

THE CYCAD HERBIVORES

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Imagine how the world looked like during the Jurassic period – dominated by huge Cycad plants. These woody plants, distinguished by their pinnate compound leaves and their huge cones filled with brightly colored seeds, still exist today in the understory of tropical rainforests (Fig. 1). Cycads survived the millennia because of their potent toxicity. In fact, human consumption of Cycad flour has been linked to endemic types of Alzheimer and Parkinson diseases in the Pacific Islands (Borenstein *et al.* 2007). Paradoxically, throughout the tropics Cycad seeds, stems and roots have been ancestrally used as a source of sustenance and some communities still collect and use this food resource today (Thieret 1958). Even though cooking techniques may reduce some of the toxicity, there are residual effects since intoxication from over ingestion of Cycad plants is well known. How people have been able to survive on Cycads is still a mystery but one that may be unravelled by looking at parallelisms between the Cycad-human story and that between Cycads and insects.

The fossil record of Cycads (order Cycadales) reaches back over 270 million years (Mamay 1969; Zhifeng and Thomas 1989). This striking plants are believed to have reached peak abundance and diversity in the Mesozoic Era (150 mya) (Jones 1998). During these millions of years of evolution, they have established important relationships with insects some of which are still present today.



Figure 1. Female *Dioon edule* (Cycadales: Zamiaceae) plant exhibiting a cone bearing toxic (but edible) seeds. Note the heavily damaged leaves by *Eumaeus debora* (Lepidoptera: Lycaenidae).

According to the fossil record, plant-herbivore interactions were established early in the evolution of insects. Fossils showing insects with mandibles capable of feeding on pollen, spores or ovules appeared as early as the Carboniferous period (Crepet 1979; Jolivet 1998). In this intertwined evolutionary arms-race, plants have protected themselves from insect herbivory by structural and chemical defenses, and yet, in many cases, pollination is insect-mediated (Vovides *et al.* 1993). Insect pollination has existed since the Mesozoic Era and beetles were probably among the earliest pollinators (Jolivet 1998). Pollen is nutrient-rich, so a trade-off exists in the nutritional value of the pollen and its toxicity as the plant benefits from attracting and rewarding pollinators, but it will also benefit by protecting the pollen from non-pollinating herbivores. In many systems, pollen is protected with the plant's secondary metabolites (Praz *et al.* 2008).

In contrast to most gymnosperms, Cycads are insect-pollinated (Terry 2001) with most evidence involving weevils (Curculionidae) (Vovides 1991), and less commonly, members of other beetle families (Tenebrionidae, Languriidae, Anthribidae, Boganiidae, and Nitidulidae) (Jones 1998). Thrips (genus *Cycadothrips*: Thysanoptera) also play a major role in the pollination of the Australian Cycad, *Macrozamia* (Terry *et al.* 2007). Similarities in the vectors and the pollination procedures between species of different genera and on different continents suggest that these pollination systems were established long before the break-up of the super-continent of Gondwana (Jones 1998). Cycad-associated Boganiidae beetles have phylogenetic links back to the Lower Cretaceous Period (about 100 mya) when Africa and Australia were still joined, and are still intimately associated with these plants in both Africa and Australia (Labandeira 2000). Thrips, and some beetle pollinators reported so far, belong to basal lineages within their specific phylogenies, suggesting the possible antiquity of important insect-Cycad relationships (Jones 1998).

In the Cycad-pollinator paradigm, which is an example of a primitive pollination interaction, plant volatiles attract pollinators that congregate, mate and oviposit on the plant's reproductive structure (Jones 1998; Vovides *et al.* 1993). Schneider *et al.* (2002) speculate that this pollination strategy evolved from a situation of "accidental pollen transfer" into a symbiotic relationship where initially beetles would visit cycad cones to feed on their nutrient-rich pollen.



Figure 2. Aulacoscelinae beetles feeding voraciously on *Zamia elegans* (Cycadales: Zamiaceae).

However, Cycads do not represent a source of food to all, as they are protected with highly toxic compounds. Only the insects that have the ability to detoxify or control the plants compounds can feed upon them. This toxicity has always been regarded as a defense strategy towards herbivores.

Cycad Toxicity

Cycads contain two major classes of defensive compounds against herbivory: azoxyglycosides (AZGs) which are produced as secondary metabolites, and non-protein amino acids, such as BMAA, produced by cyanobacteria on the cycads roots (Cox and Windsor 1999a; Cox *et al.* 2003; De Luca *et al.* 1980; Kisby *et al.* 1992; Yagi 2004).

