection for succulence—a trait typical of primary growth—has favoured the retention of juvenile ancestral characteristics in the

descendant adults. Thus, adult succulent cacti can be regarded, in a way, as 'giant seedlings' from the point of view of the anatomy of their vascular bundles. The obvious adaptive cost of this transition has been the loss of mechanical resistance as the capacity to store water was selected into the group.

ACKNOWLEDGEMENTS

Larry Venable, from the University of Arizona, Tucson, first directed our attention to the importance of allometric neoteny in some desert plants. We are indebted to him for the initial stimulus that triggered this study. We would also like to thank Esthela Sandoval for her support and for giving us access to the anatomy laboratory of the Botanical Garden at the University of Mexico. We are also indebted to Santiago Arizaga for technical support and to Consuelo Bonfil for assistance with the translation of the manuscript.

REFERENCES

- Alberch P, Gould S, Oster G, Wake D. 1979.** Size and shape in ontogeny and phylogeny. *Paleobiology* **5**(3): 296–317.
- Bailey IW. 1960.** Comparative anatomy of the leaf bearing Cactaceae. I. Foliar vasculature of *Pereskia*, *Pereskopsis* and *Quiabentia*. *Journal of the Arnold Arboretum* **41**: 341–356.
- Bailey IW. 1962.** Comparative anatomy of the leaf bearing Cactaceae. VI. The xylem of *Pereskia sacharosa* and *Pereskia aculeata*. *Journal of the Arnold Arboretum* **43**: 376–388.
- Bailey IW. 1963.** Comparative anatomy of the leaf bearing Cactaceae. IX. The xylem of *Pereskia grandifolia* and *Pereskia bleo*. *Journal of the Arnold Arboretum* **44**: 222–231.
- Bailey IW. 1964.** Comparative anatomy of the leaf bearing Cactaceae. XI. The xylem of *Pereskopsis* and *Quiabentia*. *Journal of the Arnold Arboretum* **45**: 140–157.
- Carlquist S. 1962.** A theory of paedomorphosis in dicotyledonous woods. *Phytomorphology* **45**: 29–45.
- Carlquist S. 1988.** *Comparative wood anatomy. Systematic, ecological and evolutionary aspects of dicotyledon wood*. New York: Springer-Verlag.
- Cheverud J. 1982.** Relationships among ontogenetic, static and evolutionary allometry. *American Journal of Physical Anthropology* **59**: 139–149.
- Gibson AC. 1973.** Comparative anatomy of secondary xylem in Cactoideae (Cactaceae). *Biotropica* **5**(1): 29–65.
- Gibson AC. 1976.** Vascular organization in shoots of Cactaceae. I. Development and morphology of primary vasculature in Pereskioideae and Opuntioideae. *American Journal of Botany* **63**(4): 414–426.
- Gibson AC. 1977.** Wood anatomy of Opuntias with cylindrical to globular stems. *Botanical Gazette* **138**(3): 334–351.
- Gibson AC. 1978.** Architectural designs of wood skeletons in Cacti. *The Cactus and Succulent Journal of Great Britain* **40**(3): 73–80.
- Gibson AC, Nobel P. 1986.** *The Cactus Primer*. Cambridge: Harvard University Press.
- Gould SJ. 1966.** Allometry and size in ontogeny and phylogeny. *Biological Reviews* **41**: 586–640.
- Gould SJ. 1977.** *Ontogeny and Phylogeny*. Cambridge: Belknap Press of Harvard University Press.
- Gould SJ. 1988.** The uses of heterochrony. In: McKinney M, ed. *Heterochrony in evolution*. New York: Plenum Press, 1–13.
- Guerrant EO. 1988.** Heterochrony in plants: the intersection of evolution ecology and ontogeny. In: McKinney M, ed. *Heterochrony in evolution*. New York: Plenum Press, 111–133.
- Himmelblau MD. 1972.** *Applied nonlinear programming*. New York: McGraw-Hill.
- Huxley J. 1932.** *Problems of relative growth*. London: MacVeagh.
- Johansen DL. 1940.** *Plant microtechnique*. New York: McGraw-Hill.
- Kellogg E. 1990.** Ontogenetic studies of florets in *Poa* (Gramineae): allometry and heterochrony. *Evolution* **44**(8): 1978–1989.
- Lord EM, Hill JP. 1987.** Evidence for heterochrony in the evolution of plant form. In: Raff R, Raff E, eds. *Development as an evolutionary process*: MBL Lectures in Biology **8**: Alan R. Liss, Inc, 47–70.
- McNamara KH. 1986.** A guide to the nomenclature of heterochrony. *Journal of Paleontology* **60**: 4–13.
- McKinney M. 1988.** Classifying heterochrony: allometry, size and time. In: McKinney M, ed. *Heterochrony in evolution*. New York: Plenum Press, 17–34.
- Raff R, Wray G. 1989.** Heterochrony: Developmental mechanisms and evolutionary results. *Journal of Evolutionary Biology* : 409–434.
- Sass EJ. 1961.** *Botanical microtechnique*, 3rd edition. Iowa: Iowa State University Press.
- Takhtajan A. 1972.** Patterns of ontogenetic alterations in the evolution of higher plants. *Phytomorphology* **22**(2): 164–171.



BIBLIOTECA
CENTRO DE ECOLOGIA

IV. Phyllotaxis and the evolution of stem succulence in cacti

Phyllotaxis and the evolution of stem succulence in cacti.

ABSTRACT

The differences in stem morphology between six cactus species were measured in the field, and their patterns of areole arrangement around the stem, or phyllotaxis, were modelled mathematically. The study was based on field data from the Valley of Zapotitlán, State of Puebla, and from the Isthmus of Tehuantepec, State of Oaxaca, Mexico. The mathematical model used only two parameters: (a) areole density in the stem (a measure of internode length or stem compaction), and (b) the divergence angle between successive areoles with respect to the central axis of the stem (a measure of the phyllotactic arrangement of leaf primordia). The stem morphology in different cactus subfamilies is correlated to changes in one or both parameters. Gradual changes in stem compaction and in the divergence angle can explain through a simple mechanism the apparently complex evolutionary transition from primitive woody and leafy plants to the more evolved, stem-succulent and densely spiny morphologies of cacti.

