Resolving the phylogenetic placement of *Blossfeldia liliputana* (Cactaceae): reticulate evolution, chloroplast inheritance, and graft-chimeras

Root Gorelick

School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, U.S.A. (email: cycad@asu.edu).

Summary: The perplexing ancestral phylogenetic placement of *Blossfeldia liliputana* based on chloroplast DNA can possibly be explained by (1) the hybrid origin and uniparental inheritance of chloroplasts in *Blossfeldia* and (2) the DNA samples originating from the grafting stock upon which the *Blossfeldia* was cultivated. The problem with the first of these hypotheses is that nobody knows whether chloroplasts are inherited from one or both parents in *Blossfeldia* nor how this inheritance pattern may have changed in ancestors of *Blossfeldia*. Phylogenetic reconstructions of species trees assume that the mode of organelle inheritance is known, and these phylogenies are confounded by reticulate evolution. The problem with the second hypothesis is that most cultivated specimens of *Blossfeldia* are grafted and nobody is certain whether or not these readily form graft-chimeras. Until both hypotheses are tested, *B. liliputana* should be considered a highly derived member of the subfamily Cactoideae, as indicated by morphological data.

Zusammenfassung: Die auf Grund von Chloroplasten-DNA-Untersuchungen rätselhafte phylogenetische Stellung von *Blossfeldia liliputana* kann möglicherweise (1) durch einen hybridogenen Ursprung von *Blossfeldia* und uniparentale Chloroplastenvererbung erklärt werden, oder (2) weil Chloroplasten untersucht wurden, die aus der Propfunterlage der kultivierten Pflanzen stammen. Das Problem mit der ersten dieser beiden Hypothesen ist die Tatsache, dass niemand weiss, ob die Chloroplasten bei *Blossfeldia* nur durch eine oder durch beide Elternpflanzen vererbt werden, und ob dieses Vererbungsmuster bei Vorläufern von *Blossfeldia* anders gewesen sein könnte. Phylogenetische Rekonstruktionen von Artverwandtschafen gehen davon aus, dass der Modus der Organellenvererbung bekannt ist, und solche Phylogenien werden durch reticulate Evolution verkompliziert. Das Problem mit der zweiten Hypothese liegt darin begründet, dass die meisten kultivierten Exemplare von *Blosssfeldia* gepfropft sind, und dass sich niemand sicher ist, ob sich nicht leicht Pfropfchimären bilden. Solange diese beiden Hypothesen nicht ausgetestet worden sind, wird *Blossfeldia* am Besten als hochabgeleitetes Glied der Unterfamilie Cactoideae betrachtet, wie es auch von den morphologischen Daten gestützt wird.

Introduction

With trepidation, Nyffeler (2002) proposed that *Blossfeldia liliputana* Werdermann is a basal member of the subfamily Cactoideae Schumann. Yet, phylogenies based exclusively on morphological characters place *Blossfeldia liliputana* as a highly derived taxon. Evidence for this placement comes from such notable characters as having fewer stomata than any other terrestrial plant (other than parasites and aquatics), stomata that are sunken in areolar pits, extremely thin epidermis, and lack of a hypodermis (Barthlott & Porembski, 1996).

The present consensus position is that *Blossfeldia* Werdermann is in the tribe Notocacteae Buxbaum, a position largely founded on a superficial resemblance to *Parodia* Spegazzini and *Frailea* Britton & Rose, with somewhat similar seeds and a hairy testa (Hunt *et al.*, 1990). Seeds, however, provide the only similarity. *Blossfeldia* lacks several important characters of Notocacteae, such as presence of acicular bristles in the receptacle areoles. *Blossfeldia* flowers are much reduced in their structures and seem to have more in common with tribe Trichocereeae Buxbaum than with Notocacteae. *Blossfeldia* flowers have a well-defined cauline zone, with a mass of white kinked uniseriate trichomes at their base, which is not

found in tribe Notocacteae, but it is present in subtribe Rebutiinae of the tribe Trichocereeae, e.g. *Rebutia* Schumann *sensu stricta* (Mottram, pers. comm.). *Blossfeldia* fruits also share many morphological characters with subfamily Rebutiinae, such as fleshy scales overtopping the rim of the umbilicus (Mottram, pers. comm.). Thus, there is morphological evidence for placement of *Blossfeldia* in either of the two closely related tribes Notocacteae and Trichocereeae, neither of which is generally considered ancestral within the Cactoideae.

