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Notes on insect diversity of Indian *Cycas* species

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Abstract

The question of an analogue evolution of plant - insect interaction in gymnosperms has remained unanswered until recently, in spite of indications of insect pollination in the extant cycad genera. Earlier observations on insect pollination have now been verified by convincing experiments with several cycads in which anemophilous pollination was excluded. Insects have long been known to visit cycad cones and now it is established that in rest of the cycad species where both male and female reproductive organs are organized in compact cones, these insects help in the pollination. But *Cycas* is the only genus where female megasporophylls do not form a cone and its pollination mechanism has not been fully comprehended. The present investigation was carried out in the natural habitats of *Cycas*species from the Western Ghats, India. Five coleopteran insects were found abundantly in the male cones of *Cycas* and our observations on the role of these insects led us to believe that *Cycas* among cycads which appear to be true remnants of pteridospermous line have somehow has also maintained mutualism with the primitive plant chewing insects like coleopterans couldbe the results of an ancient co-evolution.

Keywords: Cycadaceae, Cycas, Male cone, Pollen, Coleopterans, Pollination

Introduction

Survival of cycads with its primitive life and reproductive strategies has fascinated the biologists since a long time. All the ten genera of living cycads are obligate dioecious and reproductive parts are arranged in compact cones except for the female reproductive organ in the genus *Cycas*, where the ovule bearing leaves, the megasporophylls are loosely arranged in strobili or pseudo cones (Figure 1). Since the male cones produce a copious amount of pollen grain, the pollination in cycads was thought to be anemophilous (Norstog, 1987; Tang, 1987; Niklasand Norstog, 1989**;** Donaldson *et al*, 1995; Donaldson, 1997). The growing insurgence in the past decades to understand the reproductive biology of the cycads has proved that all the living species of cycads maintain symbiotic associations with host-specific insects especially with coleopterans (Hall et al., 2004; Terry, 2001 and Terry and Marler, 2005, Schneider, 2002). Now it is established that these insects help in the pollination of those cycad species where both male and female reproductive organs are organized in compact cones.*Cycas*is the most primitive genus among all the living cycads and have megasporophylls which are not organized into a compact cone and at maturity these spirally arranged megasporophylls spread over like the foliage leaves thus do not provide shelter to insects which cone species do. In *Cycas,* it is still speculated whether wind or insect or both are responsible for the transfer of pollen. If we agree that *Cycas* is anemophilous assessing its primitive female cone morphology then several

fundamental questions needs to be answered like the species - specific association of insect fauna in the male cones and function of secondary metabolites (odor) at the time of pollen dispersal. However, studies on the reproductive biology and ecology of South African cycads have established the fact that Coleopterans play a significant role in the pollination of cycad genera of these regions. Wild populations of *Cycas* in Southeast Asia and Australia have also been surveyed for insects and two genera of curculionidae the genus *Tychiodes*(ca 30 species)and beetles of the genus *Xenocryptus* (17 species) have been reported and believed to be pollinators of *Cycas*(Tang *et al.,* 1999; Pant & Singh, 1990). Weevils of a related Cossoninaegenus have been collected from cones of Australian species of *Cycas* in Queensland and the Northern Territory (Jones, 2002).

Indian cycads

The order Cycadales consists of ten living genera with about 305 species and subspecies distributed over the tropical and sub-tropical regions of the World (Hill, 2004). Asian cycads are represented by a single genus *Cycas*with ca 105 species and subspecies. From India,eight species of and a variety of *Cycas* were reported (*C. annaikalensis* Singh and Radha*C. circinalis*L., *C. circinalis*var.*orixensis* Haines,*C.swamyi* Singh and Radha, *C. sphaerica* Roxb, *C. beddomei* Dyer, *C. pectinata* Ham. and *C. rumphii* Miq., *C. zeylanica* Lindstorm and Hill)from the wild habitats of Western Ghats crossing the states Karnataka, Kerala, Tamil Nadu; Eastern Ghats crossing Andhra Pradesh, Orissa; Assam, Sikkim and Andaman Nicobar Islands (Singh and Radha, 2008, Lindstrom and Hill 2007).

Butterflies, moths, weevils, beetles, thrips and ants have been found in association with cycads (Schneider, 2002, Jolivet, 2005). Some of these phytophagous insects are casual visitors, while others are host–specific and perhaps obligate pollinators. Insects associated with the cycads can be broadly categorized into two groups, viz., insects on the vegetative organs and those associated with the reproductive organs.

