

## PHENOLOGY AND POLLEN STUDIES OF SOME SPECIES OF ANNONACEAE IN NIGERIA.

Azeez, S.O.\* and Folorunso, A.E.

Obafemi Awolowo University, Department of Botany, Ile-Ife, Osun State, Nigeria.

Corresponding Author; email: okikihazan@yahoo.com.

(Received: 17th April, 2014 ; Accepted: 27th May, 2014)

### ABSTRACT

Phenology and pollen studies were investigated in some species of Annonaceae in Nigeria to help in providing pollen data and record of flowering patterns of the species studied. Phenological data for flowering and fruiting were tracked once a week for both rainy and dry seasons. Pollen grains from fresh anthers of the different species of Annonaceae were collected for pollen grain stainability and morphological studies. The peak flowering period in all the species studied was between March and April. Flower production took place before flushing of leaves in *M. tenuifolia*, however, in *A. reticulata* and *A. squamosa*, flowering and flushing of leaves occurred at the same time. All the species have predictable annual fruiting periods; the harvesting of their fruits and seeds, and sustainable management is bound to be relatively easy. The percentage pollen stainability was higher than percentage pollen germination in the species studied. Low fertility was observed in *A. reticulata* and *C. patens*. Tetrad pollen observed in *Annona*, *Monodora* and *Xylopia* is an advanced character in Annonaceae and could have an advantage over the monad. The genus *Cleistopholis* now appears to be one of those genera which have retained the largest number of the characteristics of a primitive member of Annonaceae. The evolutionary development is from *Cleistopholis* (monad) to *Xylopia* (tetrad) to *Monodora* (tetrad) to *Annona* (tetrad).

**Keywords:** Phenology, Pollen, Annonaceae, Evolution, Monad, Tetrad.

### INTRODUCTION

The dearth of phenological studies in tropical plants has been pointed out by various workers (Morellato and Haddad, 2000; Keatley, 2000). Phenological studies of tropical plants aim for a better understanding of the intricate relationship of species maintenance through the processes of pollination and seed dispersal. Phenological data are of great importance for the correct use and protection of the ecosystems (Machado *et al.*, 1997). Phenological parameters could facilitate data collection from these taxa in Annonaceae if well documented. Farmers and fruit growers can use phenological data as a tool in knowing when to plant and harvest crops and in some cases when to apply herbicides and insecticides. It could also aid in resolving the confusion arising from the classification of this family.

The classification of the Annonaceae has been a herculean task to workers on this family, the largest family of primitive Angiosperms (Chatrou, 2005). Palynology has played a major role in attempts to clarify higher-level relationships in the Annonaceae. Four major clades including Annonoideae, Anaxagoreoideae, Ambarioideae and Malmeioideae have been reported for Annonaceae (Chatrou *et al.*, 2012). Granular

monosulcate pollen as in Anaxagoreoideae and Ambarioideae, was ancestral in the family. Because columellar taxa diverged earlier than previously thought, columellae may have re-originated in the common ancestor of Malmeioideae and Annonoideae, which make up most of the family, with several later reversals to granular structure (Chatrou *et al.*, 2012).

This work therefore studies the phenological parameters of *Annona senegalensis*, *Annona squamosa*, *Annona muricata*, *Annona reticulata*, *Cleistopholis patens*, *Monodora tenuifolia* and *Xylopia aethiopica* to facilitate data collection in them. Pollen parameters in these species were also studied to resolve the problem of classification in the family.

### MATERIALS AND METHODS

#### Phenological Studies

The flowering and fruiting periods of some of the species of Annonaceae studied in Obafemi Awolowo University Ile-Ife, Nigeria Campus (longitude 7° 50.70" N and latitude 4° 58.44" E) were observed and documented. These plants were surveyed once a week for both rainy and dry seasons, the trend of the flowering, fruiting and shedding of leaves were then recorded. The

phenology was first studied between year 2003 and 2005; after three years, the phenology was studied again which covered between 2008 and 2010.

