

Giselle Rôças · Cláudia Franca Barros  
Fábio Rubio Scarano

## Leaf anatomy plasticity of *Alchornea triplinervia* (Euphorbiaceae) under distinct light regimes in a Brazilian montane Atlantic rain forest

Received: 11 November 1996 / Accepted: 5 February 1997

**Abstract** *Alchornea triplinervia* trees occur in a montane Atlantic rainforest at the Macaé de Cima State Ecological Reserve (Nova Friburgo, RJ, Brazil). They are found in two adjacent secondary forests at distinct successional stages: a late-secondary (shaded and humid) and an early-secondary forest (drier, higher light intensities reaching the understory). Leaf samples collected in these environments were compared in regard to various anatomic parameters. Histochemical tests, anatomic measurements and counting indicated no significant variation for the basic leaf anatomy. Nevertheless, as compared to the late-secondary forest, the leaves of the early-secondary forest individuals showed an increased bulk of sclerenchyma at the main nervation and petiole, gelatinous fibers with hygroscopic walls all along the central vascular system, thicker cuticle, a higher abaxial stomatal density, abundance of tannin in the mesophyll, a significantly thicker palisade and spongy parenchyma, and compactation of the spongy parenchyma. This anatomical variation indicates an increased xeromorphism of leaves under the drier conditions of the early-secondary forest. Leaf anatomy plasticity may contribute to the high ecological plasticity of this widespread neotropical species.

