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GENETIC DIVERSITY AND CONSERVATION OF CERTAIN SPECIES OF RATTAN IN ANDAMAN AND NICOBAR ISLANDS AND SOUTHERN INDIA

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ABSTRACT

Rattans are economically one of the most important group of forest species after timber trees. The rampant cane harvesting in the natural forests is threatening the stability of the wild rattan population. Hence conservation of the genetic resources of rattans requires urgent action which necessitates an understanding of the genetic variation. A basic knowledge in the genetic diversity, reproductive biology and cytology is essential for formulating the conservation measures.

Phenotypic variations were studied in *Calamus andamanicus,C.thwaitesii* and *C.palustris.* In C. *thwaitesii* variations were noticed in stem diameter, leaflet arrangement, characteristics of leaf sheath and fruit size. In *C. andamanicus* the populations differ with respect to stem diameter, leaf sheath charactersitics and the length of intlorescence. In *C. palustris* no prominent phenotypic variation was noted.

Genetic diversity studies show that provenances significantly differ with regard to seedling height. The phenotypic and genotypic coefficients of variation for seedling height were found to be high. Heritability was also found to be high. Highly significant correlation between height at different ages indicates the possibility of early selection.

The species studied are dioecious and flowering is annual. The time of initiation of flowering varies slightly depending on the locality from year to year. Generally the flowering starts during July-August and the fruits mature in April-May. Three to four inflorescences are produced in a single plant during one season. In each inflorescence there will be 3-4 partial inflorescences. The order of emergence of partial inflorescences as well as rachillae on them is acropetal. The time gap between the emergence of different partial inflorescences and the rachillae is reflected on the time of maturation of fruits also.

There is no particular sequence of flouer opening. Male flower starts opening at about I a.m and female flower at about 4 a.m. Anthesis starts even before the flower is fully open. Female flower is receptive till noon. The inflorescence emits a sweet scent. The pistillate flowers do not produce nectar. The adjacent sterile staminate flower exudes nectar.

Even though the morphology of *Calamus* inflorescence suggests wind pollination insects are the main pollinating agents Bees are probably the important pollinators

Karyological studies in *C. andamanicus* and *C. palustris* revealed 26 chrmosomes in the former and 28 in the latter. Chromosome size ranged from 0.89 to 2.46 μ m in *C. palustris* In *C. andamanicus* size ranged from 0.44 to 2.22 μ m.

1. INTRODUCTION

Rattans, the spiny climbing palms with about 600 species in 13 generaare distributed in tropical and subtropical Asia and equatorial Africa. In many Asian countries, rattans are second only to timber in economic importance. A considerable portion ofthe rural population depends on rattans in one way or other. It is an important source of income and employment for people directly or indirectly. In India alone rattan industries account for 2,00,000 employees (Manokaran, 1990) Rattan contributes 25-35% of the total household income of tribal communities in North Eastern India. Rattan furniture is much valued in many countries, and its export from producer countries have steadily increased, over the years, into a multibillion dollar business.

Increase in demand of the raw material has resulted in over-exploitation of the natural resource. This, along with the change of land-use pattern, has led to erosion of the biodiveristy of rattans. Many ofthe species have become rarer in distribution and are threatened with extinction. Hence, conservation and utilisation ofthe genetic resources ofrattans require urgent action. Conservation measures require an understanding of the genetic system and spatial patterns of genetic variation. Each genetic improvement programme is based on the assumption that in the species to be improved there is enough genetic variability for the traits ofinterest, and that this variability can be exploited. Most plant traits vary among individuals under the control of both genetic and enviommental factors. The component of the trait which is under genetic control can be transmitted to the progeny. Mating system is an important factor which determines the population structure.

The utilisation of genetic resources depends on a knowledge of the amount and distribution of genetic variation. Several methods are currently available to assess genetic variation in plant species. None of these methods gives a complete picture ofthe complex structure ofgenetic variation in the wild plant species. Hence it is suggested that multiple methods should be adopted simultaneously to investigate the pattern of genetic variation in rattan species (Finkeldey, 1995). Both genetic markers and quantitative traits are expected to provide valuable information for the genetic management of rattan species. Isozyme genetic markers have already been identified for several important rattan species in Malaysia and Thailand (Bon *et al.*, 1995).

Very little data is available on the genetic diversity ofrattans in India. Hence,as a preliminary step, a study on the genetic diversity and conservation of certain species of rattans in Andaman and Nicobar Islands and Southern India was initiated. The main objectives are to study the phenotypic and genotypic variations within the species, to study the reproductive biology and cytology.

2. GENETIC VARIABILITY

Calamus is not domesticated in India and therefore it is not subjected to any selection. Hence considerable genetic variability can be expected in natural population. Phenotypic variations are noted in *Calamus* populations in India (Renuka, 1992. 1995) but whether these variations are exclusively under genetic control is not known. Such natural variations within species are not surprising, because regeneration in rattans is mainly through seeds and the dioecious flowers seem to favour outcrossing in nature with consequent heterogeneity in wild populations (Venkatesh, 1992).

Since rattans cannot be grafted or rooted by cuttings, the only possible method for improvement is through propagation of selected plus rattan plants by seed, and assembling seedlings in cane gardens. But selection of plus plants in rattans is rather difficult due to the following reasons: The plants in the natural forests are not even-aged and hence, it is very difficult to compare and assess the superiority Secondly, some of the clumps might have been partially harvested earlier and from the culms left over, the original growth cannot be assessed. (Indira, 1992). However, good clumps or piants may be selected as mother plants.