Why did Nyfeller's (2002) analysis result in a glaringly anomalous phylogenetic position for *B. liliputana* amongst the Cactoideae? His phylogeny was based solely on *trnK/matK* and *trnL-trnF* sequences of chloroplast DNA. Gorelick (2002) proposed that the problem is that *B. liliputana* is of hybrid origin and that chloroplast DNA is only maternally inherited. Here, I review and critique that argument, noting that until further cytological or molecular work with *B. liliputana* is done, we will not know whether that argument is valid. I also discuss the mechanisms and equivocal evidence for *Blossfeldia* morphology arising via allopolyploidy. Additionally, I propose an alternative hypothesis based on graft-chimeras for why analysis of chloroplast DNA may have incorrectly ascribed the phylogenetic placement of *B. liliputana*: The chloroplast DNA sampled may have been from the stock rather than from the *Blossfeldia* scion.

Polyploidy and inheritance of chloroplasts
B. liliputana is hexaploid, which is virtually a guarantee of allopolyploidy. The only parsimonious way to produce hexaploids is via hybridisation between a diploid and a tetraploid parent. Hexaploidy may have resulted from the hybridisation of a diploid and a tetraploid parent in which both parents had unreduced gametes (i.e. containing the same amounts of DNA as the parents' somatic cells). This can arise by the eggs and sperm cells not having undergone both meiotic divisions (Bretagnolle, 2001) or by them both undergoing two meiotic reduction divisions followed by a chromosomal doubling (Friedman, 1999). Alternatively, both parents could have undergone normal meiotic divisions and a triploid offspring was produced, which later produced hexaploid descendants via a subsequent autopolyploidization. Both alternatives should be considered reasonable possibilities for the evolution of *Blossfeldia* or any hexaploid flowering plants (Ramsey & Schemske, 1998). Other than allopolyploidy, the only other possible origin for hexaploidy in *Blossfeldia* is aneuploidy followed by spontaneous chromosome doubling (autopolyploidy). However, this is highly unlikely. Perusal of the *Index to Plant Chromosome Numbers* (Goldblatt & Johnson) (updated and published annually online at

http://mobot.mobot.org/W3T/Search/ipcn.html) and Ramsey & Schemske (1998) show that aneuploidy is rare or absent in cacti.

Intergeneric hybrids do occur in the Cactoideae, albeit rarely. The tetraploid *Bergerocactus emoryi* (Engelm.) Britton & Rose hybridizes with both the diploid *Myrtillocactus cochal* (Orcutt) Britton & Rose to form the hexaploid x*Myrtgerocactus lindsayi* Moran and the tetraploid *Pachycereus pringlei* (S. Watson) Britton & Rose to form the octaploid x*Pacherocactus orcuttii* (K. Brandegee) G.D.Rowley (Moran, 1962a, b; Pinkava *et al.*, 1998). The diploid *Hylocereus undatus* (Haworth) Britton & Rose has been hybridized with the tetraploid *Selenicereus megalanthus* (Schumann ex Vaupel) Moran to form a viable hexaploid (Tel-Zur *et al.*, 2003). I hypothesize that the hexaploid *B. liliputana* was similarly formed via hybridization of a diploid and tetraploid parent. My suspicion is that both parents were members of the Notocacteae or Trichocereeae, which have several tetraploid lineages (Pinkava *et al.*, 1998).