INTRODUCTION

A remarkable characteristic of the Cactaceae is the great variety of shapes and sizes that the family exhibits along its range in the American Continent, from southern Canada to Patagonia. The family is represented by arborescent, shrubby or creeping individuals with woody or succulent stems. Additionally, the succulent stems can be jointed, flat, cylindrical or globose in shape, with spines distributed uniformly around the stem or forming longitudinal ribs. The height of cacti can vary from 20 meters (*e.g. Carnegiea gigantea* in the Sonoran Desert, *Pachycereus pringlei* in Baja California and *Neobuxbaumia mezcalaensis* in the dry tropical forests of Oaxaca in Mexico), to approximately one centimeter (*e.g. Blossfeldia liliputana* in Argentina; Bravo-Hollis, 1978).

Gibson & Nobel (1986) hypothesized that the ancestors of the family were mesophyllous plants inhabiting subtropical regions with a more or less warm and humid climate, probably originated in the Caribbean region. The species of the subfamily Pereskioideae show some ancestral morphological and anatomical characteristics, like a woody stem, developed leaves and a C₃ photosynthetic metabolism. The development of succulence in the stem, the reduction or loss of leaves, and the switch to a CAM photosynthetic pathway

were the evolutionary novelties that allowed cacti to colonize new regions. With these novelties, the family also evolved the striking diversity of forms that can be observed at present. The phenotypic differences between the woody pereskioid trees and the leafless succulent species are so striking, that hypotheses on the evolutionary mechanisms that sustained these remarkable changes and generated the variety of current life-forms warrant examination. We have looked at the problem at different levels. At a physiological level, we have shown that plants belonging to different subfamilies with contrasting morphologies share the C_3 metabolic path during the early ontogeny. A few weeks after germination the succulent cacti shift to their typical CAM metabolism (Altesor et al., 1992). At an anatomical level, Carlquist (1962) described ancestral juvenile traits in the woody stem of adult cacti. More recently, we have shown that a retardation in the developmental rate of woody tissues (allometric neoteny) and the maintenance of the juvenile characteristics in the adult stems (paedomorphism) are the main evolutionary mechanisms operating in the development of succulence in the family (Altesor et al., 1994). Like most dicot seedlings, adult cacti have their vascular elements organized in discrete bundles and have a photosynthetic epidermis in the stem, while the rest of the shoot is formed by non-specialized parenchyma. Thus, from the point of

view of their stem anatomy, adult cacti are like giant seedlings, with overgrown primary wood forming giant vascular bundles surrounded by undifferentiated parenchyma, and a photosynthetic epidermis (Fig.1). Having provided some insight on the evolutionary transformation of cacti at the physiological and anatomical levels, in this paper we explore the morphological mechanisms underlying the development of stem succulence in cacti.

Almost all higher plants can be conceived as reiterative arrangements of basic stem elements as their architectural units. The most simple expression of these basic units is the stem segment formed by an internode, a node, a leaf and the axillary bud subtended by the leaf. At this level of organization, three basic morphologic variables modulate the architecture of the whole plant. The first one is the length of the internode. The second one is the relative position of the leaf in one internode with respect to the leaf in the previous internode (a phenomenon known in botany as phyllotaxis). The third variable is the probability of the axillary bud generating a lateral branch. A fourth important variable can be recognized as serially-dependent on the previous one, namely the angle that lateral branches form with the main stem. The phenomenon of branching as an important cause of plant architecture has been studied in great detail by a number of authors (Bell et al., 1979; White, 1984). In particular, a

series of formal rules, known as "L-systems" or "Lindenmeyer Grammars", have been elaborated to simulate the branching of plant stems (Lindenmeyer 1971, 1975). Although branching patterns are important elements in the evolution of cactoid forms, in this paper we shall concentrate in the first two variables described above, *i.e.* internode length and phyllotactic patterns. As we expect to demonstrate along this paper, there is reason to believe that these two variables have played a fundamental role in the evolution of stem succulence in the Cactaceae.

In the primary shoot of most dicotyledons, the vascular bundles that run along the stem perform the basic function of connecting the leaves to the rest of the plant (Gibson, 1978). Thus, vascular bundles join leaves along the length of the shoot, and their distribution inside the stem is strongly linked to the external arrangement of the leaves, *i.e.* to the phyllotactic pattern (Tomlinson & Wheat, 1979). With the appearance of secondary growth, a continuous cambial layer develops, the vascular bundles disappear and leaves (or branches that have developed from the axillary buds of pre-existing leaves) become connected to a continuous woody tissue (Fig.1). Cacti, however, retain the bundle-arrangement of vascular tissues throughout most of their lives (with the exception of the genus *Pereskia* which develops true secondary wood with a

continuous cambial layer). Additionally, the leaves and the subtended axillary buds are reduced in most cacti to a series of spine-cushions known as areoles, a distinctive feature of the cactus family. Basically, an areole is a transformed axillary bud situated over a tubercle with spines and often with hairs and trichomes. The basic vascular morphology of cacti, however, is similar to that of the seedlings of non-succulent dicots, in the sense that the vascular bundles run along the stem connecting neighbouring areoles. Thus, the phyllotactic arrangement of the areoles on the stem is strongly linked to the internal distribution of the vascular bundles (also known as "ribs" in some species).

As was discussed in a previous paper (Altesor et al., 1994), the evolution of stem-succulence has implied the need to maintain large masses of non-specialized parenchyma supported by a limited amount of vascular bundles as the main mechanical element. But the way the areoles are arranged externally has a direct effect on the way these support tissues are distributed inside the plant, and ultimately may have consequences on the ability of the whole plant to support its own weight. Thus, it may be hypothesized that selection for a given phyllotactic arrangement also implies a selection for a given internal distribution of the plant's vascular bundles, and ultimately, a selection for a certain biomechanical conformation. In cacti, phyllotaxis and plant

biomechanics seem to be strongly correlated, and hence possibly subject to a common process of natural selection.