Generally forest geneticists (tree breeders) consider the use of proper seed source or provenance as the most important step because it leads to cheapest and fastest gains. Provenance trials provide the necessary information which form a basis for the selection of suitable seed sources. Provenance trials are conducted to find out the best population for commercial planting and also for studying the extent and pattern ofvariation within the species for a variety of characters. Earlicr work on temperate trees showed that genetic differences associated with place of origin have often been several times as great *as* those between individual trees in the same stand (Wright, 1976).

Other than field trials, isozymes are currently the most frequently used genetic markers for population genetic surveys to infer patterns of genetic variation. lsozymes are enzymes catalysing similar reactions in the metabolism of an organism. Isozyme analysis, ie., the visualization of locus specific isozymes through electrophoretic separation and histochemical staining, is a relatively cheap and fast means ofanalysing single locus variation in biological organisms (May, 1994) The method was developed in the late 1960s and has been applied to

a wide range of animals, plants and fungi ever since. Prior to this, only morphological markers were available to study single gene variation.

Difference in enzyme activity or even its absence in different parts of the same plant as well as at different growth phases is a major limitation in the use of this technique. The need for carrying out the extraction of the enzymes from healthy and freshly collected tissues is often a problem when the plants of interest are growing in the wild. Maintenance of the plants in a nursery is the only reliable means of ensuring the availability of tissues in a fresh condition.

Studies using isozyme analysis in rattans have been previously carried out by Alloysius and Bon (1995) and Siti Salwana *et al.* (1996). *C. manan* and *C. .subinermis* showed a high level of genetic diversity.

2.1. Materials and Methods

An ecogeographical survey was undertaken throughout the Western Ghats and Andaman and Nicobar Islands to study the phenotypic variations of different populations of *C. thwaitesii, C. andamanicus* and *C. pulustris.*

Seeds of *C. andamanicus* from five different areas of Andaman and Nicobar Islands were collected during April to May 1995. Seeds from these five areas were treated as seed sources or provenances. The details are given in Table 1. These 5 localities are completely isolated from one another. Out of the five provenances Tarmugli and Smith Island are very small Islands.

Table 1. Provenances (seed source) of *C. andamanicus*

Provenance No.	Seed source	Locality	Latitude	Longitude
1	Baratang	Central Andamans	12°15'N	92°50'E
2.	Campbell Bay	Great Nicobar	7°N	93° 55'E
3	Mannarghat	South Andamans	12°N	92°40'E
4.	Smith Island	North Andamans	13°N	93°E
5.	Tarmugli	South Andamans	1l°30'N	92°15'E

C. palustris is seen only in Andaman islands. Most of the populations were seen in the Tribal Reserve area inhabited by the hostile Jarawa Tribe and hence extensive collection of material was not possible. Seeds that could be collected were not sufficent for a provenance trial.

Fruits were collected from healthy clumps. As soon as the fruits were collected, they were depulped, cleaned and stored in moist saw dust.

A nursery was raised in the Kerala Forest Research Institute campus at Peechi. Seeds were sown in the moist sawdust. The seeds of *C. andamanicus* were found to be dormant till the following year. Species from Western Ghats germinated within 1-2 months. These plants were transplanted to polythene bags filled with soil and sand.

In the nursery, for each provenance, 3 replications each with 80 seedlings were put apart for genetic studies. However, for the Mannarghat provenance of *C. andamanicus* the number of seeds obtained and the seedlings raised were lesser.

Morphological and height variations were monitored at the age of 6 and 18 months. The data were analysed for the variation between provenances using the statistical package SPSS/PC+Advanced statistics V2.0. The phenotypic and genotypic coefficients of variation (PCV and GCV) were estimated as

$$PVC = \frac{\text{Phenotypic variance X 100}}{\text{Mean}} \qquad GCV = \frac{\text{Genotypic variance X 100}}{\text{Mean}}$$

Provenance heritability was computed as $\frac{\text{Genotypic variance}}{\text{Phenotypic variance}}$ and the mean comparison test was done following Calinski and Corsten (1985)

In isozyme studies poly acrylamide gel electrophoresis (Non-SDS, non denaturing gel) was used since the equipment for starch gel electrophoresis was not readily available. Extraction and staining procedures for selected enzymes as described by Wendel and Weeden (1989) and Sadasivam and Manikam (1992) were followed.

The following eight enzymes were tested for suitability for studying genetic variation:

Peroxidase, Esterase, Catalase, Malate dehydrogenase (MDH), Alcohol dehydrogenase (ADH), Formate dehydrogenase (FDH), Glutamate dehydrogenase (GDH) and Superoxydismutase (SOD). Seedlings raised from seeds of individual plants from various provenances of *Calamus andamanicus* and *C. thwaitesii* and maintained in the nursery were used as the source of tissues for isozyme analysis. Leaflets of the unopened terminal leaf of two year old seedlings were collected immediately before extraction.

Tissues were extracted using a pre-cooled mortar and pestle kept in a bath of ice. Extraction was carried out in a Tris HCI buffer using 1 g of leaf tissue.

50 mM Tris-HCI buffer (pH 7 0)
50 mM $MgCI_2$
50 mM EDTA

A Bio-Rad Miniprotean unit was used far the study and electrophoresis was carried out at 200 V and 6OmA for 45 minutes. Gels were run in a refrigerator to maintain a temperature of4oC.

2.2. Results

Phenotypic variations:

C. *thwaitessii*

C. *thwaitesii* is a large diameter rattan; spread throughout the Western Ghats of peninsular India. It is a high climbing rattan. The inflorcscence is long and flagellate.

The species shows very distinct phenotypic variations between the populations. The populations vary in stem diameter, characteristics of leaf sheaths, leaflet arrangement and fruit size(Figs.1-6).

Stem diameter

There are thick stemmed and slender stemmed populations. The stem of thick stemmed plants are about 3 cm in diameter without sheaths, Slender stemmed plants are about 1.5 cm in diameter.