Azoxyglycosides

Cycad-associated AZGs have a common aglycone named methylazoxymethanol (MAM), which is the toxic compound (Kobayashi and Matsumoto 1965). MAM is highly reactive and has the ability to alkylate nucleic acids, such as DNA, which leads to potent mutagenic properties (Laqueur and Spatz 1968). These neurotoxin and carcinogenic properties have been well documented in numerous animal models (Laqueur and Spatz 1968; Matsushima *et al.* 1979; Seawright *et al.* 1990; Smith 1966; Teas and Dyson 1967).

The two most common Cycad AZGs, cycasin and macrozamin, are found in the seeds and leaves of all Cycad genera (De Luca *et al.* 1980; Yagi 2004; Yagi and Tadera 1987). These glycosylated (sugar bound) storage forms are less toxic, and MAM is released by the action of either endogenous plant glycosylases upon herbivory or enzymes in the herbivore digestive system (Schneider *et al.* 2002; Yagi 2004). In the herbivore gut, enzymes may be of insect or microbial origin (Laqueur and Spatz 1968).

Several butterfly species belonging to the *Taenaris*, *Luthrodes* and *Eumaeus* genera and also the pollinating weevil *Rhopalotria mollis* Sharp, which are Cycad specialists, have been shown to sequester the AZG toxins (Castillo-Guevara and Rico-Gray 2002; Nash *et al.* 1992; Rothschild 1992; Schneider *et al.* 2002). Once sequestered, the toxins are now used by the insects as a defense mechanism against predators. Cater-

pillars of the tiger moth *Seirarctia echo* (J.E. Smith), when reared on MAM-enriched food, catalyze the conversion of MAM back to the less-toxic cycasin form using a gut glycosyltransferase and store the cycasin in their haemolymph (Teas 1967). This suggests a specialized mechanism involved in the control and translocation of the toxins.

Non-protein amino acids (BMAA)

The second important class of toxic compounds found in Cycad is the non-protein amino acid β -methyl-amino-alanine (BMAA) which acts on neural systems (Seawright *et al.* 1990). This metabolite originates from cyanobacteria that form a symbiotic relationship with the Cycad coralloid roots (Cox *et al.* 2003), and is believed to be produced by all cyanobacteria lineages in symbiosis or free-living (Cox *et al.* 2005). BMAA is known to accumulate in the plant tissue, and has been identified in idioblasts in Cycad cones (Vovides *et al.* 1993); however, the presence of this amino acid and other Cycad secondary metabolites in the pollen is unknown. BMAA is known to accumulate in the tissue of seed predators like bats (Cox *et al.* 2003), but no Cycad specialist insect has ever been analyzed for its presence.

Insect adaptations to Cycads

The insect mechanisms involved in detoxifying and/or sequestering the Cycad toxins are evidence of the ancestral arms-race between herbivores and plant defense. One of the least studied Cycad specialists are the Aulacoscelinae, a basal lineage of leaf beetles (*Chrysomelidae sensu lato*) (Cox and Windsor 1999b). The aposematically colored Aulacoscelinae beetles, voraciously feed on young Cycad leaves (Fig. 2), and when threatened they can reflexively bleed cycasin and macrozamin (Prado unpublished). The reflexive bleeding mechanism consists of exposing haemolymph between the tibia and femur. More information on the nature of the Aulacoscelinae-Cycad relationship is needed to support the idea that these beetles have been related to Cycads since the Jurassic period, as suggested by fossil evidence (Crowson 1991; Zhang 2004). A key aspect in resolving the nature of the herbivore beetle-Cycad story is the mechanism involved in controlling the Cycad toxicity.

One important strategy used by insects to cope with plant defensive compounds is preferential excretion of the toxic compounds and/or detoxification (Després *et al.* 2007). When the cycad is fed upon, plant or insect-derived glycosidases cleave the sugars from cycasin and macrozamin to generate MAM, which may then be converted to the aldehyde (MAMAlD) by an NAD(P)⁺-dependent alcohol dehydrogenases and/or choline dehydrogenase, present in the insect saliva and/or gut increasing the compounds solubility (Candas *et al.* 2003). However, specialist herbivores, such as these Aulacoscelinae beetles, feeding on the Cycad may have a myriad of enzymatic mechanisms to metabolize the AZGs. Like in the Lepidopteran Cycad-specialist, a glycosyltransferase enzyme may add a sugar back to the toxic MAM convert-



ing it back to its safe cycasin and macrozamin form. Another possibility is a direct uptake of the compounds from the gut to the haemolymph through an active transport mechanism.