**Key words** *Alchornea triplinervia* · Atlantic rain forest · Leaf anatomy · Phenotypic plasticity

### Introduction

Phenotypic plasticity – the capacity of an individual to change its morpho-anatomy and/or physiology in response

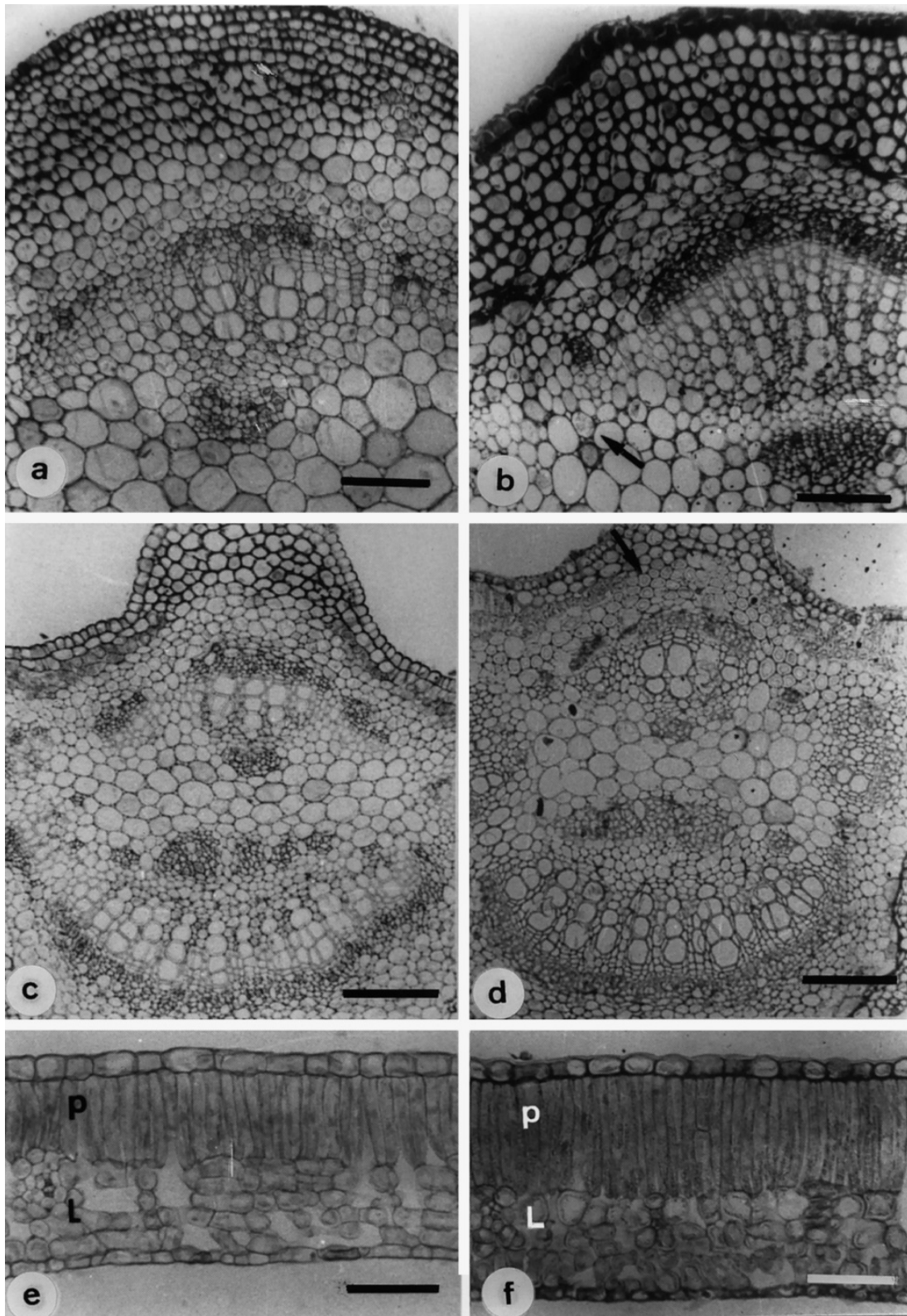
to environmental changes – is a feature of high ecological relevance particularly to sessile organisms such as plants. Phenotypic plasticity is often, but not always, related to adaptive advantages (Schlichting 1986). Despite the review of Bradshaw (1965) regarding the key role of phenotypic plasticity in plant evolution, until recently it has been seen as a buffer to natural selection and as an impediment to evolutionary changes (Levin 1987). This and the difficulties inherent to plasticity measurements (Thompson 1991, 1992; Via 1992) have contributed to the controversial literature on this subject. Schlichting (1986) has listed a number of suggestions to fill the gaps in phenotypic plasticity theory and highlighted the need of comparative studies among native plant populations. Although the ecological effects of distinct light regimes on leaf anatomy have often been studied (e.g. Chabot et al. 1979; Mott and Michaelson 1991), these effects have only recently been examined in native tropical populations (e.g. Chazdon and Kaufmann 1993).

