

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

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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.

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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 paedomorphosis 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

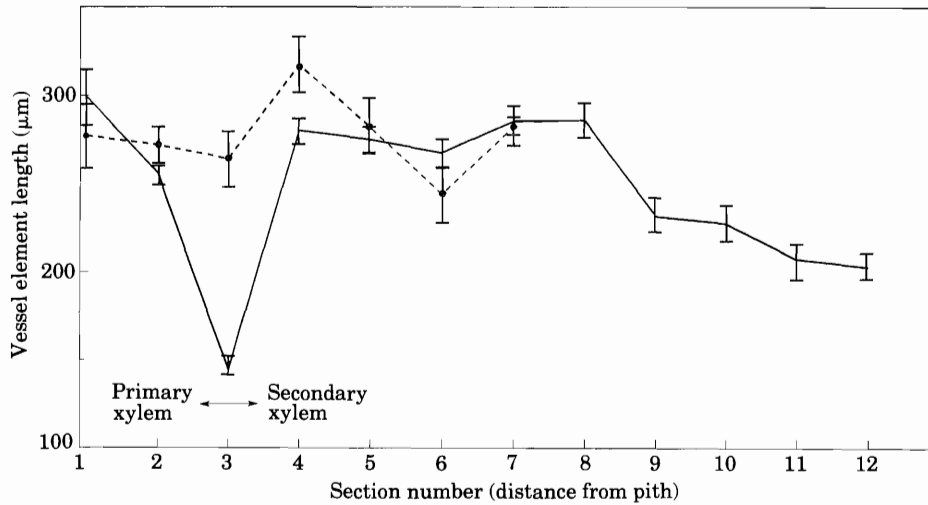


Figure 1. Age-on-length curve of the vessel elements of *Pereskia lychnidiflora* (—) and *Neobuxbaumia tetetzo* (·····). The abscissa shows the ranked order of the anatomical sections (each section is separated from the previous one by 3 mm), and is thus a measure of the distance from the pith of the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

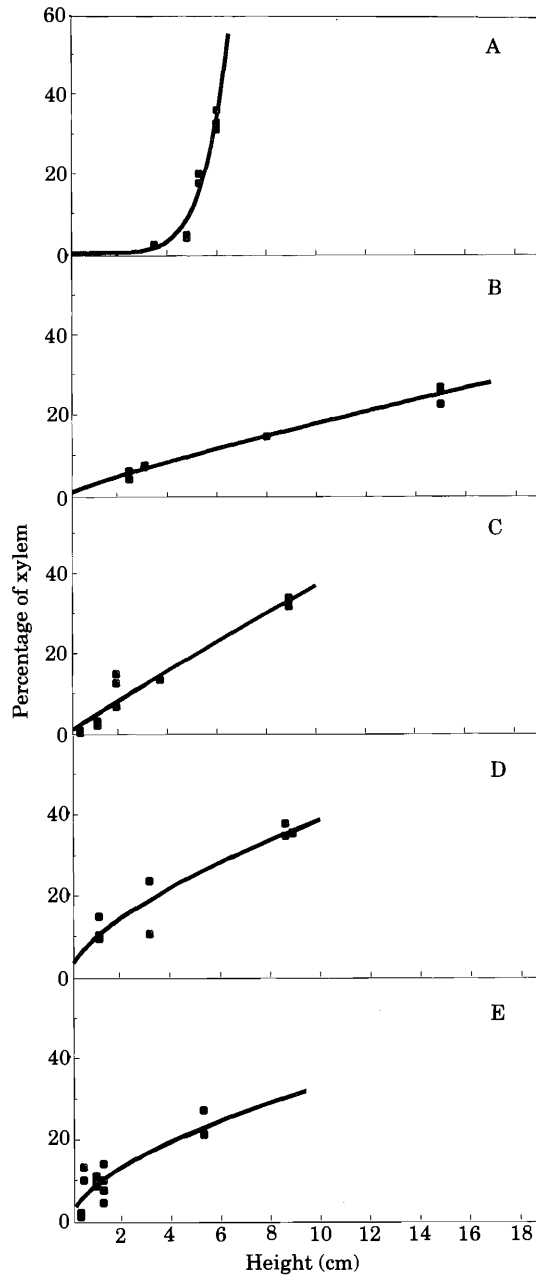


Figure 2. A–E. Allometric growth curves showing the percentage of xylem at the base of the plant vs. length. A, *Pereskia aculeata*. B, *Opuntia pilifera*. C, *Neobuxbaumia tetetzo*. D, *Mammillaria collina*. E, *Ferocactus latispinus*.