Leaf sheath

The colour of the leaf sheath and the colour and arrangement of spines on the leaf sheath vary between populations. The colour of the leaf sheath may be green or yellowish green. In majority of the populations the spines on the sheath are arranged in well defined series and are jet black in colour. But some of the populations have brown spines which are arranged in closely packed series. (Fig. 5 & 6)

Leilflets

In thick stemmed populations the leaflets are distinctly grouped along the rachis. In thin stemmed plants the grouping of leaflets are not clear. The drooping of the leaflets is more prominent in thin stemmed populations (Figs. 1-2)

Fruits

In thick stemmed populations the fruit size also is greater, as compared to the fruit size of thin stemmed plants.

C. andamanicus

This is a large diameter rattan with a wide distribution in Andaman and Nicobar Islands (Fig. 7).

Phenotypic variations are noticed with respect to the diameter of stems, nature and colour of leaf sheath, the arrangement of spines on the leafsheath and the length of the inflorescence (Figs 9-10)

Stem diameter

Some of the populations have stems of 3 cm diameter while others are 6 cm in diameter

Leaf sheath

The leaf sheaths are usually yellowish green turning to reddish brown with minute bristle like, deciduous spines arranged in comb like crests. Some of the populations have triangular spines of about 5 x 0.5 cm and the sheaths are reddish brown even at younger stages.

Length of inflorescence

Some of the populations produce long flagellate inflorescence while others have short, non flagellate inflorescences (Figs. 9 & 10).

C. palustris

This is a medium diameter rattan. No prominent phenotypic variation was noted in this species.

Genotypic variations

In the seedlings there are not much phenotypic variations between *C.* andamanicus provenances. However, the Campbell Bay provenance (Great Nicobar) shows black, longer and thicker spines while others from Andamans have brown and comparatively smaller spines. In Campbell Bay provenance the leaflets are smaller whereas in others the leaflets are slightly bigger. On analysis of the height growth it is seen that there is significant difference between provenances at 6 and 18 months age (Table 2).

Table 2. Analysis of variance for height at 6 arid 18 months

Source	DF	Mean sum of squares	
		Height 1	Height2
Provenance	4	48 968**	265 302**
Replication	2	1814^{ns}	368 115**
Error	263	2354	49671

** Significant at 0.01 level

The mean performance of the provenances is given in Table 3

Table 3 Mean height performance

Provenance No.& name	Height in cm	
	6 months	18 months
I. Baratang	4.44	23.97
2. Campbell Bay	6.87	28.57
3. Mannarghat	4.15	29.17
4. Smith Island	5.2:	27.16
5. Tarmugli	4.07	24.17
Grand Mean	4.56	26.16

The phenotypic coefficients of variation were as high as 87.03 and 42.14 percent for height at 6 and 18 months respectively. Genotypic coefficient of variation were also as high as 81.11 and 32.41 for height 1 and height 2. This shows that both phenotypic and genotypic coefficients of variations decreased. One of the reasons may be that during stabilization, the variation starts decreasing.

Heritability was also found to decrease from 86.8 to 59.13. This type of heritability decrease in the early years of growth was reported in other species as in Black Walnut (Rink, 1984) *Tecomella undulata* (Jindal *et al.*, 1992) and *Gmelina arborea* (Indira Pers. observation).

Correlation between height at 6 and 18 months was found to be highly significant with a value of 0.20. This shows that early selection is possible.

On clustering 2 groups were observed at both the periods but the number of provenances in these clusters were different as shown below.

Height 1	provenances	2 4 1 3 5
Height2	provenances	3 2 4 5 1

From the results it is seen that at an early age, the Campbell Bay provenance of Great Nicobar Island excelled over all the other Andaman provenances. But in the second year, along with Campbell Bay, Smith Island and Mannarghat provenances were also included in the best cluster. Tarmugli and Baratang provenances showed poor performance.

To get a clear picture of the genetic variations for growth. inflorescence length as well as other characters, observations need to be continued for some more years.

Isozyme analysis

Polymorphism was exhibited in MDH, ADH and peroxidases. Variation in enzyme activity was also observed between the samples. In *C. andamanicus*, the peroxidases profile (Fig. 11) had two loci, one with three alleles and the other with two alleles. In SOD (Fig. 12), two loci were observed but no polymorphism was established. Further improvement in the procedure is required for the other enzymes. The results were not consistent and hence it was not possible to assess the genetic variability using a larger number of samples.

2.3. Discussion

For the genetic conservation of the particular species, conservation of unique characteristics of geographic races is a very important factor. A few major and differing gene complexes are responsible for the unique andvantage for growth and survival of a seed source in a special environment (Zobel & Talbert, 1984).

The amount of geographic variability is influenced by factors like the species, natural range, environmental diversity, extent of range discontinuities and some other unknown factors. Phenotypic variations were observed in *C. andamanicus* and *C. thwaitesii.* In *C. thwaitesii* two distinct populations could be identified, based on the stem diameter, leaflet arrangement, characteristics of leaf sheath and fruit size. In C. *andamanicus* the populations differ with respect to stem diameter, leaf sheath characteristics and the length of the inflorescence.

In *C. andamanicus* there is clear cut range discontinuity between provenances. But the environmental diversity is rather less. However, there are geographic differences between Andaman & Nicobar Islands which reflects in the variation between the provenances from these two places like spine characteristics, leaflet size and growth in the early stage. Observations in the coming years will give a clear picture.

In Isozyme analysis the results obtained in the study was in conclusive. Further work is required to be done on the standardization of protocol for extraction and separation of isozymes. Idealy four gel bulfers viz. Histicline, Morpholine-citrate, Tris-citrate and Lithium borate buffers should be tried with all the enzyome systems. For extraction of teat tissue the Tristicl-EDTA buffer was satisfactory for control of phenetics.