In this paper we explore, through the use of a mathematical model, the changes in the divergence angle between neighbouring areoles and the changes in internode length that have occurred during the evolution of stem-succulence in different cactus life-forms. We do not analyse the actual physiological mechanisms by which the plants may attain control of these two parameters. We assume that they are controlled by the plant in some way, and thus that they may be subject to natural selection. This assumption is based on documented evidence that some plant species can plastically regulate the two parameters in response to environmental cues. In some tropical trees, for example, it has been documented that the orthotropic (vertical) shoots present spiral phyllotaxis with a divergence angle of *ca.* 137° , while the plagiotropic (horizontal) branches present distichous leaves alternating on a horizontal plane, with a divergence angle of *ca.* 180° (Fisher, 1978; Tomlinson, 1978). Changes in internode length have been well documented in plants showing "long shoots" and "short shoots" (*i.e.* elongated shoots with extended internodes and verticillate, or rosette-like, shoots with very short internodes). This phenomenon, which is very common in some deciduous desert species like

Fouquieria spp., allows the plants to produce leaves without investing heavily in woody tissues by producing short shoots, and to explore new spaces when the season is favourable and resources are abundant, by producing long shoots. Leaves produced on short shoots form axillary rosettes attached to the parent stems, while leaves produced on long shoots are separated by long internodes and explore new spaces (Bell 1991). Moreover, recent studies in plant molecular genetics have shown that the arrangement and the morphological characteristics of leaf and floral whorls in plants are genetically controlled (*e.g.* Coen and Meyerowitz, 1991; Pyke, 1994). From the above, it is possible to conclude (a) that stem compaction and the phyllotactic divergence angle seem to be independently controlled by the plant (*i.e.* one can vary substantially while the other remains constant), and (b) that variation in both parameters seems to be genetically controlled.

In short, we assume throughout this paper that both parameters can vary within one species, and hence that they may be subject to natural selection. Our main objective was to demonstrate that apparently small changes in the phyllotactic parameters, coupled with selection in favour of the juvenile vascular characteristics of the stem and with the evolutionary transformation of leaves into spines, may account for the striking morphological variation of

cacti. Computer simulations and the evaluation of the minimum number of mathematical parameters needed to obtain a given morphology have shown to be powerful tools in the study of biological form (e.g. Niklas and Kerchner 1984).

The theory of phyllotaxis

The term phyllotaxis means "leaf arrangement", but in its broadest sense it is currently meant to encompass the study of the incorporation into the individual plant of new parts (leaves, bracts, flowers, etc) that are similar to parts that are already present, and the spatial arrangement of these parts (Jean, 1984). There is a vast literature on this subject, spanning more than 170 years, that has produced a rather obscure and sometimes contradictory nomenclature which is not always easy to understand. For our specific problem, perhaps the most important fact to be borne in mind with respect to the problem of leaf arrangement is that an optimal packing of alternate leaves (or any other set of parts arranged alternately around a circular or cylindrical structure) can be achieved for a fixed leaf arrangement. This arrangement is generated when the divergence angle between neighbouring leaves is a fraction of a circumference

equal to $(3-\sqrt{5})/2$, an irrational constant value which we will refer to as the "Fibonacci angle" or " φ ", and which is equal to $137^{\circ}30'27.95\dots$ " (Jean, 1984).

The mechanisms through which the plants achieve and regulate this packing of parts around the stem have been explained through a number of hypotheses (a detailed review is given in Jean, 1984), including three important mechanistic theories. (a) The theory of contact pressures (Richards, 1951; Adler, 1977) stipulates that competition for meristematic space, manifested by physical contact between leaf primordia, generates the pattern of distribution of leaves around the stem. (b) The theory of first available space (Snow & Snow, 1962) postulates that new primordia will be generated near the apical meristem as far as physically possible from already existing primordia. (c) The theory of diffusion of an inhibitor (Hellendoorn & Lindenmeyer, 1974; Thornley, 1975 a,b; Mitchinson, 1977; Young, 1978) proposes that biochemical substances produced by pre-existing primordia inhibit the formation of new primordia in their proximity. These three theories are not mutually exclusive, and in fact the three mechanisms may operate simultaneously. Recent mathematical simulations have shown that the organization of plants parts around a stem in Fibonacci angles can be obtained as the result of collisions between primordia (Fowler, Prusinkiewicz and Battjes, 1992). A detailed review of possible

mechanisms controlling organogenesis at the shoot apex is given in Lydon (1994).

Apart from the mechanistic theories, which have tried to explain the morphogenetic mechanisms of leaf arrangement in plants, some authors have tried to find a functional explanation to this phenomenon. The functional theories of phyllotaxis have based their emphasis on the adaptive value of different phyllotactic distributions for the adult plant, arguing that a leaf arrangement in Fibonacci angles permits the optimal interception of light (Wiesner, 1875; Jean, 1984; Niklas, 1988), or a minimal overlap between neighbouring leaves (Wright, 1873).

The Fibonacci divergence angle ($\varphi=(3-\sqrt{5})/2$) is related to the Fibonacci series ($\{0,1,1,2,3,5,8,13,21\dots\}$); a mathematical series where the term $f_{n+1} = [f_n+f_{n-1}]$. The limiting value for the ratio (f_{n-1}/f_{n+1}) when $n \rightarrow \infty$, is φ (Niklas, 1992). This means that, in any plant that has a Fibonacci phyllotactic angle, leaves that are f_n leaves away from any given one (where f_n is the n th term of the Fibonacci series) will tend to coincide nearly above the original one. A set of nearly-coinciding leaves forms what is known as a "contact parastichy", or series of leaves forming spirals along the stem. The number of contact parastichies that are visible on a plant is a function of the degree of compaction

of the stem (*i.e.* of the internode length; Fig.2). Additionally, because of the numerical proximity of the value of φ with the ratio between terms of the Fibonacci series, it follows that the number of spirals in a contact parastichy will be a Fibonacci number.



METHODS

Phyllotaxis

We chose five cactus species: *Pereskia lychnidiflora* De Candolle, *Opuntia pilifera* Weber, *Neobuxbaumia tetetzo* (Coulter) Backeberg, *Ferocactus latispinus* (Glass) Taylor, and *Mammillaria collina* Purpus, representing typical and contrasting morphologies within the family (a woody, leaf-bearing cactus, and four fully-succulent life-forms: a cladode-stemmed cactus, a giant columnar cactus, a barrel cactus and a globose cactus, respectively). In several individuals of each species we measured indicators of the two morphological parameters under study (internode length and divergence angle). Internode length was evaluated through a measure of stem compaction, calculated as the number of areoles per unit stem length. To make plants of different sizes comparable, the unit length of the stem in each species was arbitrarily defined as equal to the diameter of the stem. We also counted the

number of ribs or areole helices (contact parastichies) to evaluate the value of the divergence angle generating the phyllotactic arrangement. Finally, photographs were taken to make further analyses in the laboratory. *Pereskia lychnidiflora* was observed in the Isthmus of Tehuantepec, State of Oaxaca, and the other species in the Valley of Zapotitlán, State of Puebla, Mexico, an area of great endemicity of cactus species.

