Breeding system and pollination ecology of two *Abrus* species (Fabaceae) from tropical West Africa

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The pollination and breeding strategies of two *Abrus* species (*A. precatorius* L. and *A. pulchellus* Wall. ex Thw.) were comparatively investigated in Nigeria, West Africa. Observations were made on the phenology, floral morphology and architecture, breeding and pollination system, as well as flower-insect interactions. During 1999–2000, *Abrus precatorius* was observed to flower twice a year (April to June and September to November) while *A. pulchellus* flowered only once, from October to December. Flowers of *A. precatorius* never opened, which indicated obligate cleistogamy in this species. The study has produced evidence showing that the type of breeding strategy in *A. pulchellus* is of the facultative type. There was no evidence of agamospermy in the two species. Fourteen insects and an arachnid belonging to eleven families in six orders were observed visiting flowers of the two species. Among these visitors, *Chrysops longicornis*, *Riptortus dentipes*, *Rhinocoris bicolor* and *Rhinocoris rapax* were the most likely to aid cross-pollination and hence are legitimate visitors. Our study suggests that low outcrossing rate occurs in both species despite the predominant cleistogamous characteristics in the genus. Occurrence of low outcrossing is a kind of evolutionary homeostasis in both species for perpetuating healthy offspring.

**Keywords:** *Abrus*, breeding, cleistogamy, insects, out-crossing, phenology, pollination

**Introduction**

The genus *Abrus* Adanson is a small pantropic genus (ISELY 1990) belonging to the Papilionoideae of Leguminosae (Fabaceae). In tropical West Africa, HUTCHINSON and DALZIEL (1958) reported three species: *Abrus precatorius* L., *A. pulchellus* Wall. ex Thw. and *A. canescens* Welw. ex Bak., all of which occur in Nigeria. *Abrus* species are lesser known and under-utilized among the legumes (NAS 1979) although the plant has great potentials for exploitation. Some members have been employed in ethnomedical, ethnopharmacological and toxicological uses (DALZIEL 1937, EL-SHABRAWY et al. 1987, AMER et al. 1989, REDA et al. 1989, DIMETRY et al. 1990, SINHA 1990, BURKILL 1995). The leaves and leafy twigs of some species are used in the preparation of liquorice (CHOI et al. 1989, JAKINOVICH et al.

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1990), while the seeds are used to simulate eyes in sculpting in southeastern Nigeria. Children have been reported to suck nectar from the flowers as a snack.

The re-emergence of medicinal plants as healthcare alternatives (Hoareau and Da Silva 1999) calls for greater interest in poorly utilized plant genetic resources. In the face of increasing threat from deforestation and uncontrolled grazing as well as unsustainable harvesting practices, any effort towards a better understanding and proper documentation of these plant genetic resources is worthwhile. Apart from the report of bees being attracted to Abrus species (Burkill 1995), literature on the breeding strategies and pollination biology of its Nigerian species is deficient. Most, especially the papilionaceous, legumes are cleistogamous (Arroyo 1981) in spite of their showy flowers; we investigated the breeding systems and pollination strategies of A. precatorius and A. pulchellus. This was an effort to ascertain whether the strategy for perpetuation in the two species is strictly cleistogamous or loosely so. The findings will be invaluable to breeding, conservation and biotechnological exploitation of these species.

Materials and methods

The fieldwork was undertaken from January 1999 to December 2000 during which seed samples were collected from the wild from different parts of southern Nigeria for each species (seeds of both species were randomly collected from Owerri Imo State – 05° 29”N, 07° 05”E; Onne Rivers State – 04° 51”N, 07° 03”E; Ibadan Oyo State – 07° 23”N, 03° 54”E; Kwale/Okpai Delta State – 05° 42”N, 06° 29”E and Kolo Creek Bayelsa State – 04° 54”N, 06° 22”E). They were planted out in plots of 50 stands per species to acclimatize them to the conditions of the experimental center. (Seeds were collected from five locations. Seeds from each location were treated as a population. Ten plants were established from each population.) Seeds harvested from the second filial generation were used for breeding studies. The experimental center was University of Port Harcourt Botanic Garden (05° 45”N, 07° 15”E), which is located in the humid rain forest belt, with conditions similar to those relating to the species in nature – A. precatorious and A. pulchellus. Stakes were also provided for the plants to climb, as they would under natural growth conditions. Seeds were planted in January 1999. Growing stands were monitored in 1999 and 2000.