Further work using starch gel electrophoreses is envisaged since it facilitates the analysis with larger number of samples and simulatances testing of different buffer systems.

3. REPRODUCTIVE BIOLOGY

The general history of palm pollination studies begins with Martius (1823), who recognised that heat, odour and nectar production in palm inflorescences attract insects, particularly curculionid beetles. Wallace (1853) also reported that the odour of palm inflorescences attract numerous small flies and beetles. Kerchove (1878) gave an extensive discussion of heat and scent in palm inflorescences and mentioned both wind and insect pollination.

Schmid (1970a, 1970b) presented one of the first detailed pollination studies of a wild palm in its native habitat, and also reviewed some of the early literature. Meeuse (1972) considered palms to have retained a primitive declinous anemophilous condition. He believed that the potential palm pollinators, such as bees and flies, evolved too late to have been pollinators of primitive palms. But Van der Pijl (1978) considered the family to be basically cantharophilous. Uhl & Moore (1977) considered that most palms were insect pollinated. Henderson (1986) made a review of pollination studies in Palmae. In Calamoideae only very little information is available on pollination (Mogea 1978, Dransfield 1979, Madulid, 1980) that too only a few scattered notes. Pedersen (1995) studied in detail the pollination biology of 3 species of rattans in Thailand.

Rattans are dioecious plant with annual flowering. An exception is *Korthalsia* which is monoecious with bisexual flowers. Two types of flowering are seen among rattans, hapaxanthic and pleonanthic. In hapaxanthic flowering, the topmost nodes of a rattan produce inflorescences more or less simultaneously and in doing so, the apex becomes exhausted and the stem dies after flowering and fruiting, eg., *Korthalsia* In pleonanthic flowering the stein continues to grow after flowering, eg., *Calamus, Daemonorops* Some of the *Daemonorops* species are however hapaxanthic (Dransfield.

In India, reproductive biology of rattans has not been studied in detail mainly because of lack of rattan plantations and the inaccessibility of the natural populations in forests. Generally the cane growing areas in forests are infested with elephants. This along with the presence of other wild animals makes it risky to stay in the field overnight to study details of floral behaviour.

3.1. Study site

A plot was selected in Mannarghat Forest Range of South Andamans to study *C. andamanicus.* Another plot was selected in Vazhachal Forest Division in Western Ghats to study C. *thwaitesii. C. andamanicus.* populations were found at an altitude of 50-75 m and C. *thwaitesii* were located at an altitude of 300m.

3.2. Materials and Methods

Frequent visits were made to the study sites starting from the onset of flowering. For recording the time of floral opening and anthesis observations were made during night hours.

To study the pollen viability and stigma receptivity, pollen was collected during anthesis and kept in small glass tubes. Branches of female inflorescences, in which the flowers had started opening were selected and bagged. The flowers of 2 rachillae were pollinated with the collected pollen, bagged and labelled. After 2 hrs the process was repeated in another 2 sets of selected rachillae. Likewise the pollination was continued for 8 hrs. Then the time gap was increased and the pollination was continued till 24 hrs. Percentage of fruit set was registered in all cases.

In vitro pollen germination was tested using different germination medium like tap water. Brewbaker medium and sucrose solution with various concentrations. Optimum germination was noticed in 0.6% sucrose solution and this was used as the germination medium. Pollen viability was also studied by germinating them in 0.6% sucrose solution at 2 hr. intervals.

A strip of transparent adhesive tape was suspended for 24 hrs near a pistillate inflorescence to test the presence of pollen in the air The strip was observed under the microscope

3.3. Results

Rattan inflorescences vary greatly in size and morphological structure. An individual stem produces 3-4 inflorescences in a flowering season.

Flowering season:

Flowering in both the species is annual. The time of flowering varies slightly with the locality and, to some extent, from year to year. In the first year *C. thwaitesii* started flowering in July and continued in August also. In C. *andamanicus* flowering started in August. In the second year observation was possible only for *C. thwaitesii*.

Inflorescence:

C. thwaitesii

The staminate and pistillate inflorescences are about 6 m long and flagellate with several partial inflorescences. In staminate inflorescence, the flowers are arranged distichously on the rachilla (Fig. 4). In the pistillate inflorescence, a sterile male flower is seen beside each female flower.

C. andamanicus

The inflorescences are erect in some populations while in others they are long and flagellate. The erect inflorescences are 1.25 m long and panicled while the flagellate inflorescences are about 6 m long(Figs. 9-10) The male flowers are arranged distichously on the rachilla. In the female inflorescence, a sterile male flower is seen beside each female flower (Fig. 8).

Development of inflorescence:

The development of the inflorescence follows the same pattern in both the species. The first indication of flowering is a slight inflation of the bracts which ensheath the basal part of the partial inflorescence. Until then the inflorescence closely resembles a flagellum. The development of the partial inflorescences and the rachillae on it is acropetal. The time gap between the initiation of a partial inflorescence and its maturation is about two months. It takes about one month to complete the emergence of all partial inflorescences and their rachillae. 3-4 partial inflorescences are produced in a single inflorescence and 3-4 inflorescences are produced in a single plant at one season.

Floral opening and anthesis

In the male inflorescence no sequence of flowering is evident during anthesis. The opening of the flowers starts at about 1 a.m. and flowers are fully open by 4 a.m. Anthesis starts even before the flower is fully open. The inflorescences emit a sweet scent. This scent is conspicuous during the morning hours. In *C. thwaitesii* the scent production begins on the day prior

to the opening of the male flowers In both the species the pollen are completely shed by noon

The female flowers also open during the early dawn around 4 a.m. and the stigmatic lobes start to recurve. There is no sequence of flowering here also. The stigmatic surface remains white and receptive till noon. During the afternoon it gradually turns brown. Female inflorescences also produce a sweet smell as the male inflorescence. The pistillate flowers do not produce nectar. The adjacent sterile Staminate flower opens and exudes nectar.