Number of ribs in barrel and columnar cacti

We counted the ribs in 44 randomly-selected individuals of *Ferocactus latispinus*, in 95 randomly-selected individuals of *Neobuxbaumia tetetzo*, and in 20 randomly-selected individuals of *Echinocactus plathyacantus*. *Ferocactus* is a barrel species with helicoidal ribs, while *Neobuxbaumia* is a giant columnar species with longitudinal ribs. *Echinocactus*, a barrel cactus with longitudinal, not helicoidal, ribs, was included in this analysis for comparison purposes. In *Ferocactus* and *Echinocactus* we counted the number of ribs at the top of the stem of large adult plants. In *Neobuxbaumia* we counted the ribs at eye height, ca. 1.7 m from the ground. The resulting distribution of rib numbers was tested for randomness by comparing it against a theoretical Poisson distribution with the same mean. With this test we

evaluated if variation in rib numbers from plant to plant could be considered a random process around a certain allometric mean, or if, alternatively, the number of ribs is dependent on some other factor rather than plant size.

Vascularization

We also selected plant "skeletons" (*i.e.* the remains of the vascular system from dead plants) from two contrasting species. The distribution of vascular bundles inside the stem can be easily observed in these dried structures, as the external photosynthetic epidermis and the internal succulent parenchyma rot quickly once the plant dies, and only the vascular tissues remain. We chose to measure the vascularization of a cylindrical opuntioid species (*Opuntia fulgida* from the Sonoran Desert) related to the flat-stemmed *O. pilifera*, as the vascular tissues from cylindrical stems are better preserved under field conditions and allow easier measurements. We also measured the vascularization of a giant columnar cactus with prominent ribs. For this purpose we used *Neobuxbaumia tetetzo*, the same columnar species that was used for the external morphological measurements explained in the previous section.

Simulation studies

A computer program was written to simulate the distribution of areoles around a cylindrical stem section. The program is based only on two parameters: (a) stem compaction (a function of internode length), and (b) the angle between successive areoles. The output of the program is a graphic representation of the resulting conformation (e.g. Fig.2).

The program allows either to enter a particular divergence angle, or to choose from a set of predefined angular values. Among the predefined values, ϕ , the Fibonacci angle, can be chosen to generate an optimally-packed and regularly-distributed set of areoles for a given stem compaction. The program also allows to choose angular values from any pair of terms of the Fibonacci series (f_{n-1}/f_{n+1}). For example, the terms (f_4/f_6)=(3/8) yield an angular divergence of 0.375 of a circumference, or $135^\circ 00' 00''$. Because these angles are not irrational numbers, they will generate sets of areoles that form longitudinally ribbed plants. For example, the value (3/8) will generate stems with 8 ribs, as the 9th areole of a series will appear exactly above the first one ($135^\circ \times 8$ is 1080° , an exact multiple of 360°).

Simulations were done by feeding the program with the phyllotactic values of the field plants. Alternatively, in cases where the exact measurement

of the divergence angle in the field had been difficult, the program was used to obtain by trial and error the parameter estimate that repeated in the computer screen the architecture observed in the field. Stem compaction was measured successfully in all plants.

RESULTS

Phyllotaxis

The Fibonacci divergence angle (φ) gave the best description of the areole arrangement in the ancestral-like species *Pereskia lychnidiflora*, in the cladode-stemmed *Opuntia pilifera*, and in the globose *Mammillaria collina*. While the generating angle in these three species was uniformly $(3-\sqrt{5})/2$, large between-species differences were observed in the degree of stem-compaction. The less compacted form was *Pereskia lychnidiflora*, followed by *Opuntia pilifera*, and lastly by *Mammillaria collina*, the most densely compacted species of this group (Fig.4). The columnar and barrel forms (*Neobuxbaumia tetetzo* and *Ferocactus latispinus*) also had high values of stem compaction. Additionally, they presented a divergence angle different from φ , the Fibonacci angle, that allowed the alignment and fusion of tubercles to form the ribs (Fig.4).

Number of ribs in barrel and columnar cacti

Individuals of *Ferocactus latispinus*, 49% had 13 ribs in the stem, 42.2% had eight ribs and 6.8% had five ribs (Fig.3).

In this species corresponded in all cases to numbers of the Fibonacci series. Their distribution departed very significantly from the expected under the assumption of a random process of rib formation. It is interesting to note that ribs in this species of barrel cacti, are not longitudinally arranged but form a

Individuals of *Neobuxbaumia tetetzo*, 29% had 13 ribs, 26.9% had 12 ribs, 7.5% had 15 ribs, 5.4% had 8 ribs, and the remaining were distributed among 7, 9, 10, 11, 16, 17 and 19 ribs (Fig.3).

The distribution shows two peaks significantly above the expected values that also correspond to Fibonacci numbers (8 and 13), and two below them (12 and 14). In this species the ribs are longitudinally arranged. It was also found that the 20 individuals of *Ferocactus platyacanthus* did not differ significantly from the expected distribution ($\chi^2=6.5$, d.f.=3, the categories had to be pooled

to show a tendency to

Opuntia fulgida and *Opuntia* species corresponding to the Fibonacci series. The arrangement of the areoles in *Opuntia* species is like its relative *O. fulgida*. In *Opuntia* species the ribs are arranged in a spiral pattern, spiraling in one direction.

Opuntia species does not have a spiral pattern. The areoles are organized in a regular pattern (Fig.5).

The tatic patterns of the spiral pattern and the spiral patterns are coincides with the

result of our model. The lowest compaction of areoles was found in *Pereskia lychnidiflora*, a species with a Fibonacci divergence angle (φ). This species belongs to a subfamily (Pereskioideae) presenting ancestral stem characteristics: they are large woody plants with sparingly succulent stems, arborescent growth, and developed leaves. Increasing stem-compaction through the simple shortening of the internodes without modifying the divergence angle, generates the apparently complex spiral phyllotactic patterns of some of the stem-succulent cacti. This is the case of *Opuntia pilifera*, a cladode-stemmed cactus with very reduced and ephemeral leaves belonging to the subfamily Opuntioideae. The highest degree of stem-compaction is present in the globose forms of the subfamily Cereoideae, exemplified in this study by *Mammillaria collina* (Fig.4). The dense spiral phyllotaxis of these globose plants allows a most efficient packing of the areoles uniformly around the stem, and results in a dense reticulum of spines that covers the plant. Nobel (1978) has stressed the importance of spines in these plants as a means of self-shading and of defense against predators (McAuliffe 1984). In stem-succulent cacti with a Fibonacci divergence angle (φ), the vascular bundles are organized in a reticular network that corresponds to the spirals connecting neighbouring

areoles (Fig.5). The separated vascular bundles allow the change of volume of the plant, and facilitate the accumulation of water in the tissues (Nobel, 1978).