### Pollen Studies

Pollen grains from fresh anthers of the different species of Annonaceae studied were collected for pollen grains stainability and pollen morphological studies. For each species, pollen grains were shaken onto a clean slide and stained with Cotton Blue in Lactophenol. Four slides were prepared for each species. The pollen grains were examined and scored for stainability at 100 magnification and percentage stainability was calculated. Well formed, intact and uniformly-stained pollen grains were considered viable while those that were only partially stained or not stained at all and with collapsed outline were scored as non-viable (Olorode and Baquar, 1976). Pollen germination was carried out using the method of Nurhan (2003). Fresh matured pollen grains were dusted on the sterilized culture medium prepared from 25ml distilled water, 0.5g agar and 6.25g saccharose. The culture medium was left for 12 hours at 18°C for pollen tubes to germinate *in vitro*. The pollen grains were then fixed in 1:3 acetic-alcohol. 1 drop of this suspension was put on the slide and then stained with Cotton Blue in Lactophenol. The slides were viewed under oil and phase contrast illumination and then photographed under Lietz Dialux research microscope.

The pollen grains were acetolysed following the procedure of Erdtman (1943). The pollen grains were mounted in the dilute 25% glycerine and photomicrographs of the acetolysed pollen grains were taken with Leica camera, mounted on a Leitz Dialux research microscope.

### RESULTS

It was observed that *A. senegalensis* and *Cleistopholis patens* flowers during the dry season to the beginning of rainy season between January and April. *A. squamosa*, *A. reticulata*, *Monodora tenuifolia* flower during the early rainy season between May and August. The flowering period of *A. muricata* and *Xylopiya aethiopica* covers both the early and the late rainy season, March to October (Figure 1). The fruiting periods of the species is generally from the beginning of the rainy season to the late

rainy season. All the species studied are deciduous because, at one time or the other, there was effoliation and leaf flushing. Shedding of leaves occurred mostly during the dry season around November to January. In *Monodora tenuifolia* there was flowering after defoliation followed by leaf flush. In *A. reticulata* and *A. squamosa*, flowering and flushing of leaves occurred simultaneously. Flowering and fruiting occurs throughout the year in *A. muricata* while in *A. senegalensis* and *A. reticulata* only flowering occurs throughout the year (Figure 1). *Cleistopholis patens* flowers between January and July. *A. squamosa* flowers between February and June; *M. tenuifolia* flowers between February and August and *Xylopiya aethiopica* flowers between May and December. During the latter part of the flowering period, only the younger branches towards the top produce flowers. The peak flowering period for all the species studied except *Xylopiya aethiopica* was between March and April while the peak flowering period in *Xylopiya aethiopica* was July.

The fruiting period in *A. senegalensis* falls between December and September with peak period between May and July (Figure 2). *A. reticulata* produces fruit between June and December having a peak period between November and December. *Cleistopholis patens* has its fruiting peak period between April and May; *Xylopiya aethiopica* produces fruits between June and January with the peak fruiting period between August and September while that of *Monodora tenuifolia* occurs between May and June (Figure 2). The peak fruiting period in *A. squamosa* is in May. The peak flowering and peak fruiting period of *A. muricata* occurred three to four times in a year. It was noted that there was variation among years in time, length and abundance of flowering and fruiting.

Compound pollen grains in form of tetrad occurred generally in all the species of Annonaceae studied except in *Cleistopholis patens* (Figure 3). *A. muricata* has the largest pollen diameter and *C. patens* has the smallest diameter (Table 1). It was observed that the tetrad structure of *Xylopiya aethiopica* could be easily broken up when it was acetolysed. *Cleistopholis patens* has oblate pollen structure and it is surrounded by a thin wall. This is a pollen grain in which the polar axis is shorter than the equatorial axis. The pollen

structure of *A. squamosa* has a tetragonal tetrad in which the centres of the grains define a tetrahedron, it is transparent and surrounded by a thin wall. The pollens of *A. muricata* are also tetragonal tetrads like *A. squamosa*, however it is perforated, not transparent and surrounded by a thick wall (Figure 3).