Most flowering plants have chloroplasts that are solely inherited from the female parent (Mogensen, 1996). Hence phylogenies based on uniparentally inherited chloroplast DNA would only reflect half of the ancestry – the female half – of any plant of allopolyploid origin. Gorelick (2002) argued that *B. liliputana* could then have a highly derived paternal parent and an ancestral maternal parent and would thereby be classified as ancestral if solely using chloroplast DNA data. Furthermore, hybridization (reticulate evolution) results in the transfer of chloroplast DNA amongst lineages, which confounds phylogenetic reconstruction of species trees (Moore, 1995).

In most flowering plants, chloroplast DNA is exclusively maternally inherited because plastids are never incorporated into their sperm cells. In virtually all flowering plants, four microspore cells are produced by meiosis. Each of these haploid microspores then undergoes a mitotic division to produce a generative and a vegetative cell. During this mitotic division, plastids, including chloroplasts, are excluded from the generative cell in most flowering plants (Hagemann & Schröder, 1989; Mogensen, 1996). The generative cell later undergoes another mitotic division to form two sperm cells. However, in a minority of flowering plant species, plastids are parcelled into the generative and sperms cells (Corriveau & Coleman, 1988), hence are deposited into the zygote, and therefore paternally inherited plastids can be retained in seedlings (also note that it is possible for other mechanisms to selectively eliminate organelles of one parent or the other in the embryo, as occurs with human male mitochondria). It appears that only six species of cacti have been tested for whether chloroplasts are inherited solely from the maternal parent, pater-

nal parent, or both. Three prickly pears (*Opuntia basilaris* Engelmann & Bigelow, *O. engelmannii* Salm-Dyck, *O. vulgaris* Miller) and one hedgehog cactus (*Echinocereus engelmannii* (Parry ex Engelmann) Rümpler) have strictly maternal inheritance. Two epiphytic cacti, both in the tribe Rhipsalideae A. P. de Candolle – *Hatiora gaertneri* (Regel) Barthlott and *Schlumbergera truncata* (Haworth) Moran) – have many chloroplasts in their sperm cells and therefore most likely have chloroplasts inherited from both parents (Corriveau & Coleman, 1988). Furthermore, these two species do not have dimorphic sperm cells, providing further evidence of biparental inheritance (Corriveau, pers. comm.). With dimorphism, one sperm cell might contain no chloroplasts and always end up fertilising the egg cell, while the sperm cell with chloroplasts always fertilises the polar nuclei. It is not known whether chloroplast inheritance is uniparental or biparental in *B. liliputana* or any other member of the tribes Notocacteae or Trichocereeae.

Why might the fluidity of modes of organelle inheritance over evolutionary time pose a problem in the phylogenetic reconstruction of the Cactaceae? One reason chloroplast DNA is used in phylogenetics of cacti is that organelle DNA evolves much quicker than nuclear DNA because organelle genomes are haploid. This means that a maternally inherited haploid genome will have one-fourth the effective population size of a biparentally inherited diploid genome (Moore, 1995). The effective population size of the haploid genome doubles when the organelle is biparentally inherited. The coalescent theory underlying phylogenetic reconstructions therefore crucially relies on knowing whether inheritance is uniparental or biparental (Hudson, 1990). Yet, all phylogenetic studies of cacti have simply assumed uniparental inheritance.

Is *Blossfeldia* morphology consistent with allopolyploidy?
Polyploidy, especially in recently created polyploids, is known to result in novel morphological and developmental characteristics.

Most studies that have examined a broad swath of angiosperm taxa show that polyploids typically are larger plants with larger cells and organs than their diploid ancestors, albeit with a reduced number of parts, e.g. fewer flower parts (Ramsey & Schemske, 2002). This result, however, is probably biased by the corpus of work on domesticated crops in which artificial selection has been exclusively for larger individuals, especially for larger fruits (Hilu, 1993). By contrast, polyploid cacti seem to be of roughly the same size as their putative diploid ancestors, at least judging by the list of known polyploid taxa. Furthermore, virtually all cactus genera for which polyploidy has been documented are rela-

tively small plants, especially for those taxa with more than four sets of homologous chromosomes (Pinkava *et al.*, 1998), with the notable exception of *Opuntia sensu stricta* in which polyploids appear to be more robust (Pinkava, 2002). The evidence is therefore equivocal for whether polyploidy results in larger, smaller, or equal sized cacti.