Pollination

In both the species, the inflorescences of both sexes were visited by insects and ants during day as well as night. At sunrise insect activity increases around the inflorescences. Bees and wasps are the common visiting insects. Some of the flies were seen until sunset. Most of the observed flies were small and were found everywhere on the inflorescence. The insect activity was more in *C. andamanicus.*

The bees carried more pollen than any of the other insects captured. The wasps rarely carried pollen 1oads.Ants occasionally carried a few pollen grains.

3.4. Discussion

Inflorescence structure

In rattans, inflorescence structure is one of the most important characteristic for separating the genera (Dransfield, 1992). In *Calamus,* the inflorescence is usually long and flagellate. But in *C. andamanicus.*two types of inflorescences are seen. long flagellate as well as shrt, non-flagellate inflorescences. In *C. thwaitesii* the size of the fruit differs in some populations.

Time of flowering

The time of flowering varies slightly from year to year and also depending on the locality. In *C.* thwaitesii, the flowering started in the study site in July-August while in another locality about 200 km away from the study site young male flowers were noted in Jan-February. In Andaman islands *C. andamanicus* flowered during August In Nicobar islands young flowers were formed in May also. Raja Barizan (1992) and Manokaran (1989) suggested that flowering of rattans may be triggered by the fluctuations in temperature and weather.

Development of the inflorescence and fruit maturity

The order of emergence of partial inflorescence as well as the rachillae on it, is acropetal. This time difference is reflected in the maturity period of fruits also. The fruits in the topmost partial inflorescence ripen first and within a partial inflorescence the fruits of the top most rachillae ripen earlier.

The period from inflorescence emergence to fruit maturation in both species is about I0 months. This period varies from species to species. *C. manan* takes about 16 months (Alloysius *et al.,* 1994) while *C. subinermis* needs about 10 months (Alloysius, personal observation)

Pollination

The morphology of *Calamus* inflorescences gives no obvious indication about the pollination mode. The exposed stigmas and anthers are easily accessible to any potential insect visitor and even to the wind. Wind pollinationseems to be not important in rattan pollination. Lee *et al.* (1995) noted that 88% of the pollen was dispersed within 3.5 m from the inflorescence.

Insects are reported to be the main pollinators in rattans. The bees visit both staminate and pistillate inforescences. On pistillate flowers they are constantly in contact with the stigmas and hence can transfer pollen to the receptive stigma. Thus bees are probably the important pollinators. The ants, though they are frequent visitors, are unlikely to be pollinating agents because they cannot transfer pollen between plants which are spatially separated.

Staminate inflorescences attract insects by emitting scent and by offering pollen The anthers, pointing in all directions, can deposit pollen on the insect that crawls around the rachillae. The sterile staminate flowers resemble the functional staminate flowers. The function of thcsc flowers may be to mimic the functional staminate flowers and to produce nectar. When an inscct consumes nectar from the sterile flowers and tries to collect the pollen, it invariably touches the stigma of the adjacent female flower resulting in pollination.

The male plants have long and continuous flowering period while female plants have very short flowering period Under these circumstances the chances of an insect visiting the staminate inflorescences of the same species before it arrives at a pistillate flower are high Such differences in flowering periods have been observed in connection with bee pollination in other members in the family Palmae (Bullock, 1981, Henderson, 1986)

In Monocotyledons, the common position of the nectariferous tissue is in the ovary (Septa1 nectaries). This may develop between stipes with opening below the stigmatic lobes opening by pores on the surface of the gynoecium. But in *Calamoideae* septa1 glands are absent (Renuka,l981; Renuka & Manilal, 1986). Thus the absence of nectar glands in the female flower is compensated by the neuter flower.

4. CYTOLOGICAL STUDIES

The information on cytological characteristics is necessary to understand the evolutionary changes in detail. Cytological studies give genetic information on numerical and structural changes in the chromosomes, and also on recombination index which may bring new adaptations leading to new varieties or races.

Chromosomal studies were conducted and observations were reported in 7 species of *Calamus* by various researchers. Sharma and Sarkar (1956) conducted karyological studies in *C. arborescens* Griff,, *C. khasianus* Becc., *C. leptospadix* Griff. and *C. rotang* L. and reported 2n as 28 in all the species. In *C. caryoides,* Darlington and Janaki Ammal(1945) has reported 2n as 28 while Read (1965) noted 26 chromosomes. He also reported 2n as 26 in *C. mulleri*. In *C. scipionum* chromosome number was reported to be 2n = 28 (Eichhorn, 1953). Since then no karyological studies has been .conducted in this genus.

4.1. Materials and Methods

For the study of somatic chromosomes, root tips of *C. palustris* and C. *andamanicus* were collected from nursery-grown seedlings. For better fixation, the caps were removed from the root tips. The root tips were collected at different intervals from 10 a.m. to 2 p.m.

Various methods were tried to study the mitosis but spreading of chromosomes was unsatisfactory. Later pretreatment with 2-4 dichloro benzene gave encouraging results. Root tips were pretreated with saturated 2-4 dichloro benzene for 15 to 30 minutes. Then they were washed thoroughly in running water. The root tips were heated in a mixture of 2 percent Aceto-orcein and 1 normal Hydrochloric acid in 9:1 ratio for a few seconds directly over the flame. Then the extreme tips of the roots were squashed in Aceto-orcein and slides were prepared. Photographs of well scattered metaphase plates were taken.