*Alchornea* (Euphorbiaceae), a potentially economically important genus for its wood and medical properties (Mainieri and Chimelo 1989), is widely distributed in Brazil from the Amazon (Prance 1979) to the southern-most State of Rio Grande do Sul (Reitz et al. 1978). This genus poses many taxonomic problems (Oliveira et al. 1988) for its wide ecological and phenotypic plasticity. *Alchornea triplinervia* (Spreng) Muell.Arg. occurs in the seasonally flooded várzea forest in the Amazon (Campbell et al. 1992), in the gallery forests of central Brazil (Luchi 1990), in the montane Atlantic rain forest (Guedes-Bruni and Lima 1994) and in swamp forests of the sandy coastal plains locally called *restingas* (Sá 1992). Individuals show a marked morphological variation ranging from 3–5 m tall and small yellow leaves at the semi-exposed conditions of the coastal swamp forests to 15–20 m tall and large green leaves in the shaded conditions of montane forests.

We have examined the effect of light on leaf anatomy of *A. triplinervia* trees, by comparing leaves of two secondary forests at distinct successional stages, and consequently different light and humidity regimes, in a montane Atlantic rain forest in southeastern Brazil.

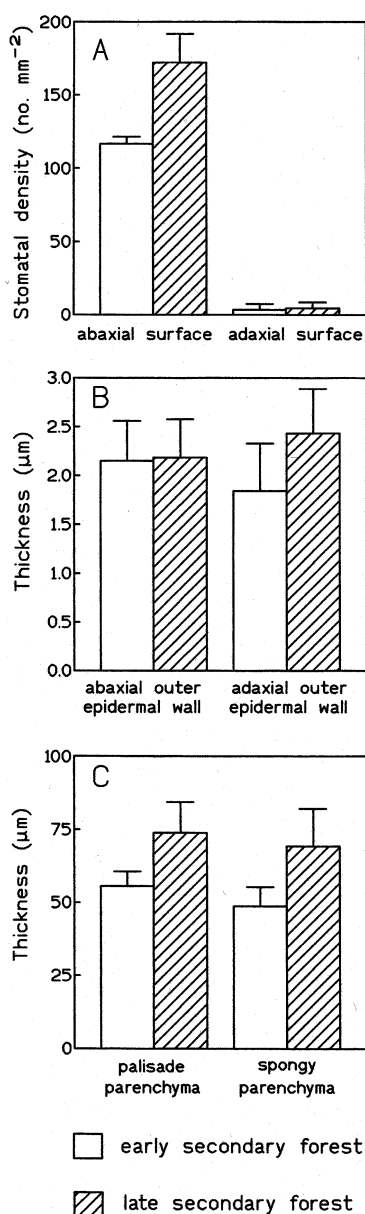
G. Rôças · F. R. Scarano (✉)  
Universidade Federal do Rio de Janeiro, Departamento de Ecologia,  
IB, CCS, Ilha do Fundão, Caixa postal 68020; 21941-590,  
Rio de Janeiro, RJ, Brazil

C. F. Barros  
Jardim Botânico do Rio de Janeiro, Laboratório de Botânica Estrutural,  
22460-030, Rio de Janeiro, RJ, Brazil



**Fig. 1** Cross-sections of leaves of *Alchornea triplinervia* from montane Atlantic rain forest: **a** petiole (median level) – late-secondary forest (*LS*, less light and more humidity in the understorey); **b** petiole – early-secondary forest (*ES*, more exposed and drier understorey). The *arrow* indicates a calcium oxalate druse; **c** mid-rib (median level)

– *LS*; **d** mid-rib – *ES*. The *arrow* indicates the presence of fibers; **e** leaf lamina (median level) – *LS* and **f** leaf lamina – *ES*: the palisade cells (*P*) have a larger anticlinal diameter and the spongy parenchyma (*L*) is more compact. Scale bars **a–d** = 100  $\mu$ m; **e, f** = 50  $\mu$ m



**Fig. 2** Leaf anatomy variation between leaves of *A. triplinervia* under distinct light and soil humidity regimes of adjacent secondary forests: **a** stomatal density; **b** thickness of the outer epidermal cell walls; **c** thickness of spongy and palisade parenchyma. Bars indicate standard deviation. All values differed significantly between habitats (paired *T*-test;  $P < 0.05$ ) except stomatal density in the adaxial surface and thickness of the abaxial outer epidermal wall

## Materials and methods

Leaf samples of *Alchornea triplinervia* (Spreng) Muell. Arg. (Euphorbiaceae) were collected in a late-secondary forest (dense canopy cover, shaded and humid) and in a neighbouring early-secondary forest (large canopy gaps, higher light penetration and less humid) of a montane Atlantic rain forest at the Ecological Reserve of Macaé de Cima, municipality of Nova Friburgo, State of Rio de Janeiro, southeastern Brazil. The altitude here ranges from 880 to 1720 m, the mesotermic climate is always humid and mean annual rainfall ranges from 1500 to 2000 mm. The mean temperature is 17.8 °C with January and February the warmest months and July the coldest.

We collected five leaves of each of five randomly chosen individuals at each site. The mean diameter of the trees at breast height was approximately 15 cm and mean height approximately 20 m. Histochemical tests were carried out for free-hand sections of newly collected material: phloroglucinol test to lignin (Johansen 1940); Hoepfener-Vorsatz test to tannins (Reeve 1951); acetic or chlorohydric acids for crystal calcium oxalate (Johansen 1940).

Stomatal density was estimated by using epidermal prints of colorless nail polish or dissociated material using Jeffrey's reagent (Jensen 1962) stained by hydroalcoholic safranin. For measurements of palisade and spongy parenchyma and outer epidermal cell wall of the adaxial and abaxial surfaces we used paraffin embedded sections stained by astra blue-basic fuchsin (Braga 1977). Counting and measurements took place on both surfaces using a sample size of 25 fields. The significance of statistical differences between samples was determined by a paired *T*-test ( $\alpha = 0.05$ ; Zar 1996).