Insects on Photosynthetic leaves

The evidences have been gathered caterpillars of Plain Cupid cycad blue butterfly *Edalespandava* (syn. *Euchrysopspandava*Horsefield, Order: Lepidoptera, Family : Hesperioidea) feed on leaflets of various *Cycas* species growing in their natural habitat as well as in the plants cultivated in the gardens. *Edalespandava*(*Euchrysopspandava*)found to be hovered around the *Cycas* plants, they were seen copulating and laying eggs in a great number on the adaxial side of the new emerging, still unfolded soft pinnae of *C.annaikalensis*, C.circinalis, *C, swamyi*, *C. spherica*, *C.rumphii*and *C. circinalis*var. *orixensis*. The eggs hatch into larvae of about a cm length. Larvae vary in color from green to dark brown as they mature (Figure 2, D). As the pinnae uncurl these caterpillars (grubs) move on the lower side of the pinnae and devour the lower cuticle and mesophyll tissue of the young and soft pinnae leaving behind the thick upper cuticle which appear blistered as if they have been infected by some pathogen (Figure 2, A). Sometimes these voracious larvae also penetrate and feed on the soft rachis of young fronds (Figure 2, B, C) thus, leaving behind hollowed rachises- with scarred bunch of blistered leaves which are inefficient to carry out any photosynthesis. .

In the laboratory, young and mature larvae were collected reared in the artificial plastic chamber by feeding the young pinnae. Except the upper cuticle (which is comparatively thick). The whole tissue of the pinnae of *Cycas* species have been eaten by the larvae. After 20-48 hrs larvae move and pupated with in secured and tightly clasped the leave bases. The pupa metamorphosed into adult butterflies within three days (Figure 2,

E,F).

Insects on the *Cycas*male cones

The insects associated with the male cone and young megasporophyll were collected in a large number most of them are identified as coleopterans. Generally Coleopterans and Dipterans are attracted by the unpleasant odor emitted by plant species (Porsch, 1956). The raised temperature and emanation of musky smell of *Cycas* male cones during early evening has been attributed to the alternate cyanide pathway, which attracts coleopterans and stimulates them for different activities.

The observations on the probable pollination mechanism was carried out in the natural habitats of two *Cycas*species in the Western Ghats i.e., *C. circinalis*and *C. annaikalensis,* Close examination during afternoon and early evenings when the musky smell emission become strong, the movement of small insects was seen. Field observations during the time from the initiation till the maturation of pollen cones have clearly shown that pollenivorous weevils invade generally male cones though they have also been observed on the megasporophylls when female strobili are young and compact and ovules were at the receptive stage. Observations at various levels of periodicity of male cone maturation were seen to have some insect visitors initially which later on grew in number as they feed on the copious amount of pollen grains produced by these cones and their starchy, fleshy tissue of microsporophyll and peduncle (Figure 3, A-E). The maximum movement of insects was seen in the cones prior to pollen shedding when usually a strong musky odor is emitted, which initiates in early evening hours and reaches its peak in the late evening with rise of cone temperature from $3\text{-}4^0$ C above atmosphere temperature. During this, the color of cone also changes from deep yellow to orange. The progenies of these beetles and weevils use the male cones as homes and multiply to subsequent generations and feed on abundant starchy tissue of almost every part of the cones till the hollowed and dried cone crumbles down (Figure 3, E, F). About 150 individuals have been collected and identified. These insects have been collected repeatedly from the young, mature and dried cones of the *Cycascircinalis*L., and *Cycasannaikalensis* localities (Southern Western Ghats) and have been identified as Coleopterans. Out of these five types of coleopterans collected, two of them have been identified as weevils are *Calandra*spp (Family: Rhynchophoridae), and *Derelomus*spp. (Family: Curculionidae), and three are beetles of the genera *Pharaxonotha? nigra* (Gorham)- (Family: Languriidae), *Tribolium*spp. (Family: Tenebrionidae), and *Carcinops troglodytes* (Paykull) (Family: Histeridae) (Table 1) (Figure 3 G-L). All these insects are pollenivorous and feed on the copious amount of pollen. Once the insects enters the male cone, they take shelter inside the male cone and start their life cycle by laying eggs, which hatched and gave rise to next generations**.**