*Annona senegalensis* has tetragonal tetrad, a unipolar tetrad in which all four members are in contact at the centre of the tetrad, so that in the correct orientation, the adjacent walls form a cross. It is

also perforated. The pollen forms of *Monodora tenuifolia* (Figure 3) and *A. reticulata* are similar to that of *A. senegalensis*, though malformation was observed in some pollens of *A. reticulata*. A spheroidal pollen grain structure was observed in *Xylopia aethiopica* and it is perforated. In this pollen type, the polar axis and the equatorial diameter are approximately equal (Figure 3). The pollen is tetraporate. However, when the pollen had not broken up, it had oblate pollen grain structure.

**Table 1:** Pollen Stainability and Germination in Relation to Pollen Diameter.

Species	Pollen stainability %	Pollen germination %	Mean ( $\mu\text{m}$ ) diameter
<i>A. muricata</i>	81.56	37.21	23.43
<i>A. reticulata</i>	43.64	12.45	6.30
<i>A. senegalensis</i>	66.26	21.32	10.50
<i>A. squamosa</i>	74.38	12.88	7.00
<i>C. patens</i>	38.53	0.94	1.75
<i>M. tenuifolia</i>	92.24	65.83	10.15
<i>X. aethiopica</i>	66.50	22.05	11.55

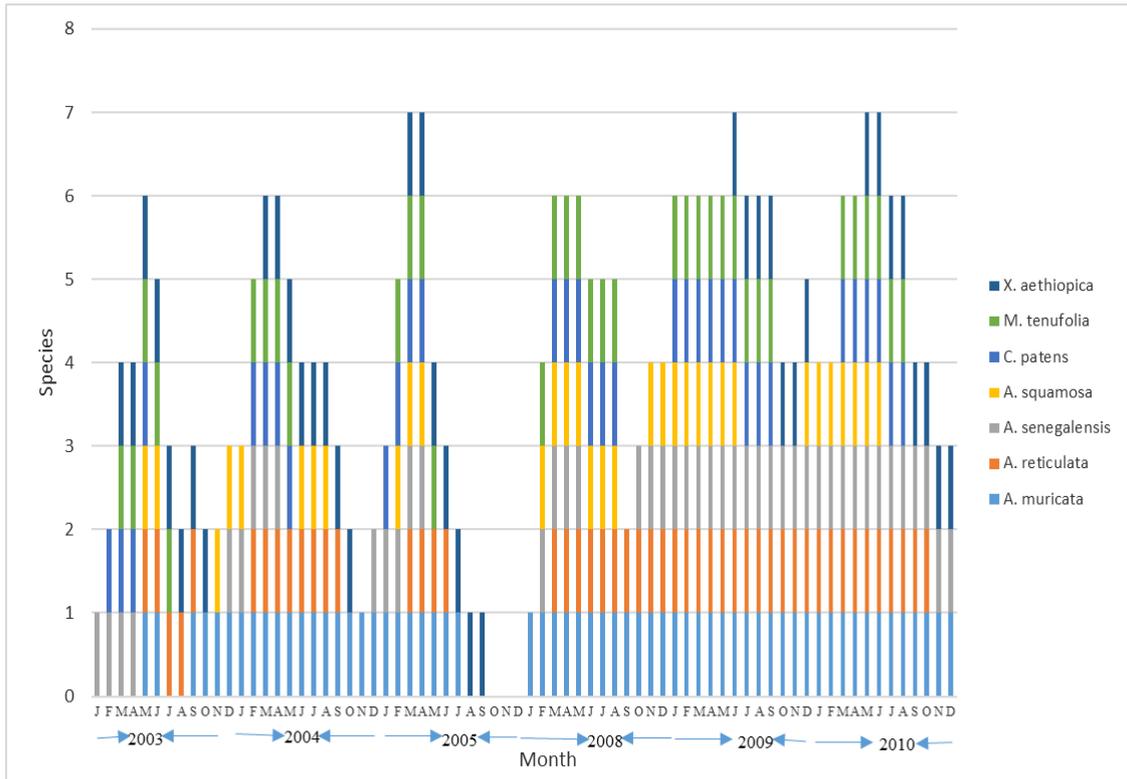


Figure 1: Flowering Patterns in Annonaceae Species Studied in Relation to Seasons