4.2. Results

C. palustris

Twenty eight chromosomes were present in the complements (Fig. 13). Based on size differences they can be grouped into

1. Three pairs of comparatively long chromosomes each having two constrictions, primary and secondary, one nearly median and other sub-terminal in position.
2. Three pairs of comparatively long chromosomes with sub-median primary constriction.
3. Four pairs of medium sized chromosomes with median primary constriction.
4. Three pairs of medium sized chromosomes with sub-median primary constrictions.
5. One pair of short chromosomes with median primary constriction.

Chromosomes were in the size range of 2.46 to 0.89μ m.

In addition to normal complement, cells with abnormal number of chromosomes were also met with. A cell with 14 somatic chromosomes, evidently a haploid set, was also observed. (Fig. 14)

C. andamanicus

Twenty six chromosomes were observed in this species (Fig. 15). The chromosome size ranged from 2.22 to 0.44 μ m. The chromosomes can be grouped into 4 categories as given below

1. Two pairs of comparatively long chromosomes with 2 constrictions, primary and secondary, one nearly median and other nearly subterminal in position.
2. Six medium sized chromosomes with median constrictions.
3. Four medium sized chromosomes with sub-median constrictions
4. One pair of very short chromosomes.

In addition to the normal chromosome complement, cells with variations in number were seen. A cell with 20 somatic chromosomes was also met with (Fig. 16).

4.3. Discussion.

In palms, except in a few cases, a general homongenity is seen, where the different species of the same genus are having same chromosome number (Sharma and Sarkar, 1956). But in some genera different chromosome numbers are reported. In *Licuala grandis* and *L. paludosa*, n is reported to be 8 while in *L. peltata* and *L. spinosanis* 14. In *Calamus* two haploid sets are reported so far, n as 14 and n as 13, In *C. rotang, C. arborescens, C. leptospadix, C. khasianus*

Janaki Ammal, 1945) and 2 n as 26 (Read, 1965). Our observations also agree with this. In *C. palustris* 2 n is 28 while in *C. andamanicus* 2 n is 26.

Moreover, somatic cells with varying chromosome numbers are observed in addition to the normal number in the same individual. These types of abnormalities were reported in many other species of palms like *Chrysalidocarpus* and *Rhapis.* In *Arenga sacchariferra* abnormal nuclei with 6 and 36 chromosomes against a normal cell with 32 chromosomes were reported and such abnormalities were reported to arise by non-disjunction of chromosomes (Sharma & Sarkar, 1956). They also opined that the widespread and universal occurrence of such behaviour obviously imply that they play some significant role in the life process of the plant. Such irregularities in chromosome behaviour in somatic cells have been met with in a number of vegetatively reproducing plants specially belonging to monocot families such as *Amaryllidaceae, Liliaceae,* etc. It has been pointed out that this behaviour has got a distinct role in evolution.

5. CONCLUSION

Conservation measures require an understanding of the genetic system of the target species and particularly an understanding of spatial patterns of genetic variation. For the genetic conservation of a particular species, conservation of unique characteristics of geographic races is needed.

In *C. andamanicus* it is evident that the Andaman and Nicobar provenances are genetically varied. Within Andamans also several distinct phenotypic variations are present in the population. In *C. thwaitesii* 2 distinct populations are evident phenotypically based on the stem diameter, leaf arrangement and fruit size. It is essential to conserve these variations among population.

A good knowledge of reproductive biology is essential for conservation and genetic improvement programme. The mating system regulate the transmission of genes from one generation to the next. Species which are naturally outcrossing can hide in their genome a number of deleterious recessive alleles, each at low frequency. These alleles cannot be eliminated by selection because they are heterozygous. But these alleles will express themselves in the homozygous state which will happen when the population size decreases resulting in crosses between relatives (consanguinity). In outcrossing species, mating among relatives generates depression in the progeny that affects it vigour and its reproductive value The inbreeding depression is linearly linked to the rate of consanguinity (Eriksson, 1972). There exists a positive relationship between the outcrossing rate and the genetic divesity of the populations (Hamrick and Godt, 1989).

Eventhough rattan is dioecious, biparental inbreeding (when the mother and father are half sibs) and more general kinds of inbreeding can occur. Again, outcrossing species are particularly prone to inbreeding depression. When establishing artificial plantations of rattans, care should be taken to collect seeds from the highest possible number of mother plants preferably growing about 100 m apart and to mix all the progenies together before planting.

Conversely, there is a tendency for insect pollinated species to have stronger genetic differentiation among stands than wind pollinated species (Brotschol *et. al.* 1986). The scattered distribution of rattans and the time difference in flowering in different areas can contribute to increase the differentiation Moreover the irregularities in chromosome number in somatic cells noticed in rattan species studied has got a distinct role in evolution.

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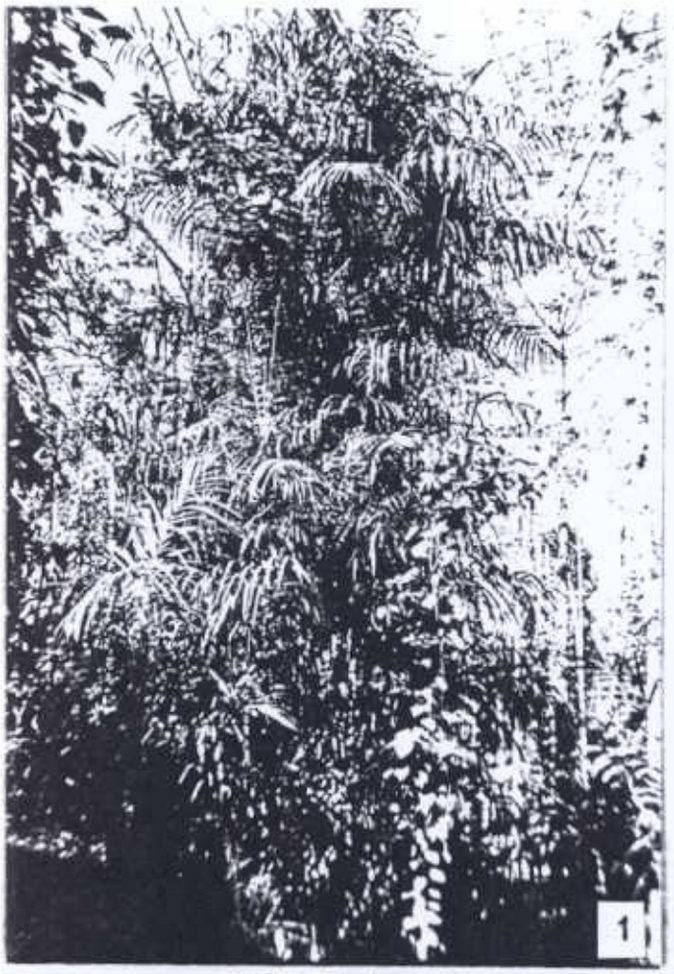
Figures