Like many of the modern day curculionoids, Calandrinae have been found to live on Palmae. In one of the Western Ghats *Cycas*species, *Cycascircinalis*whose wild localities are in palm dominated area that is rich in humus, large number weevils of *Calandra* spp. has been found in their male cones. It is presumed that in due course of time *Calandra*might have found palm as an alternative secondary host to cycads, as palms also produce copious amount of monocolpate pollen grain, very much similar to that of cycads in morphology .In long association the species of *Calandra* associated with *Cycas* and palms in this regionmight have evolved as a generalized pollinator. The other insect was, *Carcinops troglodytes* is basically a larvae predator. This species

might have evolved physiology to check to the *Calandra* invasion on the male inflorescence of palm and male cones of *Cycas* by devouring their caterpillars along with pollen debris since other group of insects have refrained themselves to devour the toxic pollen grains. The other palm weevil *Derelomus*has also been reported on *Cycasannaikalensis*, which grows in the undestroyed forest where palms populations are not found. Recently, Raju and Jonathan (2010) reported the presence of *Derelomus*weevils in the male cones of *Cycassphaerica*from the Eastern Ghats.

Another beetle *Phraxonotha*has been reported in other cycads (Tang, 1987),it is for the first time reported from any of the Indian *Cycas*. Yet another insect *Tribolium*spp., which is a common flour weevil, has also been reported from the male cones of *C. annaikalensis.* These insect which have been in association with male cones and young female cones though suggest their involvement as probable pollinator but it requires further confirmation.

Except for laxedmegasporophylls which usually are quite compact at the time of receptivity, *Cycas*has all the characters exhibited by insect pollinated plants. Strong pollen odor and color of the pollen cone have been described mainly in plants pollinated by pollen-feeding insects, especially beetles and weevils by cycads (Porsche, 1956; Faegri et al., 1979). Major presence of alpha methyl ketones in flowers and pollen odors may be associated with anemophily or with reduced entomophilies if these compounds are not countered by the presence of volatiles that are attractive to insects. Comparative studies of plants odors in plants pollinated by different animal groups are few, but they reveal patterns that are associated with the occurrence of pollen feedings(Dobson and Bergström, 2002). A similar trend was apparently found in *Cycasrumphii* by Pellmyr*et al*.,1991, which is supposedly wind pollinated, where male cones displayed a preponderance of alpha methyl ketones or alcohols in the odors of males from the insect pollinated cycads.

Odor arising from anthers in flowering plants is often considered to represent the oldest food attractants for flowers visitorsand to have preceded other olfactory and visual cuescycads (Porsch, 1956; Faegri et al., 1979). Perhaps this phenomenon began much earlier in the bisexual cones of Cycadeoideales and continued to the extant cycads. Pollen odors most likely evolved as a defense against the pathogens and pollen feeding animal taxa prior to the development of animal pollination. As flowering plants became dependent on various agencies for pollen transfer there would have been increasing selection pressure for pollen odor to include volatiles attractive to the pollinators, which may have included both food related and mating-related chemical cues among cycads, which led the authors to suggest that pollination in *Cycas*species .

Evolutionary studies have revealed that insect-plant association most probably started from Pennsylvanian period in the Carboniferous age. Perhaps therefore pollination through beetles is the oldest form of insect pollination. Paleobotanical evidences suggest that cycads came into existence during Permian time together with the beetles, and they flourished during the Mesozoic which is also thought to the period of the great diversification of the beetles. Though the beetle-cycad association is primitive it is not necessary that cycads and insects are preserving this association since Mesozoic era. In recent revision on phylogenetic relationship of cycad associated weevils and co-evolution of weevils has suggested that weevil – cycad association evolved on a continental or regional scale, and this association might have evolved as shifts from angiosperms. The

beetle families which are specialized in feeding on cycads are either neotropical or Southern Gondwanian in distribution, but some are cosmopolitan (Jolivet, 2005). The beetles frequenting cycads are found mostly on the male cones and on the leaves some weevils however have been reported from leaves but rarely do they visit the trunks. A few have been observed on the female cones but none has been found on the roots though some larvae have adapted to the ridiculous life and probably the larvae of aulacoscelines are seed borers, as those of bruchids, but these probabilities remain to be confirmed or infirmed. (Crowson, 1991).