Rapid genetic and epigenetic changes following polyploid formation are believed to disrupt development (Osborn *et al.*, 2003) and could thereby explain general size reductions. Genetic changes include gains or losses of DNA fragments (Osborn *et al.*, 2003). The developmental pathways of most organisms, including plants, are largely regulated by epigenetic signals. Epigenetic signals are often mediated by molecules that are attached to DNA, such as cytosine methylation, heterochromatin formation, and histone acetylation (Holliday & Pugh, 1975; Riggs, 1975; Russo *et al.*, 1996). These epigenetic signals act by suppressing gene products (see Gorelick, 2003a, b for review of mechanisms). The proportion of the genome modified by epigenetic signals, especially of cytosine methylation and heterochromatin, increases dramatically following formation of a new polyploid (Matzke & Matzke, 1998; Liu *et al.*, 2000), thereby disrupting regulatory pathways. Such disruptions could mean that only neotenous (paedomorphic; reproducing in a juvenile state) individuals could survive and successfully reproduce (see Ahokas & Manninen, 2000). Spontaneous changes in these epigenetic signals can cause reversion in all or part of a plant from adult to juvenile growth forms, or vice versa (Brink, 1962). In fact, the additional cytosine methylation and chromatin formation caused by polyploidization may itself be the cause of neoteny (cf. Golubovsky, 2002) or other changes in development and its timing (Dremen, 1936; Ficq & Pavan, 1957; Delevoryas, 1980; Levin, 1983 citing Gottschalk, 1976). Neoteny is known to confound phylogenetic inferences in cacti (Barthlott & Hunt, 1993).

Polyploidy may also disrupt the proper formation of wood fibres because of the increased volume needed to hold the extra chromosomal material within a cell (Stebbins, 1938; Otto & Whitton, 2000). Polyploidy causes nuclei and cells to be larger, which is believed to disrupt cambial activity (Darlington, 1932). When wood fibres are not produced or are deformed, stems will be short. Polyploid-induced disruption of developmental pathways may also have been the cause of stomatal loss in *Blossfeldia*.

I sincerely believe that allopolyploidy is the cause for the derived morphology of *Blossfeldia*. However, because I have not conclusively demonstrated this, I propose another hypothesis – that is due to Roy Mottram (pers. comm.) – that explains the phylogenetic relationship inferred by Nyfeller (2002).

Graft-chimeras

Most specimens of *Blossfeldia* that are outside of their native habitat are grafted. When any pair of plants are grafted, there exists the possibility that the scion may be a chimera. A chimera means that a shoot contains a mixture of cells from both stock and scion. These chimeras are often classified as periclinal, in which one layer of cells is from one plant and the remaining layers from another plant (e.g. *Echinopsis* 'Haku-Jo'), or sectorial, in which there is a transverse division between the cells of different origin (Tilney-Bassett, 1986). Such graft-chimeras have been formed between species in many families and can be formed naturally or artificially (Tilney-Bassett, 1986). Natural occurrences of chimeras include haustoria of parasitic plants infiltrating their host's cells (Mauseth *et al.*, 1992), which occurs in some cacti (Mauseth *et al.*, 1984, 1985). Artificial graft-chimeras of cacti have been reported between cacti in a few tribes of the subfamily Cactoideae (Heath, 1992; Rowley, 2003), including the tribe Trichocereeae, but not Notocacteae. Heath (1992) lists the following such graft chimeras in cacti: *Ariocarpus kotschoubeyanus* + *Trichocereus* (*Echinopsis*) *spachianus* = +*Ariocereus* `Ewaldii'; *Echinopsis eyriesii* + *Gymnocalycium mihanovichii* var. *friedrichii* = +*Echinocalycium* `Japonicum'; *Gymnocalycium* + *Hylocereus* (no specific epithets listed) = +*Hylocalycium*. If grafted specimens of *Blossfeldia* are used for molecular phylogenetic analysis, care must be taken to insure that the genomes being analysed are of *Blossfeldia* and not of the grafting stock. Even if clones of *Blossfeldia* were initially grafted and then removed from the stock (typically, *Pereskiopsis*), they may still retain vestiges of the graft-chimera, as appears to be the case with *Echinopsis* 'Haku-Jo'. Three of the four samples of *Blossfeldia* that Nyfeller (2002) used for his chloroplast DNA analysis were grafted.