The different adaptations to Cycad toxicity by the specialist herbivores not only determine their relationship with the plant, as pollen or leaf predators, but also limit the possible uses the plant's secondary metabolites can gain in the insect's life cycle after sequestration.

Final Remark

How does this relate back to humans? Cycads have formed an important part of the diet of many tropical indigenous groups for thousands of years (Thieret 1958). The steps and primitive tools involved in Cycad flour production are exactly the same of those used for maize flour. So, ethnobotanists are now relating the human process involved with Cycad seed detoxification with the origins of maize flour production (Fortanelli, personal comm. 2008) The mechanisms insects are using for the detoxification of the Cycad tissues could prove to be as informative as the cooking techniques that relieve the tissue from its toxicity. From these ancestral cooking techniques, ethnobotany has now essential clues about the origins of flour production. We hope to gain insights on the origins of the Cycad-herbivore relationships by studying their different detoxification mechanisms.

References

Borenstein, A.R., J.A. Mortimer, E. Schofield, Y. Wu, D.P. Salmon, A. Gamst, J. Olichney, L.J. Thal, L. Silbert, J. Kaye, U.L. Craig, G.D. Schellenberg, and D.R. Galasko. 2007. Cycad exposure and risk of dementia, MCI, and PDC in the Chamorro population of Guam. *Neurology* 68 : 1764-1771.

Candas, M., O. Loseva, B. Oppert, P. Kosaraju, and L.A. Bulla Jr. 2003. Insect resistance to *Bacillus thuringiensis*: Alterations in the indianmeal moth larval gut proteome. *Mol. Cell Proteom.* 2 : 19-28.

Castillo-Guevara, C., and V. Rico-Gray. 2002. Is Cycasin in *Eumaeus minyas* (Lepidoptera: Lycaenidae) a predator deterrent? *Interiencia* 27 : 465-470.

Cox, M.L., and D.M. Windsor. 1999a. The first instar larva of *Aulacoscelis appendiculata* n. sp. (Coleoptera: Chrysomelidae: Aulacoscelinae) and its value in the placement of the Aulacoscelinae. *J. Nat. Hist.* 33 : 1049-1087.

Cox, M.L., and D.M. Windsor. 1999b. The first instar larva of *Aulacoscelis* sp. and *Megascelis puella* Lacordaire (Coleoptera: Chrysomelidae: Aulacoscelinae, Megascelinae) and their value in the placement of the Aulacoscelinae and Megascelinae. Pages 51-70 in M.L. Cox (ed.), *Advances in Chrysomelidae Biology*. Backhuys Publishers, Leiden, The Netherlands.

Cox, P.A., S.A. Banack, and S.J. Murch. 2003. Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. *Proc. Natl. Acad. Sci. USA.* 100 : 13380-13383.

Cox, P., S. Banack, S. Murch, U. Rasmussen, G. Tien, R. Bidigare, J. Metcalf, L. Morrison, G. Codd, and B. Bergman. 2005. Diverse taxa of cyanobacteria produce β -N-methylamino-L-alanine, a neurotoxic amino acid. *Proc. Natl. Acad. Sci. USA.* 102 : 5074.

Crepet, W.L. 1979. Insect pollination: A paleontological perspective. *BioScience* 29 : 102-108.

De Luca, P., A. Moretti, S. Sabato, and G. Siniscalco Gigliano. 1980. The ubiquity of cycasin in cycads. *Phytochemistry* 19 : 2230-2231.

Després, L., J.-P. David, and C. Gallet. 2007. The evolutionary ecology of insect resistance to plant chemicals. *Trends Ecol. Evol.* 22 : 298-307.

Jolivet, P.H. 1998. *Interrelation between Insects and Plants*. CRC Press, Boca Raton, Fl. 309 p.

Jones, D.L. 1998. *Cycads of the World*. Reed Natural History, Sydney, Australia. 312 p.

Kisby, G.E., M. Ellison, and P.S. Spencer. 1992. Content of the neurotoxins cycasin (methylazoxymethanol β -D-glucoside) and BMAA (β -N-methylamino-L-alanine) in cycad flour prepared by Guam Chamorros. *Neurology* 42 : 1336-1340.