Phenology

Observations on phenology of the different populations of the two species commenced at the beginning of their flowering until the end. During this period, the pattern of perianth colour, time of anthesis (i.e. time of opening of male and female flowers), and the total period of flowering of the two species from the different populations were recorded. Floral shape changes from the closed bud to the papilionaceous form in each species were monitored. The total number of buds (buds that eventually opened i.e. flowered and those that shriveled, fell off or were devoured by predators) produced by a plant from each population was monitored and counted during the flowering period. The flowering percentage for each species was determined as a proportion of the actual number of buds that flowered compared with the total number of buds produced by that species.
Floral morphology and architecture

The structure of flowers, their position on the inflorescence, the morphology of the separate whorls and the possible effects they have on breeding strategies were assessed. Flower morphology was described using five inflorescences from each population. Names of floral parts follow those of RADFORD et al. (1974) and WEBERLING (1992).

Breeding and pollination systems

In order to analyze the occurrence of within-inflorescence fertilization (autogamy and geitonogamy), twenty-five immature buds (five buds each were chosen from an individual plant in each population; each inflorescence produces 17 to 22 buds) were isolated in insect-proof nylon bags for each species, excluding pollen and pollen visitors from other plants and monitored until fruit set. Another twenty-five immature buds per species randomly chosen from ten inflorescences of the same age were left un-bagged as controls and monitored till fruit set. The number of fruits that set and survived was checked and used as index for successful pollination. A set of ten flower buds from each population was emasculated one day prior to anthesis and bagged without hand pollination to test fruit development in the absence of pollen grains.

Flower-pollinator interactions

A total 40 and 28 hours was spent observing floral visitors and monitoring their behaviour while searching for floral resources on A. precatorius and A. pulchellus during the 1999–2000 flowering seasons. Observation times were between 0800 and 1100 hours during anthesis and between 1600 and 1700 hours post anthesis. The diversity, abundance, frequency of visit and the timing of visitation of each insect visitor were recorded. At different times during anthesis, five of each insect species were captured (the same species were captured in the same container) as they were about to leave the flowers with the aid of a hand net, killed in a glass container containing cotton saturated with CCl4 and sent to curators at the Entomology Research Laboratory, Animal and Environmental Biology Department, University of Port Harcourt for identification. The number of pollen grains adhering to the bodies of the identified insects was counted under a stereomicroscope to assess their comparative efficiency for carrying pollen. Pollen carrying efficiency of identified visitors was determined following the method of TANGMITCHAROEN and OWENS (1997) where the relative amount of pollen per insect is: 0, none; 1 – very few grains (individual pollen grains periodically scattered on the bodies of insects); 2 – a few grains (a small number of pollen grains can be found individually or in small clusters); 3 – many pollen grains (pollen grains found in clusters); 4 – abundant (clusters and individual pollen grains are noticeable even with the naked eye).

Results

Floral characteristics

Inflorescences are both pedunculate and clustered in A. precatorius but solitary in A. pulchellus. The flowers in the two species are racemose, with very short pedicels and always matured in acropetal order. An individual flower is made of a campanulate, green ca-
lyx, which in *A. precatorius* is glabrous with 5 short broadly triangular teeth (Fig. 1) whereas in *A. pulchellus*, the campanulate calyx was pubescent or sparingly so with 5 short but acutely pointed triangular teeth (Fig. 2). Five free petals make up the corolla, which may be of different colours even within the same species but are invariably of different shapes and sizes between the two species. The corolla is $1.11 \pm 0.20 \times 0.51 \pm 0.26$ in *A. precatorius* and $0.84 \pm 0.1 \times 0.43 \pm 0.23$ in *A. pulchellus*. The standard is fairly obovate with an acute and short-clawed base in both species. The wings are falcate with short claws while the centrally located keels though similarly falcate are also boat-shaped. These floral whorls are interlocked with themselves to enclose the gynoecia and androecia.