Because of their large size, the columnar forms, and to a lesser degree the barrel ones, face the problem of erect growth, and with it also face a major adaptive cost of the development of succulence: the loss of mechanical resistance. Vascular bundles organized in longitudinal ribs seem to have emerged through natural selection as a solution to this problem. In ribbed species, the vascular bundles form separated rods that run under the external ribs of the plant. This longitudinal arrangement allows the support of higher amounts of live tissues than the reticular arrangement of opuntoid and globose plants. Additionally, separated vascular ribs also allow the rapid increase of stem volume when water is quickly absorbed after abundant rains. Thanks to this system, some giant columnar cacti can absorb water totalling *ca.* 10% of their standing mass (representing as much as 50 litres in large adult plants) in only four days after receiving adequate rainfall, and in approximately 20 days they can store water representing as much as 50% of their initial standing mass(*i.e.* 250 litres; Nobel, 1988). Thus, the evolutionary transition to columnar growth seems to have been directed by the change from a reticulate phyllotaxis generated by a Fibonacci divergence angle (ϕ), to a ribbed system

of parastichies generated by a rational divergence angle that allows the development of areoles immediately above previously existing ones.

The rib numbers of *Ferocactus latispinus* correspond to terms of the Fibonacci series (Fig.3). It was also observed that seedlings usually had 3 ribs, followed by small plants with 5 ribs, intermediate plants with 8, and large plants with 13 ribs. These results agree with data reported by Gibson & Nobel (1986) for other 15 species of *Ferocactus*. According to our model, the divergence angle in *Ferocactus latispinus* corresponds approx. to $137^{\circ}39'$ (Fig.4). The variation in the mathematical value of the divergence angle is not quantitatively very large from the Fibonacci value ($\phi=137^{\circ}30'27.95\dots$) that generated the non-ribbed spiral phyllotaxis in the case of the first three species. Both angles differ in approximately $9'$, but that small parametric difference was enough to produce a very important change in the architecture of the whole plant. It is interesting to note here that *Ferocactus latispinus*, like many other barrel cacti, has spiral ribs, and not true longitudinal ribs like all columnar cacti. When stem compaction is high, spiralling rows of areoles (the contact parastichies) are formed in Fibonacci numbers (Fig.2). It is therefore straightforward to postulate that spiral ribs can easily originate through the

joining of parastichies by means of underlying vascular bundles, accompanied at the same time by very small changes in the divergence angle.

The giant columnar species *Neobuxbaumia tetetzo* presents longitudinal ribs in numbers which do not always coincide with the Fibonacci series. In theory, once a plant develops a longitudinal arrangement of areoles, there is no adaptive reason why it should show ribs in Fibonacci numbers. Any number of ribs proportionate to the size of the plant will serve the adaptive purpose of providing mechanical support. The fact that *Neobuxbaumia tetetzo* presents ribs in Fibonacci numbers more frequently than in any other number, seems more likely to be the result of some sort of evolutionary "memory" of the plant. That is, if the hypothesis that results from our analysis, in the sense that ribbed morphologies have derived from ancestral Fibonacci (φ) patterns by joining adjacent contact parastichies is true, then it could be expected that some columnar plants would still produce ribs preferentially in Fibonacci numbers, even if those ribs were perfectly longitudinal. In support of this idea, we have observed that the seedlings of *Neobuxbaumia tetetzo* are not ribbed but follow a Fibonacci pattern (*i.e.* their divergence angle is approximately φ) during their very early ontogeny (less than 6 months of age). This means that the same transition that the adult individuals of the species have made in evolutionary

time can also be observed within one individual during the early ontogeny. In some cacti with non-spiralling longitudinal ribs, like *Echinocactus plathyacantus*, the number of ribs is randomly distributed around a mean value, and does not follow a distribution in Fibonacci numbers. It seems that once a species has developed longitudinal ribs, natural selection may operate basically on the number of ribs, and the evolutionary "memory" of the Fibonacci numbers may become eventually lost.

Summing up our conclusions, two morphological variables seem to have changed together with the development of stem succulence in cacti and the replacement of leaves for spines. On the one hand, stem compaction and the packing of areoles seems to have played an important role during the transition from woody and leafy ancestral species into the modern stem-succulent plants. On the other hand, the transformation from non-ribbed to ribbed species seems to have been accompanied by changes in the phyllotactic angle, from the ancestral Fibonacci value (φ) to other values that permitted the evolutionary transformation of contact parastichies into true ribs. Finally, our model shows that when stem compaction is high, small changes in the phyllotactic divergence angle are enough to produce dramatic transformations in both the external morphological appearance and in the internal arrangement of vascular bundles.

These changes may have an extraordinary effect on the mechanical properties of the whole plant.

REFERENCES

- Adler, I. 1977. The consequences of contact pressure in phyllotaxis. *J. Theor. Biol.*, **65**: 29-77.
- Altesor, A., Ezcurra, E., Silva, C. 1992. Changes in the photosynthetic metabolism during the early ontogeny of four cactus species. *Acta Oecologica*, **13**: 777-785.
- Altesor, A., Silva, C., Ezcurra, E. 1994. Allometric neoteny and the evolution of succulence in cacti. *Bot. J. Soc. Linn.*, **114**:283-292.
- Bell, A.; Roberts, D. & Smith, A. 1979. Branching patterns: The simulation of plant architecture. *J.Theor. Bio.*, **81**: 351-375.
- Bell, A. 1991. *Plant form. An Illustrated Guide to Flowering Plant Morphology*. Oxford University Press, Oxford.
- Bravo-Hollis, H. 1978. *Las Cactáceas de México. Vols. I, II and III*. Universidad Nacional Autónoma de México, México D.F.
- Carlquist, S. 1962. A theory of pedomorphosis in dicotyledonous woods. *Phytomorphology*, **12**: 29-45.