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

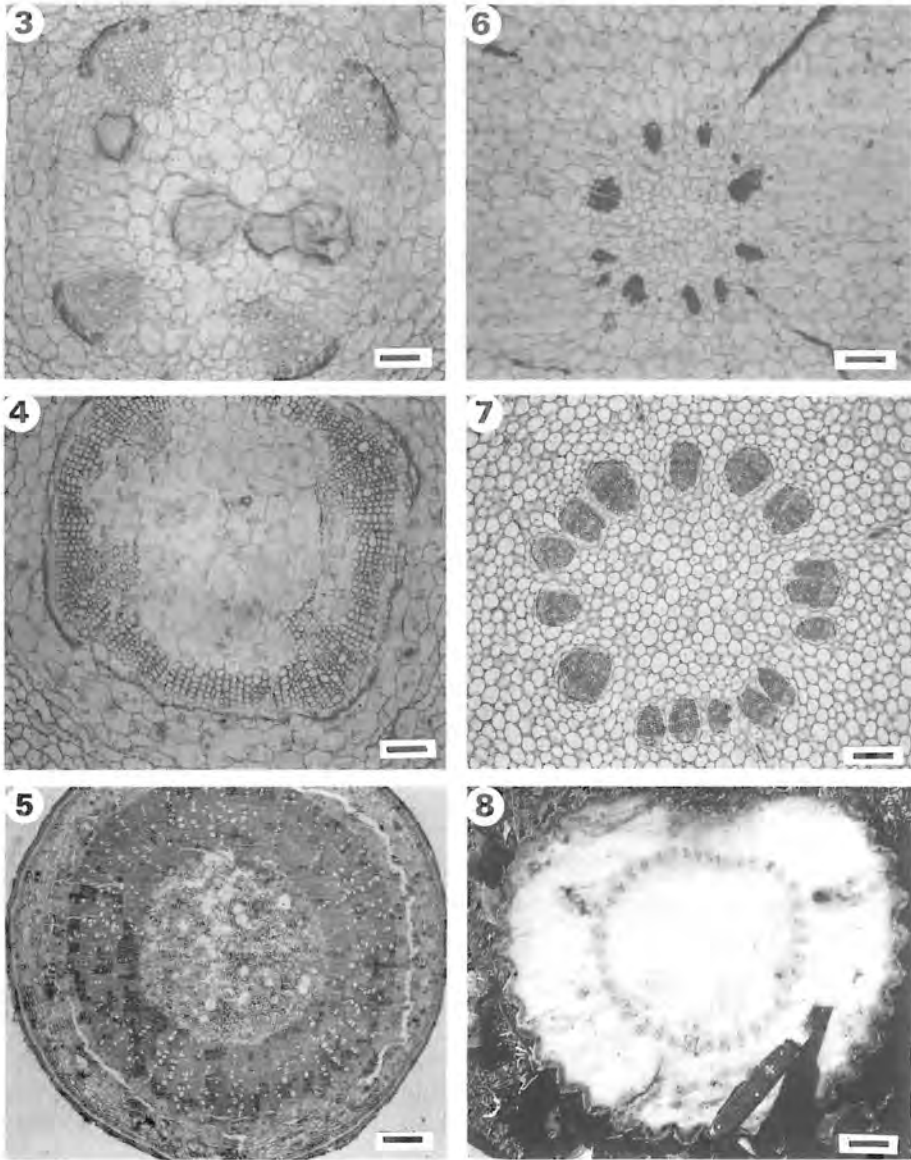
was to increase the proportion of xylem as they grew, some species increased their woody tissues at a significantly higher rate.

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.

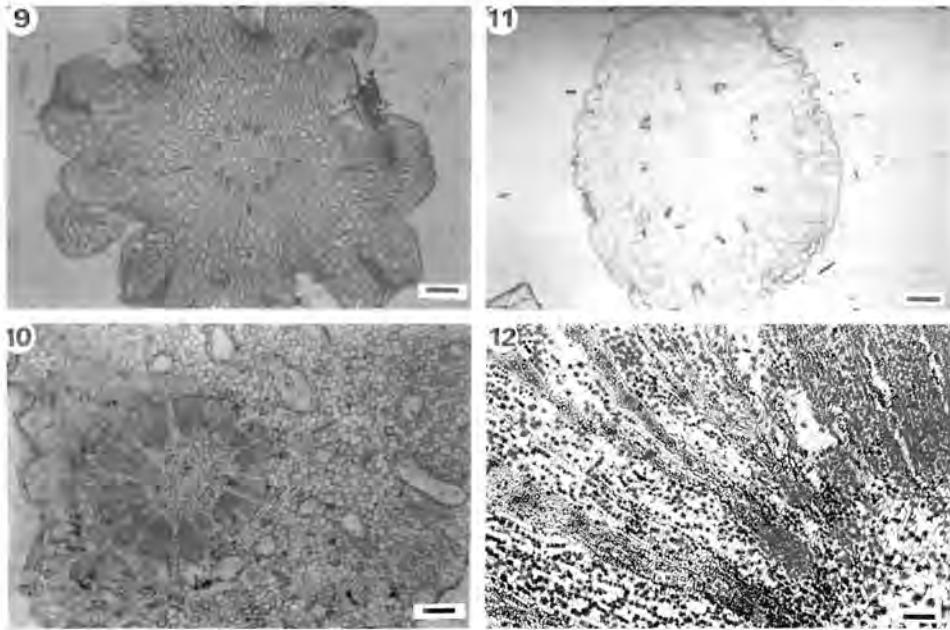
Our results show that, from many points of view, the evolution of succulence in cacti has implied a selection for juvenile characteristics in the woody tissues. Firstly, the change in lateral wall pitting that occurs in most plants during the transition from primary to secondary growth did not occur 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 pedomorphosis, or juvenilism, may be the



Figures 9–12. Transverse 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 *Carnegiea 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.

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