If *Blossfeldia* specimens are periclinal chimeras with the stock's cells on the outer layers, then these should be readily identifiable. If the specimen has a hypodermis, a thick cuticle or layer of epidermal wax, or a relatively large number of stomata (especially if they are not sunken in stomatal pits), then this is diagnostic of the stock, and not the *Blossfeldia* (Barthlott & Porembski, 1996).

Roy Mottram (pers. comm.) has not found any evidence for periclinal chimeras in grafted specimens of *Blossfeldia*. All five specimens that he examined had a thin epidermis with no visible stomata. This does not rule out the existence of periclinal chimeras, but only eliminates the possibility of those in which the stock contributes cells to the epidermal layer of the scion. One possible way to detect graft-chimeral tissues in the interior of scions is to graft *Blossfeldia* on top of a variegated chimera such as +*Hylocalycium* (=*Gymnocalycium* 'Hibotan' + *Hylocereus*), i.e. three plants grafted together with the variegated *Gymnocalycium* sandwiched as an 'inter-stem' between the *Hylocereus* stock and *Blossfeldia* scion. The distinctive colours of the variegated *Gymnocalycium* tissues would then be obvious in the *Blossfeldia* scion if a periclinal chimera forms.

Conclusion

I have presented evidence that *B. liliputana* is of likely allopolyploid origin. Whether the morphological characters that set *Blossfeldia* apart from other members of the subfamily Cactoideae could be novel and a result of allopolyploidy is still an open question, but I have outlined why this is plausible.

I have presented two hypotheses for why chloroplast DNA would erroneously implicate *Blossfeldia* as an ancestral member of the subfamily Cactoideae: (1) maternally inherited chloroplasts in conjunction with allopolyploidy and (2) contamination of samples via cells from a grafting stock. Ironically, these hypotheses are related. Much information about plastid inheritance has been garnered from chimeric plants in which a layer of tissue has incurred a somatic mutation coding for chlorophyll deficiencies making the entire plant variegated (Kirk & Tilney-Bassett, 1978). Both hypotheses need to be tested before definitive inferences regarding the phylogenetic placement of *B. liliputana* are made. The mode of inheritance of chloroplasts – whether they be maternal, paternal, or biparental – should also be tested using cytological (Corriveau & Coleman, 1988) or molecular (Milligan, 1992) techniques. The allopolyploidy and graft-chimera hypotheses are not mutually exclusive. Both could independently contribute to erroneous phylogenetic placement of *B. liliputana* as an ancestral taxon.

The graft-chimera hypothesis can be tested by grafting *Blossfeldia* onto a variegated plant, which will (by necessity) itself be a chimera. More sophisticated genetic techniques (from allozymes to microarrays) could also be used to detect otherwise cryptic graft-chimeral tissues.