## Results

Figure 1 allows a comparison between the anatomic structure of individuals from the late-secondary forest with those of the early-secondary forest. The basic structure of leaf petiole was very similar for both sites (Figs. 1a, b). The uniseriate epidermis had tubular-shaped cells and some stellate multicellular trichomes. Under the epidermis an angular collenchyma occurred followed by a ground parenchyma with druses idioblasts. The leaves of the late-secondary forest showed a vascular system arranged in an open circle of bicollateral bundles with an accessory bicollateral bundle in the center. Near the lamina, the central bundle divided itself in two isolated bundles. In contrast, the leaves under the drier conditions of the early-secondary forest showed a vascular system arranged in a closed circle. The accessory bundles, however, were similar to their late-secondary forest counterparts. Leaf mid-rib was similar for both areas. The angular collenchyma occurred under both abaxial and adaxial epidermis. The arch-shaped vascular system showed five bundles disposed near the adaxial surface. The main difference found was that all leaf samples of the early-secondary forest showed a marked occurrence of sclerenchyma around the vascular system and gelatinous fibers which were not seen in the late-secondary forest leaves (Figs. 1c, d).

The amphistomatous leaves of *A. triplinervia* showed a similar proportion of paracytic stomata in the adaxial surface for both sites, in contrast with a significantly higher (paired *T*-test;  $P < 0.05$ ) occurrence in the abaxial surface of early-secondary forest leaves in comparison with the late-secondary forest leaves (Fig. 2a). The mean width of the adaxial cuticle was higher in the early- than in the late-secondary forest leaves whereas no significant difference was found for the abaxial surface (Figs. 1e, f, 2b). Stellate trichomes occurred in both epidermis in leaves of both sites.

The dorsiventral mesophyll in the late-secondary forest leaves showed a one-layered palisade parenchyma (mean width 48.68 µm) and 3–5 layers of spongy parenchyma (mean width 55.65 µm). Early-secondary forest leaves showed a higher cell number and a higher width of both tissues (Figs. 1e, f, 2c): 1–2 cell layers of palisade parenchyma (mean width 69.37 µm) and 4–5 layers of

spongy parenchyma (mean width 73.87  $\mu\text{m}$ ) plus a reduction of intercellular spaces. In the latter, the cell layer which was closer to the abaxial epidermis showed a higher anticlinal diameter than in the palisade cells. Druses of calcium oxalate and of phenolic substances were found in both tissues.

The margins of leaves from both sites were toothed and slightly down-curved with palisade and spongy parenchyma and a small vein. In the sinus of the teeth there was an extrafloral nectary. Its epidermal cells were palisade-like and the mesophyll cells were rounded and compactly disposed.

---

## Discussion

In addition to the wide morphological variation found between *A. triplinervia* specimens along altitudinal, latitudinal, light and humidity gradients in the State of Rio de Janeiro and in Brazil, our results indicate that there is also a marked individual variation in leaf anatomy under distinct light and humidity regimes, even for neighbouring sites.

Leaves at the drier, semi-exposed early-secondary forest showed a tendency towards xeromorphism when compared anatomically with leaves at the humid and shaded late-secondary forest. The larger leaf mid-rib and petiole sclerenchyma of the early-secondary forest leaves possibly increases leaf hardness and contributes to avoiding deformation under drought and strong winds (Tschirch 1881; Turner 1994). The occurrence of gelatinous fibers with hygroscopic walls surrounding all the central vascular system is also a possible adaptive mechanism against water stress (Fahn 1985; Mauseth 1988). The thicker outer epidermal cell wall may induce a more humid microclimate, reducing the gradient of water diffusion between leaf and air (Tenberge 1992). Thin cuticles, such as those of the late-secondary forest leaves, are often found in mesic habitats (Cutler et al. 1982). The wider cuticle and higher sclerenchyma formation in the early-secondary forest leaves is possibly a response to both soil water shortage and to higher exposure to light, since the biosynthetic pathway for lignin and cutin production is directly dependent on light (Jensen 1986; Hrazdina and Jensen 1992). The significantly higher stomatal density in the abaxial surface of the early-secondary forest leaves than in the late-secondary forest leaves suggests a better control over transpiration in the former, possibly avoiding excessive water loss (Weyers and Meidner 1990).