**Fig. 1.** Appearances of flowers during anthesis in *A. precatorius*. A – initiation of anthesis. B – early anthesis. C – full anthesis. D – post anthesis

**Fig. 2.** Appearances of flowers during anthesis in *A. pulchellus*. A – unopened flower. B – early anthesis. C & D – full anthesis. E – post anthesis
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Breeding and pollination systems

In *A. precatorius* both bagged and unbagged flowers produced fruits; while in *A. pulchellus* up to 80% of the bagged flowers only produced fruits. Some of the fruits produced later aborted. Similarly, all emasculated but non-pollinated flowers did not develop into fruits.

Floral morphology/architecture and phenology

*Abrus precatorius* and *A. pulchellus* possess purple and pinkish-white flowers respectively. However, the standard, wings, and keel of the two species showed variation in colour, intensity of such colours, as well as the spread of the colours on these whorls (Tab. 1).

<table>
<thead>
<tr>
<th>Species</th>
<th>CALYX (Sepal) Initiation</th>
<th>Full anthesis</th>
<th>Unopened bud</th>
<th>STANDARD Initiation</th>
<th>Full anthesis</th>
<th>Unopened buds</th>
<th>WINGS Initiation</th>
<th>Full anthesis</th>
<th>Unopened buds</th>
<th>KEEL Initiation</th>
<th>Full anthesis</th>
<th>Colour of standard</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. precatorius</em></td>
<td>Green</td>
<td>Light green</td>
<td>Deep purple</td>
<td>Purple</td>
<td>Light purple</td>
<td>Light purple</td>
<td>Light purple</td>
<td>Light purple</td>
<td>Pink</td>
<td>Pink to Pinkish white</td>
<td>Light pink</td>
<td>White</td>
</tr>
<tr>
<td><em>A. pulchellus</em></td>
<td>Light green</td>
<td>Light green</td>
<td>Pink</td>
<td>Light pink</td>
<td>Pink</td>
<td>White</td>
<td>Orange</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

In *A. precatorius* anthesis started between 6 and 6.30 a.m. Mid anthesis and the opening of the standard and wing were attained between 9 and 9.30 a.m. of the first day (Fig. 1). On the second day, the keel still had not opened. After the second day, the keel started rupturing from base of the style exposing an ovary wall that was developing into fruit. Thus the reproductive organs (stamens, anthers, and style-stigma) were never exposed in this species. In *A. pulchellus*, anthesis started between 5 and 5.30 a.m. and lasted till the next day. Flower buds assumed characteristic changes in shape as anthesis progressed as shown in Fig. 2. Between 7.30 and 8.30 a.m. the standard and wings opened and remained so until the second day. Full anthesis was attained between 6 and 8 a.m. of the second day. During this period, the keel opened exposing the reproductive organs. Closure was initiated at about 10.30 a.m. of the second day. Anthesis in this species lasted for 2 hrs 30 mins to 4 hours. Anthesis started and ended later in *A. precatorius* than in *A. pulchellus* although there were overlaps in duration (Fig 3).