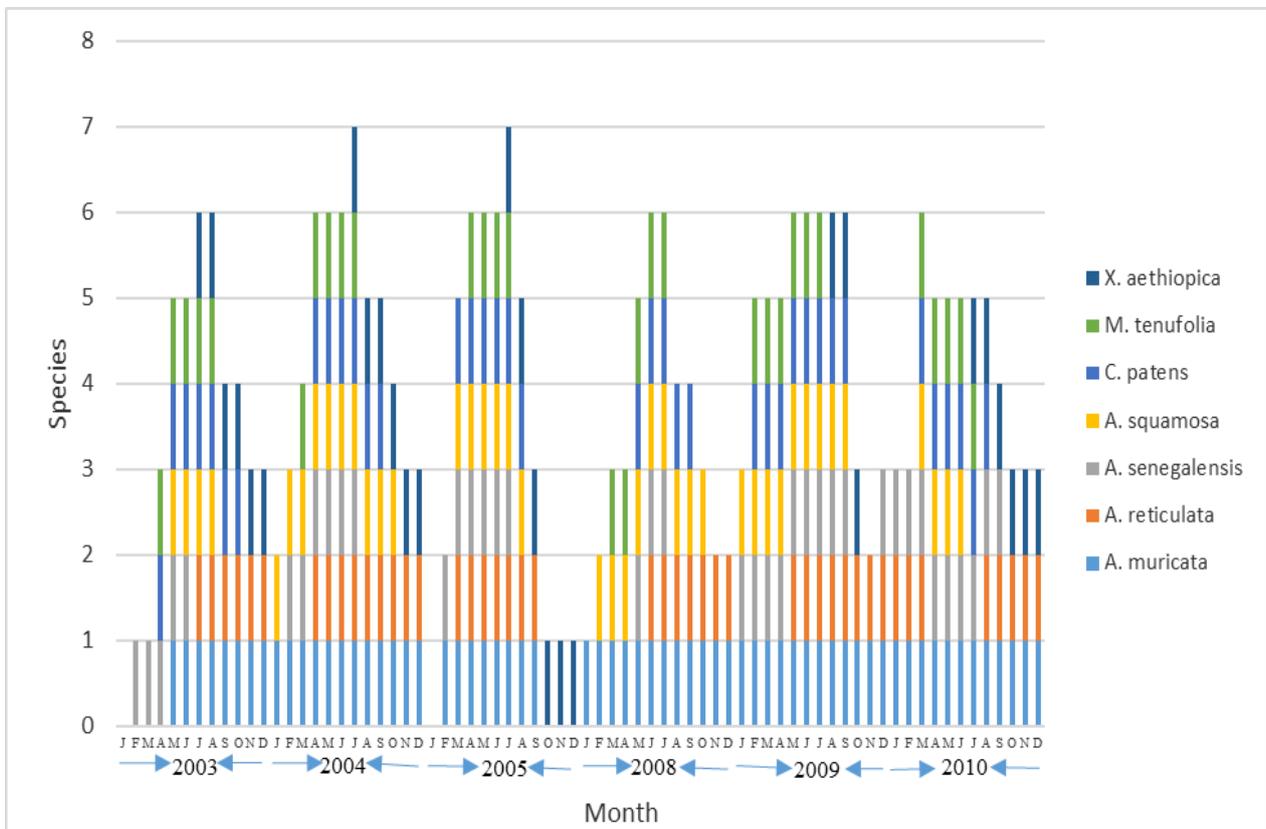
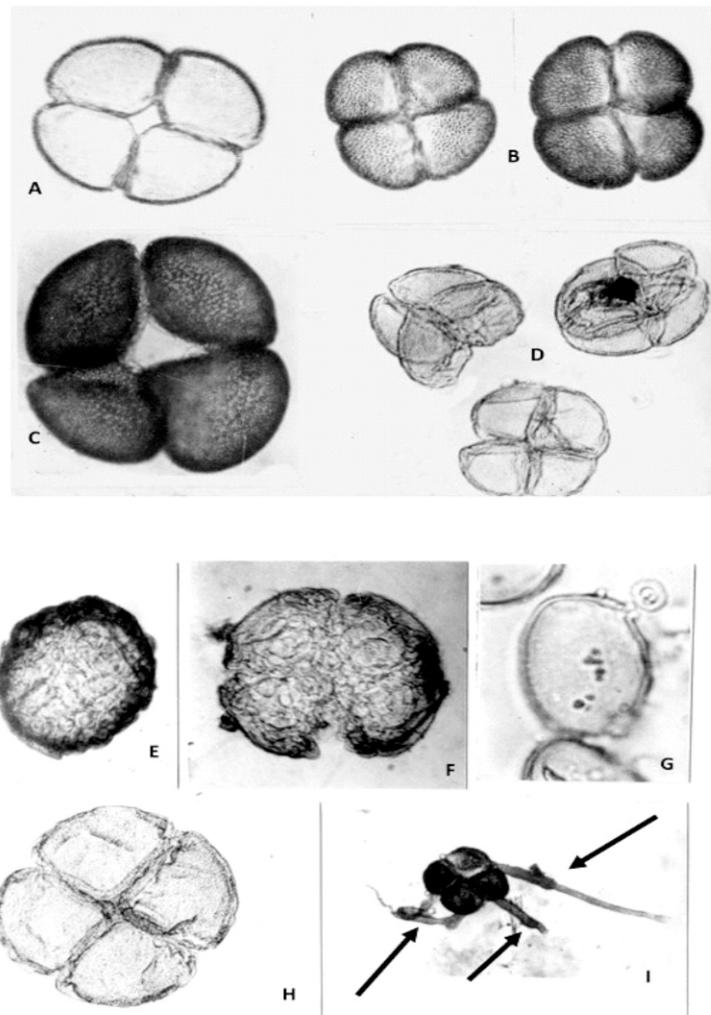