Tannins and other phenolic compounds found in higher concentrations in the mesophyll of early-secondary forest leaves than in the late-secondary forest leaves may offer protection against desiccation (Fahn 1985), against herbivory (Feeny 1976; Rhoades 1979) and maintain cytoplasmic homogeneity (Esau 1977).

The direct relationship between leaf thickness and light intensity has been observed in a number of species (Björkman 1981) and has also been found for *A. triplinervia*. Furthermore, leaves of the early-secondary forest showed a

compactation of the spongy parenchyma which, according to Levitt (1980), is due to water deficit which hinders the normal cell growth during growth period, leading to smaller and thicker cells due to the excess of photosynthetic products. Isanogle (1944) proposes that light does not affect the number of cell layers but, instead, it affects the length of the palisade cells. *A. triplinervia* showed a proportional increase in both cell size and number for both sites, which disagrees with the hypothesis that cell number is genetically determined and that the environment acts solely on cell size.

As pointed out by Strauss-Debenedetti and Berlyn (1994), acclimation cannot be predicted from anatomy plasticity measurements alone since assimilation rates can be independent of such modifications. Thus, in order to explain the high ecological plasticity of *A. triplinervia* indicated by a wide and diverse geographic distribution, the leaf anatomy plasticity here described is possibly related to physiological plasticity which deserves future studies.

**Acknowledgements** We thank C.G.Costa, A.C.Franco, A.Hagler and G.Wilson Fernandes for critical readings of the manuscript; the staff at the Plant Anatomy Laboratory of the Rio de Janeiro Botanic Gardens; CNPq (Brazilian Research Council) for research grants nos. 520494/93-8 and 301056/92-7; and the Atlantic Forest Program (Shell, Margaret Mee Amazon Trust, MacArthur Foundation) for funding.

---

## References

- Björkman O (1981) Responses to different quantum flux densities. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) *Encyclopedia of plant physiology*, vol 12A. Springer, Berlin Heidelberg New York, pp 57–107
- Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. *Adv Genet* 13: 115–155
- Braga MMN (1977) Anatomia foliar de Bromeliaceae da campina. *Acta Amazonica* 7: 5–74
- Campbell DS, Stone JL, Rosas A Jr (1992) A comparison of the phytosociology and dynamics of three floodplain (várzea) forests of known ages, Rio Juruá, western Brazilian Amazon. *Bot J Linn Soc* 108: 213–237
- Chabot BF, Jurik TW, Chabot JF (1979) Influence of instantaneous and integrated light-flux density on leaf anatomy and photosynthesis. *Am J Bot* 66: 940–945
- Chazdon RL, Kaufmann S (1993) Plasticity of leaf anatomy of two rain forest shrubs in relation to photosynthetic light acclimation. *Funct Ecol* 7: 385–394
- Cutler DS, Alvin KL, Price CE (eds) (1982) *The plant cuticle*. Academic Press, London
- Esau K (1977) *Anatomía Vegetal*. Omega, Barcelona
- Fahn A (1985) *Anatomía Vegetal*. Ediciones Pirámides, Madrid
- Feeny PP (1976) Plant apparency and chemical defense. *Rec Adv Phytochem* 10: 1–40
- Guedes-Bruni RR, Lima MPM (1994) Abordagem geográfica, fitofisionômica, florística e taxonômica da Reserva Ecológica de Macaé de Cima. In: Lima MPM, Guedes-Bruni, RR (eds) *Reserva Ecológica de Macaé de Cima*, Nova Friburgo, RJ: Aspectos Florísticos das Espécies Vasculares, vol 1. Jardim Botânico do Rio de Janeiro, Brazil, pp 17–54
- Hrazdina G, Jensen RA (1992) Spatial organization of enzymes in plant metabolic pathways. *Annu Rev Plant Physiol Mol Biol* 43: 241–267