Calamus twaitesii

Fig. 1. A plant with thick diameter rattan and distinctly grouped leaflets.
Fig. 2. A plant with thin diameter rattan and more or less regular leaflets
Fig. 3. Long flagellate female inflorescence with fruits.
Fig. 4. Male inflorescence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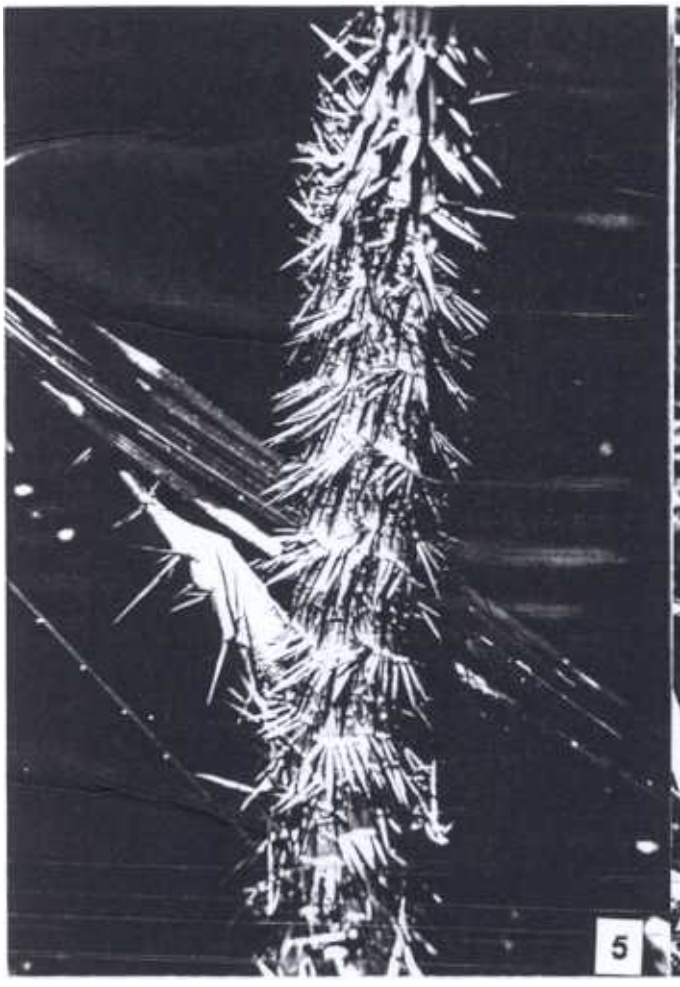
Calamus thwaitesii

Figs. 5-6. Leaf sheaths showing variations in colour and arrangement of spines.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6

C. andamanicus

Fig. 7. Habit
Fig. 8. Female inflorescence
Fig. 9. A long flagellate infiuctescence
Fig. 10. A short infiuctescence


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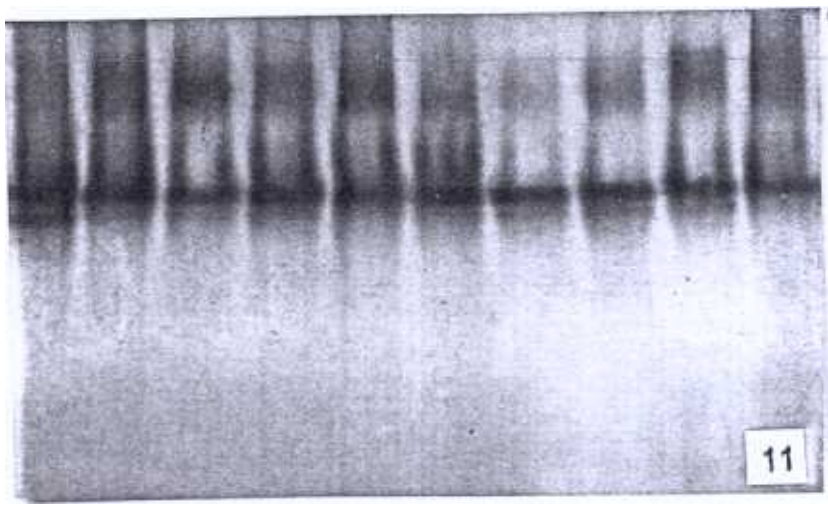
8