Kobayashi, A., and H. Matsumoto. 1965. Studies on methylazoxymethanol, the aglycone of cycasin : Isolation, biological, and chemical properties. *Arch. Biochem. Biophys.* 110 : 373-380.

Laqueur, G.L. and M. Spatz. 1968. Toxicology of cycasin. *Cancer Res.* 28 : 2262-2267.

Mamay, S.H. 1969. Cycads: Fossil evidence of late Paleozoic origin. *Science* 164 : 295-296.

Matsushima, T., H. Matsumoto, A. Shirai, M. Sawamura, and T. Sugimura. 1979. Mutagenicity of the naturally occurring carcinogen cycasin and synthetic methylazoxymethanol conjugates in *Salmonella typhimurium*. *Cancer Res.* 39 : 3780-3782.

Nash, R.J., E. Arthur Bell, and P.R. Ackery. 1992. The protective role of cycasin in cycad-feeding lepidoptera. *Phytochemistry* 31 : 1955-1957.

Praz, C., A. Muller, and S. Dorn. 2008. Specialized bees fail to develop on non-host pollen: do plants chemically protect their pollen. *Ecology* 89 : 795-804.

Rothschild, M. 1992. Egg protection by the atala hairstreak butterfly (*Eumaeus atala florida*). *Phytochemistry* 31 : 1959-1960.

Schneider, D., M. Wink, F. Sporer, and P. Lounibos. 2002. Cycads: their evolution, toxins, herbivores and insect pollinators. *Naturwissenschaften* 89 : 281-294.

Seawright, A., A.W. Brown, C.C. Nolan, and J.B. Cavanagh. 1990. Selective degeneration of cerebellar cortical neurons caused by cycad neurotoxin, L- β -methylaminoalanine (L-BMAA), in rats. *Neuropharmacology* 16 : 153-169.

Smith, D.W.E. 1966. Mutagenicity of cycasin aglycone (Methylazoxymethanol), a naturally occurring carcinogen. *Science* 152 : 1273-1274.

Teas, H.J. 1967. Cycasin synthesis in *Seirarctia echo* (Lepidoptera) larvae fed methylazoxymethanol. *Biochem. Biophys. Res. Commun.* 26 : 686-690.

Teas, H.J., and J.G. Dyson. 1967. Mutation in *Drosophila* by methylazoxymethanol, the aglycone of cycasin. *Proc. Soc. Exptl. Biol. Med.* 125 : 988-990.

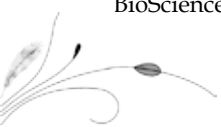
Terry, I. 2001. Thrips and weevils as dual, specialist pollinators of the Australian Cycad *Macrozamia communis* (Zamiaceae). *Int. J. Plant Sci.* 162 : 1293-1305.

Terry, I., G.H. Walter, C. Moore, R. Roemer, and C. Hull. 2007. Odor-mediated push-pull pollination in Cycads. *Science* 318 : 70.

Thieret, J.W. 1958. Economic botany of the Cycads. *Econ. Bot.* 12 : 3-41.

Vovides, A.P. 1991. Insect symbionts of some Mexican Cycads in their natural habitat. *Biotropica* 23 : 102-104.

Vovides, A., K.J. Norstog, P.K.S. Fawcett, M.W. Duncan, R.J. Nash, and D.V. Molsen. 1993. Histological changes during maturation in male and female cones of the cycad *Zamia furfuracea* and their significance in relation to pollination biology. *Bot. J. Linn. Soc.* 111 : 241-252.





- Yagi, F. 2004.** Azoxyglycoside content and [beta]-glycosidase activities in leaves of various cycads. *Phytochemistry* 65 : 3243-3247.
- Yagi, F., and K. Tadera. 1987.** Azoxyglycoside contents in seeds of several cycad species and various parts of Japanese Cycad. *Agric. Biol. Chem.* 51 : 1719-1721.
- Zhang, J.-F. 2004.** The first find of chrysomelids (Insecta: Coleoptera: Chrysomeloidea) from Callovian-Oxfordian Daohugou biota of China. *Geobios* 38 : 865-871.
- Zhifeng, G., and B.A. Thomas. 1989.** A review of fossil cycad megasporophylls, with new evidence of *Crossozamia pomel* and its associated leaves from the lower Permian of Taiyuan, China. *Rev. Palaeobot. Palyno.* 60 : 205-223.

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