Insects and Conservation of cycads in their natural habitats

From the point of view of conservation there are very great implications, the cases, where plants are lost from the natural environment it is often possible to take cultivated plants and replace them into the habitat they once occupied. With cycads this may not be feasible. The insect pollinators appear to be very specific in their association with cycads, often living in a symbiotic relationship with them. When the population of cycads of a given species in a locality falls to very low level, the insect may become extinct. When cultivated cycads are reintroduced into the area to replenish the population they may do well and it may appear that the procedure has been very successful. The problem is that without the insect pollinators there will be no natural regeneration and in the long run it will all be a waste of time unless there is human intervention on a continual basis. Another consequence is that in cases where the population of cycads in an area becomes too low and the insect pollinator cannot survive there is not much point in trying to protect the remainder of the plants and thereby hope that the population will recover. Without the pollinator this will never happen. Perhaps it is possible that a pollinator insect may be found somewhere that will pollinate cycads indiscriminately. If this was possible it would be a significant help in saving natural cycad populations from total extinction. It is certain that the subject of cycad pollinators is a vital one for those who wish to conserve these fascinating plants.

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References:

1. Crowson, R.H. The relations of Coleoptera to Cycadales. *In* M. Zunino, X. Beeltes and M.Blas.eds.,*Advances in Coleopterology*,1991,13-28.
2. Dobson, H.E.M. and G. Bergström. The ecology and evolution of pollen odors,*Plant Systematics and Evolution*,2002, 222: 63-67.
3. Faegri, K and L. van der Pijl. *The Principles of Pollination Ecology*, ed. E, Pergamon Press, New York,1979.
4. Hall, J. A., G. H Walter, D. M. Bergstrom and P. Machin. Polli-nation ecology of the Australian cycad *Lepidozamiaperoffskyana*(Zamiaceae),*Austr. J. Bot*.,2004, 52, 333–343.
5. Hill, K.D. *In: Cycad Classification: Concepts and Recommendation (eds)* Walters T, Osborne R. CABI.2004.

6. Jolivet, P. Cycads and Beetles. Recent views on pollination,*The Cycad Newsletter,*2005,28: (3-4) 1-7.

7. Jones, D.L. *Cycads of the World*, Smithsonian Institution Press, Washington.2002.

8. Lindstrom, A.J. and K.D. Hill. The genus Cycas (Cycadaceae) in India,Telopea,2007,11: 463–88.

9. Oberprieler R.G. "Evil weevils' – the key cycad survival and diversification? *Proceedings of the sixth International Conference on Cycad Biology, Thailand,*2004,17-194.

10. Pant, D.D and R. Singh. Preliminary observations on insect plant relationships in Allahabad plants of *Cycas. Palms & cycad.* 1990**.**28:10-14.

11. Pellmyr, O. Evolution of insect pollination and angiosperm diversification,*Trends Ecology and Evolution,* 1992, 7: 46-49.

12. Porsch, O. Windpollen and Blumeninseket. *Österr.Botan., Z* 1956,103: 1-18

13. Raju, A.J.S and K.H. Jonathan. Reproductive ecology of *Cycasbeddomei* Dyer (Cycadaceae), an endemic and critically endangered species of southern Eastern Ghats,*Current Science,*2010, 99(12):1833-39.

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

15. Singh, R., and P. Radha. A new species of Cycas (Cycadaceae) from Karnataka, India, Botanical Journal of the Linnaean Society,2008, 158: 430–5.

16. Tang, W. Insect pollination in the cycad *Zamia pumila*(Zamiaceae), *American Journal of Botany*,1987, 74 (1): 90-99.

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

18. Terry, T and T. Marler. Paradise last? Tipping the scales against Guam's *Cycasmicronesica,Cycad Newsletter,* 2005, 28(3-4): 21-23.

19. Donaldson, J.S., I. Nänni and de Wet Bösenberg.. The role of insects in the pollination of *Encephalartoscycadifolius.* In P. Vorster, ed., Proceedings of the Third International Conference on Cycad Biology, Cycad Society of South Africa, Stellenbosch,1995, 423 -434.

20. Donaldson, J.S. Is there a floral parasite mutualism in cycad pollination? The pollination biology of *Encephalartosvillosus*(Zamiaceae)m*American Journal of Botany* 1997, 84(10): 1398 1406.