*Abrus pulchellus* flowered and fruited from October to December with flowering peak in late November, while *A. precatorius* flowered and fruited between April and June and between September and November (Fig 4a and b). The flowering peak in *A. precatorius* came in October and it was observed that *A. precatorius* flowered and fruited more between September and November than between April and June. In fact, most of the April to June flower buds aborted.
Flower pollinator interactions

Fourteen insects and an arachnid (Tab. 2) belonging to the orders Hemiptera, Coleoptera, Diptera, Hymenoptera, Lepidoptera and Diplopoda were identified visiting the flowers of the species. Most were observed between 8 and 11 a.m. *Lutus terrestris* (Diplopoda), which emerge from the mature fruits of the species (they are oviposited into the ovaries by the adult insects during nectar feeding and subsequently develop with these ovaries) forage on the entire inflorescence devouring floral parts especially the petals. Upon emerging from the fruits, *L. terrestris* moves to young flower buds, positions its body on

Fig. 3. Timing of anthesis in two *Abrus* species

Fig. 4. Mean flowering percentage in *A. precatorius* (a) and *A. pulchellus* (b) samples between 1999 and 2000. A-1 to A-5 and B-1 to B-5 represent samples collected from different parts of southern Nigeria.

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the inflorescence stalk and gradually eats up first the petals before devouring the other reproductive organs. While rhythmically moving from one flower cluster to the other, it picks up pollen grains with its numerous appendages. The ladybird, Cheilomenes lunata, was observed associating with these species (strolling round the entire plant and making stops at leaf and stem axils) throughout the life of the plants. This insect species lives within the leaf angles and flower clusters and begins to stroll down the entire plant from about 6 a.m. each day. They recede between 11 a.m. and noon. The number (abundance) of ladybirds increases to more than 10 on mature plants as the Abrus plants mature.

Chrysops longicornis was second in species abundance and number of visits and they foraged for pollen and nectar every day of observation. They visit the flowers between 9.30 and 10.30 a.m., landing on the flower with a minute vibrational buzzing sound. They suck nectar by pushing their short proboscis to the base of the keel petals, spending about 5 to 10 minutes on each open flower before dashing to the next flower cluster. In A. precatorius, Chrysops were able to depress the keel petals covering the anthers and other reproductive
organs with a slight search pressure. On any visit, Chrysops spends about the same time in both A. pulchellus and A. precatorius foraging for nectar and pollen materials. Heavy pollen loads were observed on its appendages, thoracic and abdominal regions because the mouthparts are short, thus almost the entire body lands on the open flower anthers. This enhanced pollen transfer and deposition on other inflorescence clusters. The Hemiptera, Rhinocoris sp (R. bicolor and R. rapax) and Riptortus dentipes were classified as minor visitors to Abrus species due to the scarcity of their visits. They forage on Abrus nectar and pollen with their pointed mouthparts and move from one plant to another within the population. Deposition of pollen from one plant on another plant is possible in the course of their feeding. Apart from Abrus pollen, foreign pollen was observed on Rhinocoris bicolor and Riptortus dentipes. The ant Camponotus ligniperda was observed to form nests around buds or fruit pods and a few occasionally strayed to the nectar region of the flowers.

It was observed that the number of pollen grains on insect bodies was quite variable among insect groups. Generally, only Hemiptera, Diptera, Hymenoptera and Coleoptera had pollen on their bodies. The Formicidae (ants – Camponotus ligniperda and Dorylus nigricans) and Lepidoptera (butterfly – Acraea zetes) apparently played little or no part in transfer of Abrus pollen.

It was further observed that after flower closure in A. pulchellus, the stigmatic ends and anthers tended to stick out from the keels. Slight pressure on the keels by the possible pollinators at such points could lead to cross-pollination.

Insect activity on both A. precatorius and A. pulchellus was highest during the morning (8 to 11 a.m.), which coincided with the peak of pollen and nectar production. The majority (seven out of the fifteen insects) of the insects visiting the species belong to the Hemiptera; however the only Dipteran species, Chrysops, seems to constitute the most efficient pollinators, going by their behavioural pattern and the pollen load they carry on their body parts. Though they were not in large numbers, they visited regularly on observation days, moving from one inflorescence cluster to the other with slight vibrational buzz. Chrysops has a proboscis small as compared to body length, and they therefore alight on the corolla and anthers of each flower with their entire body, thus collecting heavy pollen materials on most parts of their body. This is also what happens when this insect species succeeds in depressing the keel of A. precatorius exposing the anthers and stigma.