- Church, M.A. 1920. *On the Interpretation of Phenomena of Phyllotaxis*.
Hafner Publishing, N.Y. & London.
- Coen, E.S. & Meyerowitz, E.M. 1991. The war of the whorls: genetic interactions controlling flower development. *Nature*, **353**:31-37.
- Fisher, J.B. 1978. A quantitative analysis of *Terminalia* branching. In: P.B. Tomlinson & M.H. Zimmermann (eds.). *Tropical trees as living systems*. Cambridge University Press, Cambridge. pp. 285-320
- Fowler, D.R., Prusinkiewicz, P. & Battjes, J. 1992. A collision-based model of spiral phyllotaxis. *Computer Graphics* 26:361-368.
- Gibson, A.C., Nobel, P. 1986. *The Cactus Primer*. Harvard University Press, Cambridge, Mass.
- Gibson, A.C. 1978. Architectural designs of wood skeletons in Cacti. *The Cactus and Succulent Journal of Great Britain*, **40**: 73-80.
- Hellendoorn, P.H., Lindenmayer, J.A. 1972. The shoot apical ontogeny of the *Picea abies* seedling. I. Anatomy, apical dome diameter, and plastochron duration. *Am. J. Bot.*, **59**: 587-597.
- Jean, R. 1984. *Mathematical Approach to Pattern and Form in Plant Growth*. Wiley Interscience, New York.

- Jean, R. 1988. Phyllotactic Pattern Generation: A Conceptual Model. *Annals of Botany*, **61**: 293-303.
- Lindenmayer, A. 1971. Developmental Systems without Cellular Interaction: Their Languages and Grammars. *J.Theor. Biol.* **30**: 455-484.
- Lindenmayer, A. 1975. Developmental Algorithms for Multicellular Organisms: a Survey of L-systems. *J.Theor. Biol.* **54**: 3-22.
- Lydon, R.F. 1994. Control of organogenesis at the shoot apex. *New Phytol.* **128**:1-28.
- McAuliffe, J. 1984. Prey refugia and the distributions of two Sonoran Desert Cacti. *Oecologia*, **65**:82-85.
- Mitchison, G.H. 1977. Phyllotaxis and the Fibonacci series. *Science* **196**: 270-275.
- Niklas, K.J. 1988. The role of phyllotactic pattern as a "developmental constraint" on the interception of light by leaf surfaces. *Evolution*, **42**:1-16.
- Niklas, K.J. 1992. *Plant Biomechanics. An Engineering Approach to Plant Form and Function*. The University of Chicago Press, Chicago and London.

1984. Mechanical and photosynthetic
of shape. *Paleobiology*, **10**:79-101.

temperatures of cacti, influences of environmental
Ecology, **59**:986-996.

Evolutional Biology of Agaves and Cacti. Cambridge
University Press. 270 pp.

use in the genetic and molecular analysis of
Phytol. **128**:19-37.

its quantitative expression and relation to
R. Soc. Lond., **235B**: 509-564.

theory of the regulation of phyllotaxis based on
R. Soc. Lond. **224 B**: 483-513.

phyllotaxis. I. A Mechanistic Model. *Ann. Bot.*, **39**:

phyllotaxis. II. A description in terms of intersecting
Ann. Bot., **39**: 509-524.

phyllotaxis and axis differentiation in Tropical trees. In:
Zimmermann (eds.). *Tropical trees as living
forests*. Cambridge University Press, Cambridge. pp. 285-320.

phyllotaxis in Rhizophoreae.

J. Theor. Biol., **71**:

phyllotaxis. In: (eds.) *Plant*

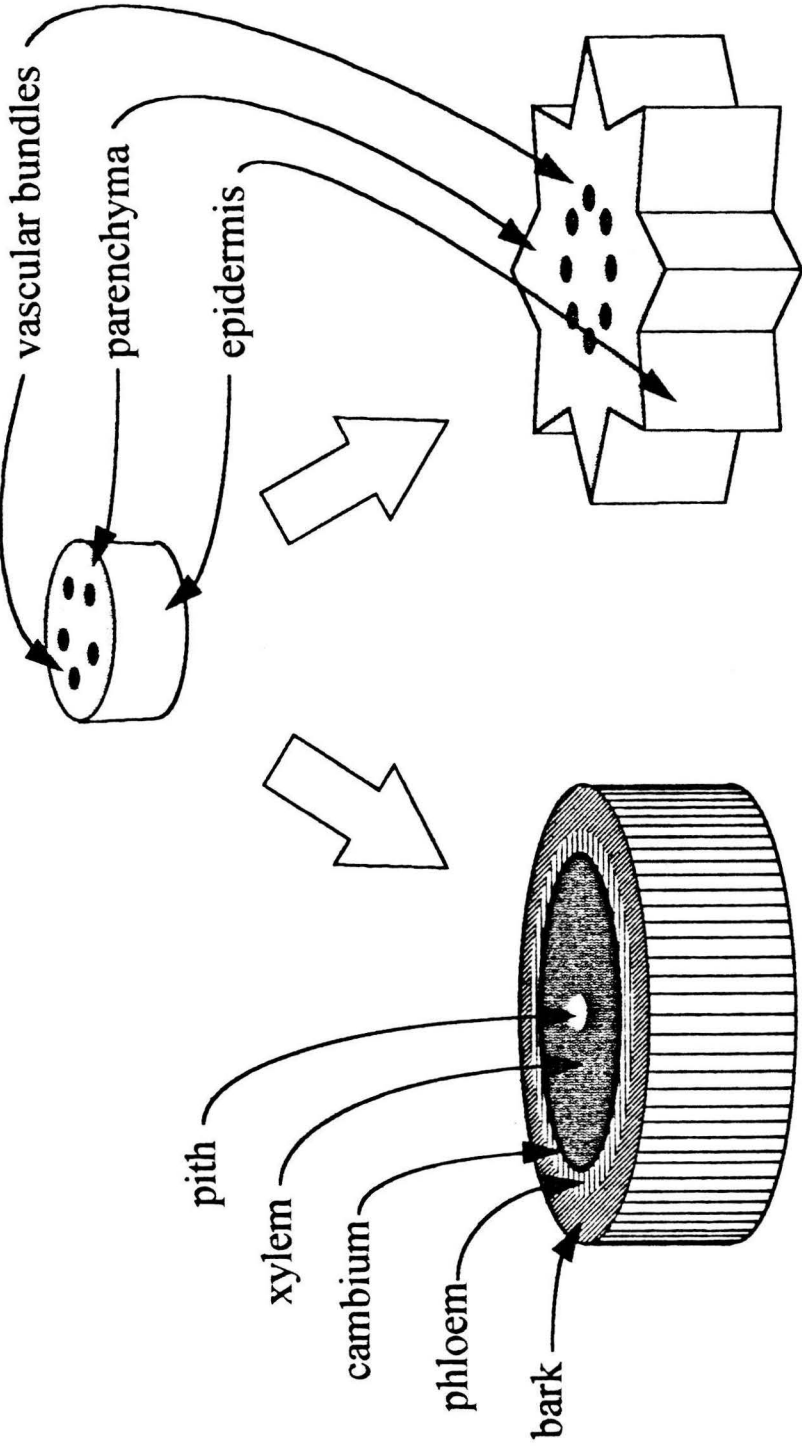
phyllotaxis of leaves in plants.