21. Niklas, K.J and K.J. Norstog. Aerodynamics and pollen grain depositional patterns on cycad megastrobili; implication on the reproduction of three cycad genera (*Cycas, Dioon,* and *Zamia),Botanical Gazette,* 1984,145 : 92 –104

22. Norstog, K.Cycads and the origin of insect pollination,*American Scientis,*.1987, 75: 270- 279.

Figure 1. ***Cycas beddomei*** **A. Female megasporophylls B. Dried Male cone**

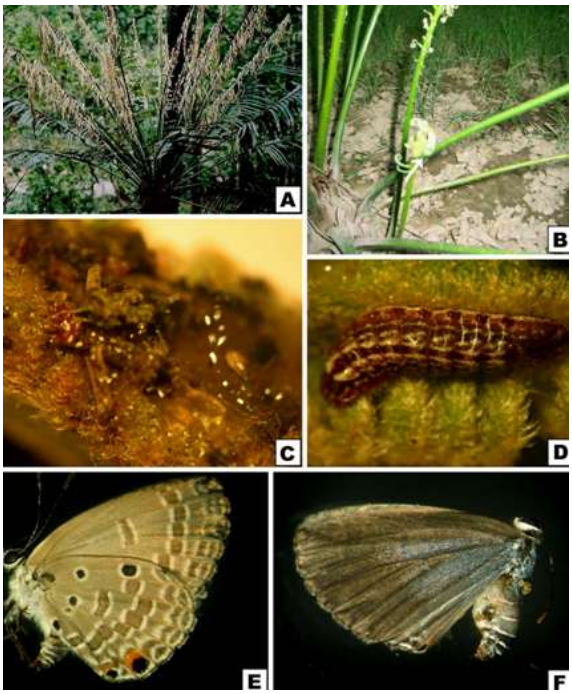

Figure 2.Insects associated with leaf: A. Showing remaining of upper cuticle, B. Young leave of *Cycasswamyi* incultivation is invaded by caterpillar,C. Close-up of young leaves devoured by caterpillar, D. Caterpillars, E, F, Adult butterflies

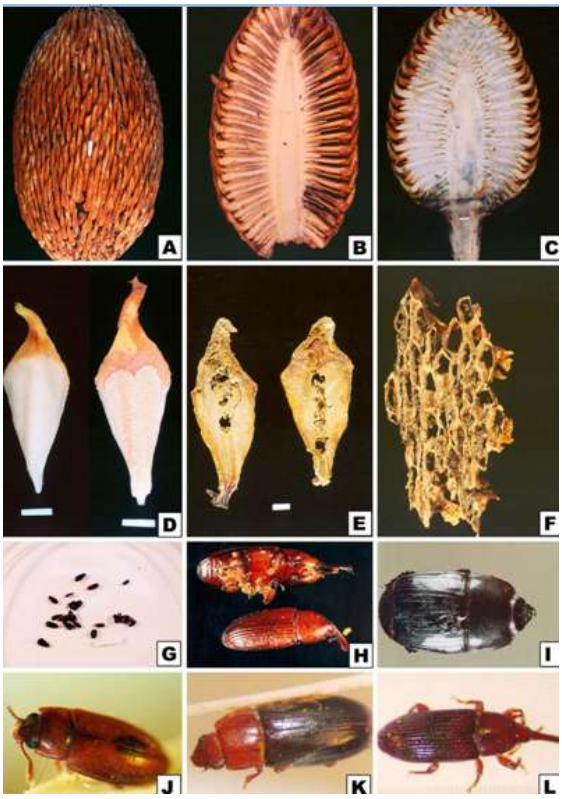

Figure 3.Insects associated with male cone: A. L.S of *Cycascircinalis* male cone;B. L.S of *Cycasannaikalensis* male cone; C. Male cone of *C. circinalis* is showing insect movements; D. Microsporophylls of *Cycas*;E. Chewed adaxial portion of microsporophylls; F. Hollowed peduncle;G. Collected insects from *Cycas* male cones;H. Weevil *Calandra* with pollens and trichomes; I.*Carcinops troglo*dytes; J. *Tribolium weevil;* K. ?*Pharoxonotha?nigra*;L. *Derelomus sp.*

Table 1. Details on Reported insect diversity of Indian *Cycas*species male cone so far

Name of the insects	***Name of the plant* /locality**	**Visited plant parts**	**Ref.**
*Calandra*sp.	*C. circinalis*/Kerala	Male cone	Radha and Singh
*Derelomus*spp.	*C. annaikalensis, C. circinalis*/Kerala *C.sphaerica* /Andhra Pradesh	Male cone	Radha and Singh Raju and Jonathan (2010)
*Tribolium*sp.	*C. annaikalensis,* *C. circinalis*/Kerala	Male cone	Radha and Singh
Alphilobus sp.	*C. beddomei*/Andhra Pradesh	Male cone	Raju and Jonathan (2010)
Carcinops troglodytes	*C. annaikalensis,* *C.circinalis*/Kerala	Male cone	Radha and Singh
Pharoxonotha?nigra	*C. annaikalensis,*	Male cone	Radha and Singh