Discussion

Breeding systems, according to Davis and Heywood (1973) determine not only the immediate reproductive survival of a plant but also the evolutionary predisposition of such species. The breeding system determines the spread of genetic variability in the population and this in turn is usually reflected to some extent in the floral morphology of the population. We observed that the two species of Abrus studied are autogamous. In A. precatorius, obligate cleistogamy was the norm (the keel did not open in any sample to expose the pollen grains and stigma, self-pollination being the rule, Fig. 1), a scenario that restricted access to pollen (Freitas and Sazima 2003). It was not exactly the same in A. pulchellus. For this plant some flowers were observed in which the anthers were exposed (Fig. 2) while some still remained unopened. It appeared though that much of the self-fertilization, in this case, took place during the course of anthesis. Thus A. pulchellus probably has the faculta-
tive kind of cleistogamy as the evidence in this study suggests. DAVI and HEYWOOD (1973) observed the type of cleistogamy found in *A. precatorius* in *Pisum sativum* L and *Hordeum vulgare* L. Though differences existed in types of cleistogamy between the two species, it was observed that their floral structure and architectural design were interestingly similar (Fig. 1, 2).

The floral architectural design favoured a high degree of autogamy in the two species as evidenced by high fruit set. It is a mechanism that checks pollen wastage by limiting pollen movement in time and space. The importance of inflorescence structure in angiosperm breeding system has been reported a number of times (WYATT 1982, OLOWOKUDEJO and HEYWOOD 1984, PELLMYR et al. 1990, OBUTE 1998, LLO and LI 1999, FREITAS and SAZIMA 1990, OBUTE 1998). Part of the design for maximum pollination, which is necessary in cleistogamous species, is the bent nature of the pistil to make it cuff around the anthers. This feature, though not a prerequisite for cleistogamy, has also been reported in another legume, *Vigna unguiculata* (OBUTE 1998). Furthermore, the enclosure of the androecium and gynoecium by the keel especially in *A. precatorius*, indicates another design for maximum self-pollination that contributes to cleistogamy.

Morphological appearances and architectural design alone are not enough to suggest cleistogamy or chasmogamy (DAVI and HEYWOOD 1973). However, the observation that post-anthesis flowers could be further opened by slight pressure from relatively heavy visitors might make the difference in the promotion of out-crossing in *A. precatorius*. Similar designs have been reported in *Vigna* (OBUTE 1998) and *Viola* (FREITAS and SAZIMA 2003). Indeed HAGERUP and HAGERUP (1953) opined that the architectural design of the flower of *Erica tetralix*, though supposedly cleistogamy-inclined, did not obstruct the role of thrips as pollinators. Similarly, the observed bright pink colour at anthesis and the post anthesis bright orange to yellow colour in *A. pulchellus* may be attractive to pollinators and could favour outbreeding, while the dull purple colour in *A. precatorius* may not.

Production of colourful flowers in angiosperms does not only serve taxonomic purposes but also plays important roles in breeding strategy of plants (DAVI and HEYWOOD 1973, UGBOROGHO and OBUTE 1993). Equally important in this regard, is the phenology of the flowers. As noted by OBUTE (1998) the phenology of flowers is significant of the type of breeding system a plant adopts. Flowers produced by *A. precatorius* between April and June (Fig. 4) had a high rate of abortion and scanty fruit set. Why this is so is not clear at present. The similarities and overlaps in the flowering patterns and anthesis durations in the two species (Fig. 3) are noteworthy. These features as noted by OBUTE (1998) suggest strategies whereby chances are created for exchange of genetic material between species even with the slightest possibility of outcrossing.