Figure 2: Fruiting Patterns in Annonaceae Species Studied in Relation to Seasons



- A.) *Annona squamosa*
- B.) *Annona senegalensis*
- C.) *Annona muricata*
- D.) *Annona reticulata*
- E.) *Xylopiopsis aethiopica* (unacetolysed)
- F.) *Xylopiopsis aethiopica* (acetolysed)
- G.) *Cleiophelis patens*
- H.) *Monodora tenuifolia* tetrad
- I.) *Monodora tenuifolia* showing germinated (tubes arrowed)

**Figure 3:** The Pollen Morphology of Some Species of Annonaceae Studied.

**Table 2:** Rainfall and Temperature Data from Year 2008-2010 in Osun State.

	Rainfall (mm)			Temperature (°C)		
	2008	2009	2010	2008	2009	2010
Jan	-	32.00	-	32.85	32.20	34.90
Feb	-	22.70	47.30	36.50	34.20	36.20
Mar	83.10	38.00	59.90	34.40	34.50	34.80
Apr	238.00	131.60	99.80	32.20	32.00	34.40
May	51.30	248.70	230.70	31.50	30.90	31.60
Jun	193.00	89.20	104.60	29.93	30.20	30.90
Jul	313.10	310.60	177.50	28.67	28.10	28.40
Aug	255.50	69.30	246.00	28.20	27.50	28.49
Sep	273.80	203.10	229.50	29.50	29.30	29.50
Oct	158.60	125.80	351.90	31.10	30.00	30.40
Nov	-	34.30	135.20	32.47	32.60	32.00
Dec	31.10	-	-	33.10	34.60	33.90

Source: Nigerian Meteorological Agency

## DISCUSSION

Inuoye *et al.* (2003) reported that the rainfall patterns significantly affect the plant phenology. Once the flowering has been initiated, the amount of precipitation over the growing season may affect the number of flowers and duration of flowering for a given species. *A. muricata* flowered throughout the year and this pattern has been observed in *A. senegalensis* and *A. reticulata*. Bawa *et al.* (2003) reported that most species flower several times a year. Also, closely related species in the tropics tend to flower together in the same season. Congenerics are similar with respect to overall flowering times and confamilials are similar for concentration of flowering time reflecting a temporal dispersion pattern of flowering across all months (Bawa *et al.*, 2003).