- Isanogle IT (1944) Effects of controlled shading upon the development of leaf structure in two deciduous tree species. *Ecology* 25: 404–413
- Jensen RA (1986) The shikimate irogenate pathway; link between carbohydrate metabolism and secondary metabolism. *Physiol Plant* 66: 164–168
- Jensen WA (1962) Botanical histochemistry. WH Freeman, San Francisco
- Johansen D (1940) Plant microtechnique. McGraw-Hill, New York
- Levin DA (1987) Plasticity, canalization and evolutionary stasis in plants. In: Davy AJ, Hutchings MJ, Watkinson MR (eds) Plant population ecology. Blackwell Scientific, Oxford, pp 35–45
- Levitt J (1980) Responses of plants to environmental stresses. Academic Press, New York
- Luchi AE (1990). Estudo anatômico do lenho em espécies de mata ciliar da Serra do Cipó (MG). Unpublished M.Sc. Thesis. Universidade de São Paulo, Brazil
- Mainieri C, Chimelo JP (1989) Fichas de Características das Madeiras Brasileiras. IPF. São Paulo, pp 389–390
- Mauseth JP (1988) Plant anatomy. Benjamin/Cummings, California
- Mott KA, Michaelson O (1991) Amphistomy as an adaptation to high light intensity in *Ambrosia cordifolia* (Compositae). *Am J Bot* 78: 76–79
- Oliveira AS, Silva IM, Alves MV (1988) Estudos taxonômicos sobre a família Euphorbiaceae Juss. II. *Alchornea triplinervia* (Spreng) Muell. Arg. var. *triplinervia* e *Alchornea triplinervia* var. *janeirensis* (Casar) Muell. Arg. *Sellowia* 40: 32–62
- Prance GT (1979) Notes on the vegetation of Amazonia. III. The terminology of Amazonian forest types subject to inundation. *Brittonia* 31: 26–38
- Reeve RM (1951) Histochemical tests for polyphenols in plant tissues. *Stain Technol* 26: 91–96
- Reitz R, Klein RM, Reis A (1978). Projeto Madeira de Santa Catarina. *Sellowia* 28–30
- Rhoades DF (1979) Evolution of plant chemical defense against herbivores. In: Rosenthal GA, Janzen DH (eds) *Herbivores: their interaction with secondary plant metabolites*. Academic Press, New York, pp 4–48
- Sá CFC (1992) A vegetação da Restinga de Ipitangas, Reserva Ecológica Estadual de Jacarepiá, Saquarema (RJ): fisionomia e listagem de Angiospermas. *Arq Jard Bot Rio de Janeiro* 31: 87–102
- Schlichting CD (1986) The evolution of phenotypic plasticity in plants. *Annu Rev Ecol Syst* 17: 667–693
- Strauss-Debenedetti S, Berlyn GP (1994) Leaf anatomical responses to light in five tropical Moraceae of different successional status. *Am J Bot* 81: 1582–1591
- Tenberge KB (1992) Ultrastructure and development of the outer epiderm wall of spruce (*Picea abies*) needles. *Can J Bot* 70: 1467–1487
- Thompson JD (1991) Phenotypic plasticity as a component of evolutionary change. *Trends Ecol Evol* 6: 246–249
- Thompson JD (1992) Reply from John Thompson. *Trends Ecol Evol* 7: 64
- Tschirch A (1881) Über einige Beziehungen des anatomischen Baues der Assimilations-Organen zu Klima und Standort, mit specieller Berücksichtigung des Spaltöffnungsapparates. *Linnaea* 43: 139–252
- Turner IM (1994) Sclerophylly: primarily protective? *Funct Ecol* 8: 669–675
- Via S (1992) Models of the evolution of phenotypic plasticity. *Trends Ecol Evol* 7: 63
- Weyers JDB, Meidner H (1990) *Methods in stomatal research*. Longman, London
- Zar JH (1996) *Biostatistical Analysis*. 3rd. edn. Prentice Hall, New Jersey