Figure 1.- Allometric neoteny in cacti. The basic characteristics of primary shoots (top) are conductive tissues organized in discrete vascular bundles surrounded by non-specialized parenchyma, and a photosynthetic epidermis. With secondary growth (bottom left), the cambial meristem forms a continuous ring that produces xylem towards the center and phloem towards the exterior. As it grows outwards, phloem is transformed into a non-photosynthetic, suberous bark which replaces the epidermis of primary shoots. In stem-succulent cacti (bottom right) the basic anatomy of primary shoots is maintained in the adult plants.



BIBLIOTECA
CENTRO DE ECOLOGIA

primary shoot

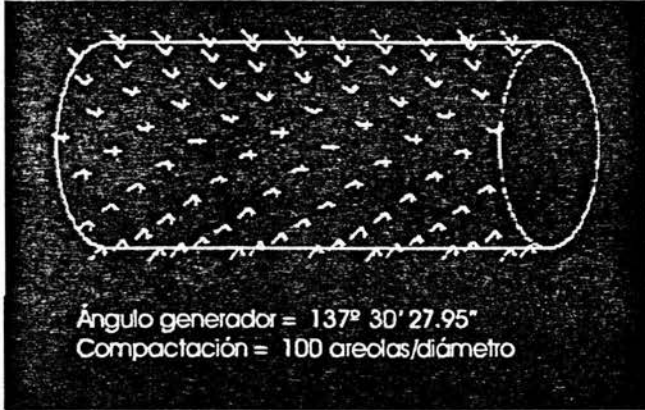


stem-succulent cacti

true secondary growth

Figure 2.- Cylindrical representation of cactus stem sections. The phyllotactic divergence angle increases from a Fibonacci value (F) to a near-Fibonacci value from left to right. The stem compaction increases from top to bottom. Note that small changes in the phyllotactic angle produce drastic changes in the plant morphology when stem compaction is high, but produce unnoticeable results when the compaction is low.

Compactación



Ángulo generador

distribution (bars) of rib numbers in selected barrel and the expected Poisson distribution (lines). (a) Distribution of rib numbers in *Perocactus latispinus* in the upper section of stem. (b) Distribution of rib numbers in *Neobuxbaumia tetetzo* at eye-level height. Both distributions differ significantly from the Poisson model ($\chi^2=81.8$, d.f.=7, $P<0.001$, respectively).

field models. (a) *Pereskia*
 ."; stem compaction = 16
 a. = $137^{\circ}30'27.95\dots$ "; s.c. =
 $37^{\circ}30'27.95\dots$ "; s.c. = 300
 39'; s.c. = 50 areoles). (e)
 = 90 areoles). In all cases,
 ated from the plant pictured

Compactación = 10 areolas/diámetro

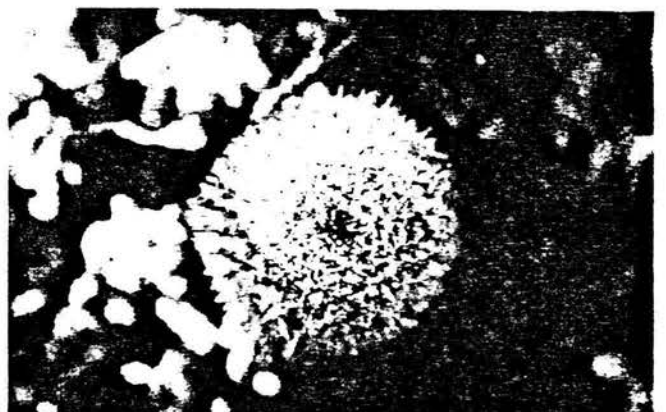
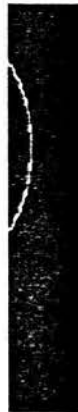
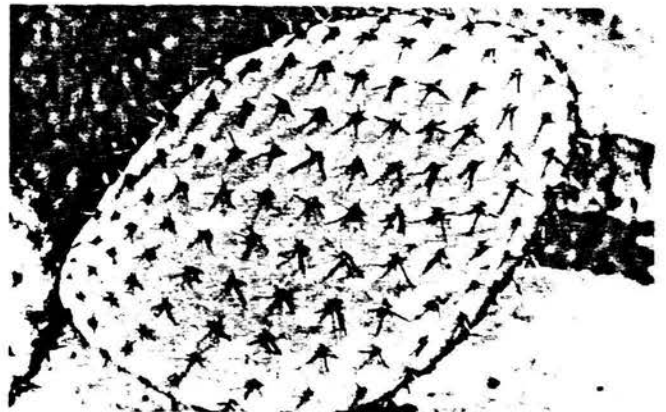
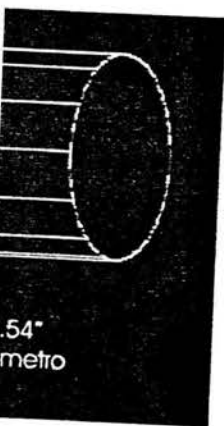
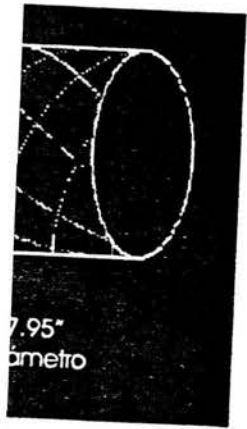


Figure 5.- (a) Vascular skeleton of *Opuntia fulgida* in the field, and the modelled representation of vascularization by generating lines ("bundles") that connect neighbouring areoles (divergence angle = $137^{\circ}30'27.95\dots$ "; stem compaction = 40 areoles per unit diameter). (b) Vascular skeleton of *Neobuxbaumia tetetzo*, and its modelled representation (d.a. = $138^{\circ}27'41''$; s.c. = 90 areoles). A small change in the phyllotactic angle predicts the formation of vascular ribs.