It was observed that the rainfall pattern in Osun State in recent years is changing which might have affected the flowering and fruiting patterns observed (Table 2). The findings of Hyesoon and Jungwoon (2004) supported what was observed in the species studied. They noted that patterns of flowering time and duration were associated with climatic factors. Both mean monthly temperature and precipitation were strongly positively correlated with the number of species in flower but not with flowering duration.

The hypothesis that related species will have similar flowering phenologies as reported by (Wright and Calderon, 1995) has been found to be

corroborated for other species of Annonaceae. All the species flowered in May and June. The peak flowering period in all the species studied was between March and April which was in line with the work of Wright and Calderon (1995). They observed that the mean flowering dates were concentrated in February and March which are the driest months of the year and in April and May when the wet season begins. Flowering is probably constrained by the dry-season water stress and the floral anthesis coinciding with the restoration of plant water status following the rain, renewed root growth, and/ or ongoing dry-season root uptake after leaf abscission (Borchert, 1980; 1983; Reich and Borchert, 1984).

Flowering was highly concentrated in time for most species hence, the mean and the variance of flowering times were similar among congenerics. The variance of flowering time was also similar among confamilials. It was observed that there was flower production before the flushing of leaves in *M. tenuifolia* however, it was noted in *A. reticulata* and *A. squamosa* that flowering and flushing of leaves occurred at the same time, though the rate at which the flowers and the leaves were produced was not very high. It was a gradual process unlike in *M. tenuifolia* where the flowering was heavy. The production of fruits at the beginning of, or during rainy season by most of the species may be an adaptation to their dispersal mechanism. All the species have predictable annual fruiting periods; the harvesting of their fruits and seeds and

sustainable management is bound to be relatively easy. Therefore, sustainable harvesting of the vegetative parts of all the species implies that exploitation should be planned so that it will not coincide with the species' peak reproductive periods.

Douglas (1997) reported that flowering period also contributed to seed set while Gross and Werner (1983) showed that the differences in the seed-set of clones flowering in *Solidago* species at different times were due to physiological and microenvironment differences among clones. There was also some degree of pollen limitation of seed-set at all times. Again pollen limitation of seed-set was mostly due to pollinators as opposed to flower predators (Gross and Werner, 1983).

The percentage pollen stainability was higher than percentage pollen germination in the species studied. This showed that the fact that a pollen grain was well formed and well stained does not mean that it is viable. There might have been a developmental sterility. Therefore, the best way to estimate the pollen viability seems to be through pollen tube germination.

Low fertility was observed in some of the species studied such as *A. reticulata* and *C. patens*. The possible evolutionary explanation for low fertility in some of the species studied is that low female fertility has evolved in long-lived organisms in which the costs of seed and fruit production are high. Another possible explanation is mutational load, such that fitness is reduced by mutations carried in the population (Charlesworth, 1989). Ovule fertilization and seed set were less following selfing than crossing, indicating partial self-compatibility (Ramsey and Vaughton, 2000) which stated that ovule fertilization and seed set in *Burchardia umbrellata* were less following selfing than crossing, showing that the plant exhibited partial self compatibility. The poor seed-set observed in *C. patens* can be explained by the observation of Ramsey and Vaughton (2000). However, natural seed-set is limited by pollen quality and quantity and varies within and among years, probably due to variation in flowering plant density. Also, inappropriate pollen deposition may contribute to pollen limitation more often than previously recognized. Moreover, interference by

self or genetically-related pollen caused pollen limitation. Both fertilization and seed-set increased in response to cross-pollination (Ramsey and Vaughton, 2000).