Apart from these designs insect visitors play significant roles in the breeding system of members of *Abrus* in Nigeria. The 5 to 10 minute duration of feeding by *Chrysops* between 0930h and 1030h, followed by its movement to another flower is mostly within inflorescence clusters of the same plant, which ensures effective self-pollination. This insect rarely visited another plant within the feeding period hence may be proposed as one of the probable pollinators of these species. The heavy pollen load seen on this insect further buttresses this assertion.

Hemipteran visitors, *Riptortus dentipes*, *Rhinocoris bicolor* and *Rhinocoris rapax* were most likely to aid cross-pollination from their behavioural pattern as they often moved...
from the proximal to the distal parts of the inflorescence before moving to other plants within the population. De FIGUIREDO and SAZIMA (2000) observed such proximal to distal movements by flies and bees that visit Piperaceae species. Such behaviour by insect visitors (BARTH 1991, RICHARDS 1997) might increase the chances of autogamy and geitonogamy. Though these species feed on floral nectar and pollen, they are less effective pollinators because in addition to occurring in small numbers, these visitors carried a low pollen load; furthermore, their visits were less frequent than those of others. These probably are not legitimate pollinators of these plants. _Lutus terrestris_, an arachnid, could bring about pollen transfer from flower to flower due to its ciliated appendages, segmented body and foraging habit; however, it was much more involved in devouring the inflorescence than in seeking for nectar. They are insect predators. The ladybird, _Cheilomenas lunata_, was observed to be associating closely with the leaf-stem angles (around the petiole) and the point of origin of branches in the two species from early growth stages to flowering. They stroll round the entire plant with little attention to the inflorescence. When they do, they spend 20 to 30 minutes in the corolla chamber feeding on nectar and pollen materials. However, their attraction may be more to extra-floral nectaries and/or abrusosides A-D, four sweet-tasting triterpene glycosides identified in the leaves and leafy-twigs of _Abrus_ species (CHOI et al. 1989, FULLAS et al. 1990, JAKINOVICH et al. 1990), for this insect species preferred young leafy twigs, leaf and stem angles to the flower clusters.

The attractiveness of _A. precatorius_ flowers to the honeybee (_Apis mellifera_, Hymenoptera) was reported by BURKILL (1995). However, _Apis mellifera_ was not encountered in the present work. A plausible existence of copious alternative pollen sources with easier reach for the bees all around the experimental area. The evidence and observed ecology of pollinators suggest the existence of both inbreeding and outbreeding in the _Abrus_ species studied. Even the obligate cleistogamous _A. precatorius_ could have insect species like _Rhinocoris bicolor, Riptortus dentipes_ and _Chrysops longicornis_ arriving from a plant, landing and depressing the keel of another plant, piercing through the nectar region with their mouthparts in continuation of routine feeding. Once this is achieved, outcrossing is made possible. These observations are not at variance with those of UGBOROGHO and OBUTE (1993) and OBUTE (1998) on _Vigna unguiculata_ and they represent an evolutionary compromise by which autogamy ensures immediate fitness of individual plants while allogamy maintains genetic variability. DAVIS and HEYWOOD (1973) noted that the evolutionary diversity of most angiosperms is predicated on such compromises. Subsumed in this compromise are the solutions to the problems of genetic fixity, which is attendant on inbreeding, and also the problem of evolutionary plasticity, which must be ensured by outbreeding.

The absence of agamospermy in the two species is probably a natural protection against upsets in the gene pool of the species due to environmental exigencies. It should be noted that though appreciable fruit set was observed in _A. pulchellus_, which is a polyploid with 2n = 44 (AGBAGWA and OKOLI 2005), fruit abortion and low seed-set was quite common in this species. Fruit abortion and low seed set occur in polyploids (SWANSON 1968). It therefore means that the existence of a modicum of outcrossing in this species apart from its taxonomic and evolutionary advantage may be a genetic and breeding strategy for self-perpetuation. Evidence was adduced in this study to conclude that the breeding system in _A. precatorius_ is of the obligate cleistogamous type.
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