The allopolyploid origin hypothesis needs to be tested via construction of phylogenies that are based on both organelle and nuclear DNA, using phylogenetic methods that account for reticulate evolution and inferred modes of organelle inheritance. This will be a highly non-trivial task. It will be essential to determine whether chloroplasts are maternally, paternally, or biparentally inherited in *Blossfeldia* and any ancestral taxa that are believed to have hybridized to form the ancestor of *Blossfeldia*, using fluorescent staining of chloroplasts and other plastids in pollen grains and sperm cells of *Blossfeldia* specimens that have never been grafted.

High ploidy levels have also been found in other diminutive geophytic South American cacti.

Tephrocactus Lemaire *sensu lato* (including *Maihueniopsis* Spegazzini, *Puna* R. Kiesling, and some species of *Austrocylindropuntia* Backeberg) have from two to fifteen times the typical diploid complement of chromosomes (Pinkava *et al.*, 1998). It will be useful to ascertain the ploidy levels of other such geophytic taxa such as *Cintia knizei* Riha and *Rimacactus laui* (Lüthy) Mottram. If high ploidy levels are found in most or all diminutive geophytic South American cacti, then this provides corroborative support for the allopolyploid hypothesis for the derived phylogenetic placement of *Blossfeldia*.

The alternative, which I have not yet discussed, is that *B. liliputana* truly is a basal/ancestral member of the subfamily Cactoideae, as indicated by molecular data. Patrick Griffith (2004) proposes that diminutive geophytic South American cacti, such as *Blossfeldia*, most closely resemble the ancestors of all cacti. His hypothesis seems plausible in light of recent molecular phylogenies showing that the nearest outgroups to cacti are diminutive members of the family Portulacaceae, many of which are geophytes (Hershkovitz & Zimmer, 1997; Applequist & Wallace, 2001). But, these phylogenies were all based on the assumptions of maternally inherited chloroplast genomes and no reticulate evolution.

It will require more data and more robust phylogenetic methods before chloroplast DNA data alone will allow us to discern whether *B. liliputana* is an ancestral or a derived member of the Cactoideae. Furthermore, there needs to be more than just chloroplast DNA evidence to justify assigning *Blossfeldia* as an ancestral member of the Cactoideae, especially in light of the preponderance of morphological evidence indicating that it is highly derived (Axsmith *et al.*, 1998). Until the above two hypotheses are tested, *B. liliputana* should probably be considered a highly derived member of the subfamily Cactoideae.

Acknowledgments

References

AHOKAS, H. & MANNINEN, M.L. (2000). Introgressive hexaploid oats from the *Avena abyssinica* (female) x *A. sativa* hybrid: performance, grain lipids and proteins. *Euphytica* **111**: 153–160.

APPLEQUIST, W.L. & WALLACE, R.S. (2001). Phylogeny of the portulacaceous cohort based on ndhF sequence data. *Syst. Bot.* **26**: 406–419.

AXSMITH, B.J., TAYLOR, E.L. & TAYLOR, T.N. (1998). The limitations of molecular systematics: a palaeobotanical perspective. *Taxon* **47**: 105–108.

BARTHLOTT, W. & HUNT, D.R. (1993). Cactaceae. Pages 161–196 in K. Kubitzki, Rowher, J.G. & Bittrich, V. (eds.) *The families and genera of vascular plants. Volume II. Flowering plants, dicotyledons: Magnoliid, Hamamelid and Caryophilid families*. Springer-Verlag, Berlin.

BARTHLOTT, W. & POREMBSKI, S. (1996). Ecology and morphology of *Blossfeldia liliputana* (Cactaceae): a poikilohydric and almost astomate succulent. *Bot. Acta* **109**: 161–166.

BRETAGNOLLE, F. (2001). Pollen production and spontaneous polyploidization in diploid populations of *Anthoxanthum alpinum*. *Biol. J. Linn. Soc.* **72**: 241–247.

BRINK, R.A. (1962). Phase change in higher plants and somatic cell heredity. *Quart. Rev. Biol.* **37**: 1–22.

CORRIVEAU, J.L. & COLEMAN, A.W. (1988). Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. *Amer. J. Bot.* **75**: 1443–1458.