Among the 56 families with compound pollen, Annonaceae, the largest family among the primitive species, is one of the families producing different pollen types, including monads, tetrads and polyads composed of 8, 16, or 32 grains (Walker, 1971). Tetrad pollen in *Annona*, *Monodora* and *Xylopia* is therefore an advanced character observed in Annonaceae. Wodehouse (1935) attributed the ontogeny of the tetrad pollen observed to the formation of a cell plate at the end of the first meiotic division after which the cell wall would be sufficiently advanced to prevent differential movement before the second division was completed. The organization of the tetrad will then depend upon the orientation of the divisions of the two daughter cells. However, simultaneous cytokineses (plate) were observed in all the species studied i.e. the cytokineses occurred at the end of second meiotic division only. The report of Echlin and Godwin (1968) corroborates the above observation. They reported that it was only at the end of second meiotic division that the evidence of incipient wall formation showed and by that time the four daughter cells had adopted the tetrahedral arrangement. At this period the entire tetrad was surrounded by a layer of callose which isolated it from other tetrads and for a brief period the four naked protoplasts had been in contact (Melville, 1980). Conversely, the tetrad structure observed in the species studied was explained by Lora *et al.*, (2009) to be as a result of delay in the dissolution of pollen mother cell wall and tapetal chamber which are the key events that hold the four microspores together in a confined tapetal chamber. These allow them to rotate and then bind them through the aperture sites, through small pectin bridges, followed by joint sporopollenin position. Another reason proposed for this permanent binding of pollen in group of 4 could be a failure in the synthesis of callose layer during microspore separation in the tetrad (Blackmore and Crane, 1998).

It was noted that up to four pollen tubes could be seen developing from some of the tetrads. This could have an advantage over the monad. This is

because a monad will produce just a pollen tube while tetrad can produce a maximum of four. At least one out of four will germinate. If a tetrad finds its way to the top of the stigma, it has higher probability of fertilizing the ovule than a monad pollen grain. Walker (1971) supported this view from his explanation that the adaptive advantages derived from the release of aggregated pollen in insect-pollinated species could increase pollination efficiency since more pollen grains could be transferred in a single pollinator visit. Moreover, Walker (1971) stated that a correlation between pollen tetrad and polyads with high number of ovules per flower has been shown in a survey of the Annonaceae.

Moreover, pollen grouping could be the result of relatively minor ontogenetic changes beneficial for pollen transfer and or protection from dessication. A comparison of these events with those recorded in the recent pollen developmental mutants in *Arabidopsis* indicates that several failures during tetrad dissolution may convert to a common recurring phenotype that has evolved independently several times whenever this group conferred advantages for pollen transfer representing an example of convergent evolution (Lora *et al.*, 2009).

The release of aggregated pollen is more advantageous in situations where pollinators are infrequent (Harder and Johnson, 2008) and in situations of short pollen viability and pollen transport periods. A short pollen viability period has been reported in *A. cherimola* (Herreno *et al.*, 1999; Lora *et al.*, 2006) and a short pollen episode is common on several Annonaceae (Ratnayake *et al.*, 2006). An additional possible benefit of aggregated pollen is protection against dessication and entry of pathogens through the thin walls of the pollen aperture sites (Le Thomas *et al.*, 1986).

Variation in the pollen grains of the Annonaceae reflects one of the evolutionary sequences in pollen of the first angiosperms (Le Thomas, 1981). Annonaceae species remain among modern plants in which a group still retains certain primitive characters of the first angiosperms. These characters include a medium-sized flower, spiral aestivation, numerous stamens and ovules, and monad pollen etc. The genus *Cleistopholis* now

appears to be one of those genera which have retained the largest number of such characters. In this study, the pollen of *Cleistopholis* is monad which shows that it is more primitive in this regard than the other genera of Annonaceae. Tetrad pollen in *Annona*, *Monodora* and *Xylopia* is therefore an advanced character observed in Annonaceae. Based on this study, the probable evolutionary development in these genera of Annonaceae is from *Cleistopholis* (monad) to *Xylopia* (tetrad) to *Monodora* (tetrad) to *Annona* (tetrad).

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