CONCLUSIONES

El desarrollo de la succulencia fue la novedad evolutiva que permitió a las cactáceas cruzar nuevas fronteras adaptativas y distribuirse a través de todo el Continente Americano. La disociación morfológica observada entre los adultos que han desarrollado la succulencia en el tallo y las cactáceas con leño y hojas, responde a un proceso de reorganización funcional que puede detectarse a diferentes niveles de complejidad.

Los tres artículos que conforman esta tesis comprenden estudios de procesos en la evolución de la succulencia, a nivel fisiológico, anatómico y morfológico.

En la introducción señalamos tres eventos interrelacionados ocurridos en el curso de la evolución de las cactáceas: la reducción de las hojas hasta la pérdida de las mismas; la formación de tejido succulento en el parénquima y médula, y el cambio de metabolismo fotosintético C_3 a CAM.

El enfoque que guió el trabajo fue el estudio comparativo del desarrollo en cinco especies que presentan características morfológicas contrastantes y pertenecen a las tres subfamilias: Pereskioideae, Opuntioideae y Cereoideae.

norias" filogenéticas o de novedades
 llo, el primer nivel de aproximación
 lismo fotosintético. Se cuantificó y
 te las primeras semanas de vida en
 a" ontogenética del carácter ancestral,
 esentaron metabolismo C₃ durante las
 eskia aculeata, las especies derivadas
 las cactáceas, entre la décima y la
 en las primeras semanas de vida, etapa
 ortantes consecuencias desde el punto
 ecimiento típica del metabolismo C₃
 PAR recibidos bajo la planta nodriza y
 iodo de establecimiento. Otro factor
 a temperatura, que en ocasiones puede
 La apertura de los estomas durante el
 3 permite la transpiración con la
 de esta ventaja adaptativa será una

osintético y leño pedomórfico
 estudiadas en dos grupos. El
lichnidiflora, reuniendo los
 la ontogenia y en el adulto;
 nillo continuo de cambium y
 ; sin lignificar; y presencia de
 o se desarrolla la succulencia,
 parece el metabolismo CAM
 los adultos del segundo grupo
 jico. Se fijó la atención en la
 as, en este caso las areolas, en
 arámetros fáciles de medir en
 as y el ángulo de divergencia
 filotaxia y la disposición del
 consecuencias mecánicas de la
 on muy baja (16 areolas por
 i filotaxia típicamente espiral
 a 137°30'27", corresponde a

Pereskia licheniflora. Un aumento en la compactación, con el mismo ángulo de generación da por resultado la morfología típica de *Opuntia*. El mayor empaquetamiento de areolas, manteniendo el ángulo de divergencia, resulta en la morfología de las cactáceas globosas como *Mammillaria*. Tal densidad de areolas les permite evitar el sobrecalentamiento de los tejidos, gracias al efecto de autosombreado, así como protegerse de los depredadores.

Para altos niveles de compactación, un cambio mínimo en el ángulo de divergencia da lugar a un cambio morfológico importante como el observado en las formas de barril como *Ferocactus latispinus*. Enfrentar el crecimiento erecto y por lo tanto soportar un gran volumen de tejido vivo se resuelve con un cambio mayor en el ángulo de divergencia. En las plantas erectas se produce el acomodamiento vertical de las areolas y en consecuencia la formación de costillas, tal como ocurre típicamente en las cactáceas columnares como *Neobuxbaumia tetetzo*. El sistema vascular se dispone en estas plantas en haces verticales separados por parénquima, permitiendo el crecimiento erecto así como el cambio de volumen en respuesta a las condiciones hídricas.

Los resultados de los tres trabajos nos sugieren la importancia del estudio del desarrollo para desentrañar los mecanismos evolutivos asociados a las grandes transformaciones morfológicas. De manera muy resumida, y a

modo de conclusión, los resultados de esta tesis nos permiten inferir que durante la evolución de la succulencia del tallo en las cactáceas operaron los siguientes mecanismos:

a.- Por un lado, con la morfología suculenta se desarrolló en las cactáceas un metabolismo fotosintético tipo CAM, aunque las especies mantuvieron la posibilidad de utilizar el metabolismo C_3 ancestral durante la etapa de establecimiento, cuando la disponibilidad de agua es más abundante y un rápido crecimiento es fundamental. Así, el mecanismo de conservación de los caracteres ancestrales en los juveniles de adultos con caracteres derivados (proceso conocido como "recapitulación"), parece estar jugando en las cactáceas un papel importante.

b.- Adicionalmente, la succulencia de las cactáceas se desarrolló en los tallos con pérdida de las hojas, a diferencia de otras suculentas del Nuevo Mundo, que la desarrollaron en las hojas como es el caso de las agaváceas. El desarrollo de la succulencia en las cactáceas implicó entonces el desarrollo de tallos de epidermis fotosintética con grandes masas de parénquima no especializado y de células ricas en vacuolas. Nuestros resultados sugieren que

el mecanismo evolutivo que operó durante esta transformación fue la conservación de los caracteres de la anatomía juvenil en los tallos adultos. Así, la neotenia alométrica, una forma de heterocronía, parece haber desempeñado también un papel fundamental en la evolución de la succulencia de los tallos en la familia.

c.- Finalmente, el desarrollo de tallos adultos con anatomía juvenil representó también un problema morfológico importante. Para sostener estos tallos ricos en parénquima y pobres en tejido leñoso, fue necesario desarrollar una nueva disposición de los haces vasculares dentro del tallo. El modelo matemático de la filotaxia presentado en esta tesis sugiere que esta transformación morfogenética pudo haberse realizado mediante la variación adaptativa de dos parámetros morfológicos que son controlados durante el crecimiento del ápice: el ángulo generador de la filotaxia y la compactación de los entrenudos. Nuestros resultados sugieren que cambios graduales y relativamente pequeños de estos dos parámetros pueden generar nuevas conformaciones de los haces vasculares en los tallos, y podrían estar asociados al desarrollo evolutivo de las costillas de leño como estructura fundamental de soporte.

En resumen, esta tesis concluye que tres mecanismos sencillos (la recapitulación, la heterocronía, y la regulación meristemática de la morfogénesis) y de variación gradual, parecen ser los elementos más importantes sobre los que operó la selección natural durante el desarrollo de la succulencia del tallo en la familia Cactaceae.