DARLINGTON, C.D. (1932). *Recent advances in cytology*, 1st edition. Churchill, London.

DELEVORYAS, T. (1980). Polyploidy in gymnosperms. Pages 215–218 in W. H. Lewis (ed.) *Polyploidy: biological significance*. Plenum Press, New York.

DREMEN, H. (1936). Aposporic parthenogenesis in a triploid apple, *Malus hupehensis*. *J. Arnold. Arboret.* **17**: 90–105.

FICQ, A. & PAVAN, C. (1957). Autoradiography of polytene chromosomes of *Rhynchosciara angelae* at different stages of larval development. *Nature* **180**: 983–984.

FRIEDMAN, W.E. (1999). Expression of the cell cycle in sperm of *Arabidopsis*: implications for understanding patterns of gametogenesis and fertilization in plants and other eukaryotes. *Development* **126**: 1065–1075.

GIBSON, A. C. (1973). Comparative anatomy of secondary xylem in Cactoideae (Cactaceae). *Biotropica* **5**: 29–65.

GOLDBLATT, P. & JOHNSON, D.E., (EDS.) (1997). *Index to plant chromosome numbers*. Missouri Botanical Garden, St. Louis.

GOLUBOVSKY, M. (2002). Paternal familial twinning: hypothesis and genetic/medical implications. *Twin Res.* **5**: 75–86.

GORELICK, R. (2002). DNA sequences and cactus classification: a short review. *Bradleya* **20**: 1–4.

GORELICK, R. (2003a). Evolution of dioecy and sex chromosomes via methylation driving Muller's ratchet. *Biol. J. Linn. Soc.* **80**: 353–368.

GORELICK, R. (2003b). Transposable elements suppress recombination in all meiotic eukaryotes, including automictic ancient asexuals: a reply to Schön and Martens. *J. Nat. Hist.* **37**: 903–909.

GOTTSCHALK, W. (1976). *Die bedeutung der polyploidie für die evolution der pflanzen.* Gustav Fisher Verlag, Stuttgart.

GRIFFITH, M. P. (2004). What did the first cactus look like? An attempt to reconcile the morphological and molecular evidence. *Taxon* (in press).

HAGEMANN, R. & SCHRÖDER, M.B. (1989). The cytological basis of the plastid inheritance in angiosperms. *Protoplasma* **152**: 57–64.

HEATH, P.V. (1992). The nothogenera of the Cactaceae. *Calyx* **1**: 93–140.

HERSHKOVITZ, M.A. & ZIMMER, E.A. (1997). On the evolutionary origins of the cacti. *Taxon* **46**: 217–232.

HILU, K.W. (1993). Polyploidy and the evolution of domesticated plants. *Amer. J. Bot.* **80**: 1494–1499.

HOLLIDAY, R. & PUGH, J.E. (1975). DNA modification mechanisms and gene activity during development. *Science* **187**: 226–232.

HUDSON, R.R. (1990). Gene geneologies and the coalescent process. *Oxford Surveys Evol. Biol.* **7**: 1–44.

HUNT, D.R., TAYLOR N.P. & IOS WORKING PARTY. (1990). The genera of Cactaceae: progress towards consensus. *Bradleya* **8**: 85–107.

KIRK, J. T. O. & TILNEY-BASSETT, R.A.E. (1978). *The plastids: their chemistry, structure and inheritance,* 2nd edition. Elsevier/North Holland, Amsterdam.

LEVIN, D.A. (1983). Polyploidy and novelty in flowering plants. *Amer. Nat.* **122**: 1–25.

LIU, B., HU, B., DONG, Y.Z., LIU, Z.L. & HE, M.Y. (2000). Speciation-induced heritable cytosine methylation changes in polyploid wheat. *Prog. Nat. Sci.* **10**: 601–606.

MATZKE, M.A. & MATZKE, A.J.M. (1998). Polyploidy and transposons. *Trends Ecol. Evol.* **13**: 241.

MAUSETH, J.D., HSIAO, S.C. & MONTENEGRO, G. (1992). Vegetative body of the parasitic angiosperm *Ombrophytum subterraneum* (Balanophoraceae). *Bull. Torrey Bot. Club* **119**: 407–417.

MAUSETH, J D., MONTENEGRO, G. & WALCKOWIAK, A.M. (1984). Studies of the holoparasite *Tristerix aphyllus* (Loranthaceae) infecting *Trichocereus chilensis* (Cactaceae). *Can. J. Bot.-Rev. Can. Bot.* **62**: 847–857.

MAUSETH, J.D., MONTENEGRO, G. & WALCKOWIAK, A.M. (1985). Host infection and flower formation by the parasite *Tristerix aphyllus* (Loranthaceae). *Can. J. Bot.-Rev. Can. Bot.* **63**: 567–581.

MILLIGAN, B.G. (1992). Is organelle DNA strictly maternally inherited? Power analysis of a binomial distribution. *Amer. J. Bot.* **79**: 1325–1328.

MOGENSEN, H.L. (1996). The hows and whys of cytoplasmic inheritance in seed plants. *Amer. J. Bot.* **83**: 383–404.

MOORE, W.S. (1995). Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution* **49**: 718–726.

MORAN, R. (1962a). *Pachycereus orcuttii*: a puzzle solved. *Cact. Succ. J. (US)* **34**: 88–94.

MORAN, R. (1962b). The unique *Cereus*. *Cact. Succ. J. (US)* **34**: 184–188.

NYFFELER, R. (2002). Phylogenetic relationships in the cactus family (Cactaceae) based on evidence from *trnK/matK* and *trnL-trnF* sequences. *Amer. J. Bot.* **89**: 312–326.

OSBORN, T.C., PIRES, J.C., BIRCHLER, J.A., AUGER, D.L., CHEN, Z.J., LEE, H.S., COMAI, L., MADLUNG, A., DOERGE, R.W., COLOT, V. & MARTIENSSEN, R.A. (2003). Understanding mechanisms of novel gene expression in polyploids. *Trends Genet.* **19**: 141–147.

OTTO, S.P. & WHITTON, J. (2000). Polyploid incidence and evolution. *Ann. Rev. Genet.* **34**: 401–437.

PINKAVA, D.J. (2002). On the evolution of continental North American Opuntioideae (Cactaceae). *Succ. Pl. Res.* **6**: 59–98.

PINKAVA, D.J., REBMAN, J.R. & BAKER, M.A. (1998). Chromosome numbers in some cacti of western North America, VII. *Haseltonia* **6**: 32–41.

RAMSEY, J. & SCHEMSKE, D. W. (1998). Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Ann. Rev. Ecol. Syst.* **29**: 467–501.

RAMSEY, J. & SCHEMSKE, D.W. (2002). Neopolyploidy in flowering plants. *Ann. Rev. Ecol. Syst.* **33**: 589–639.

RIGGS, A.D. (1975). X inactivation, differentiation, and DNA methylation. *Cytogenet. Cell Genet.* **14**: 9–11.

ROWLEY, G.D. (2003). Cristates, variegates and the asymmetric universe. *Brit. Cact. Succ. J.* **21**: 41–44.

RUSSO, V. E. A., MARTIENSSEN, R. & RIGGS, A.D. (1996). *Epigenetic mechanisms of gene regulation.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

STEBBINS, G.L. (1938). Cytological characteristics associated with the different growth habits in the dicotyledons. *Amer. J. Bot.* **25**: 189–198.

TEL-ZUR, N., ABBO, S., BAR-ZVI, D. & MIZRAHI, Y. (2003). Chromosome doubling in vine cacti hybrids. *J. Hered.* **94**: 329–333.

TILNEY-BASSETT, R.A.E. (1986). *Plant chimeras.* Edward Arnold, London.