9


10

Fig. 11. Isozyme profile of peroxidase
Fig. 12. Isozyme profile of SOD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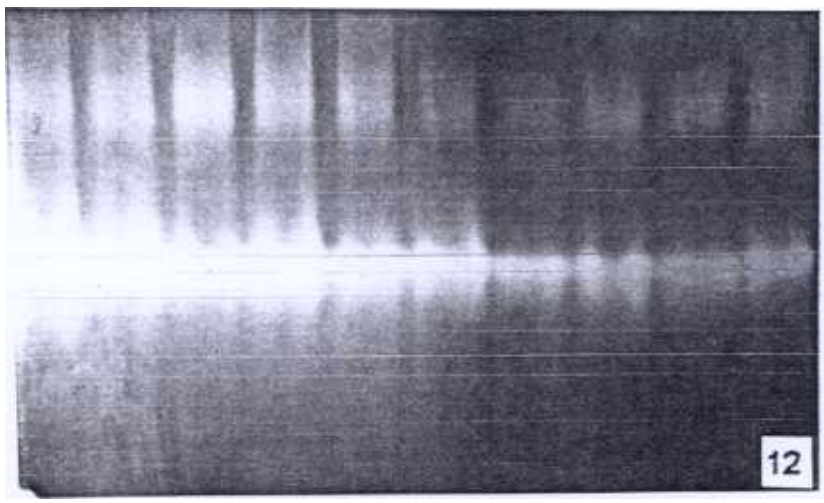
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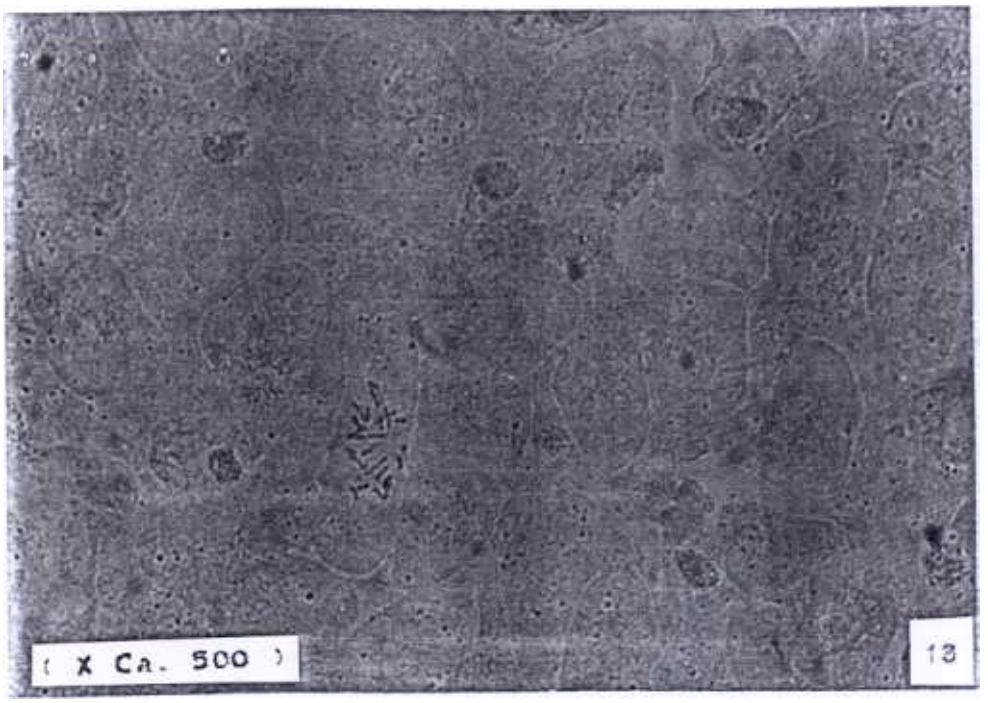
pnlustris

Fig. 13. A cell with 28 chromosomes
Fig. 14. A cell with 14 chromosomes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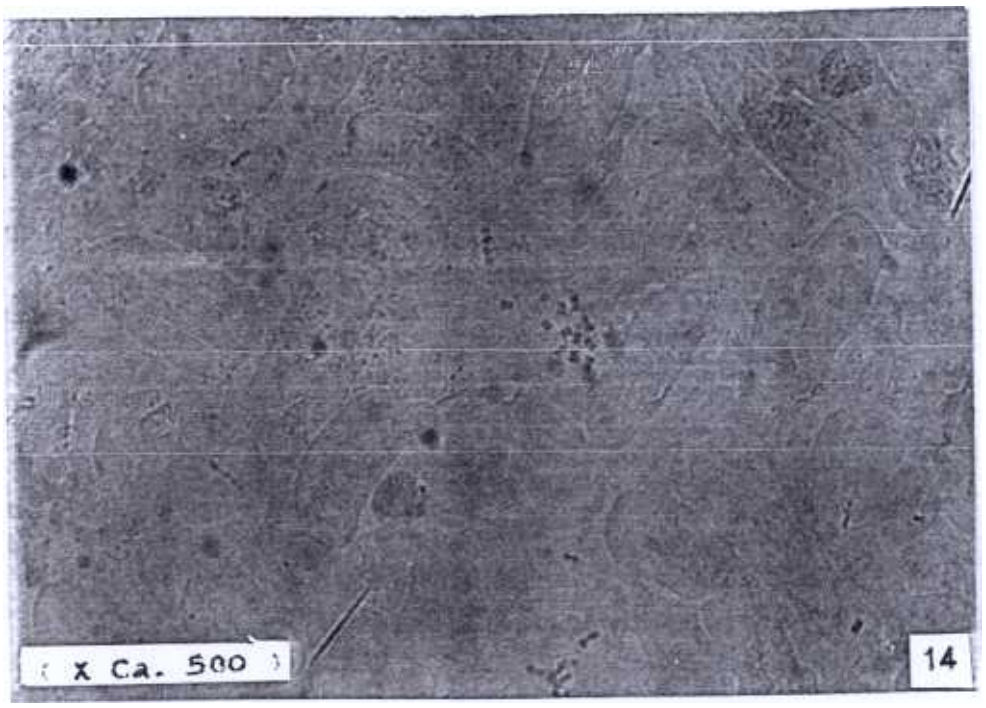
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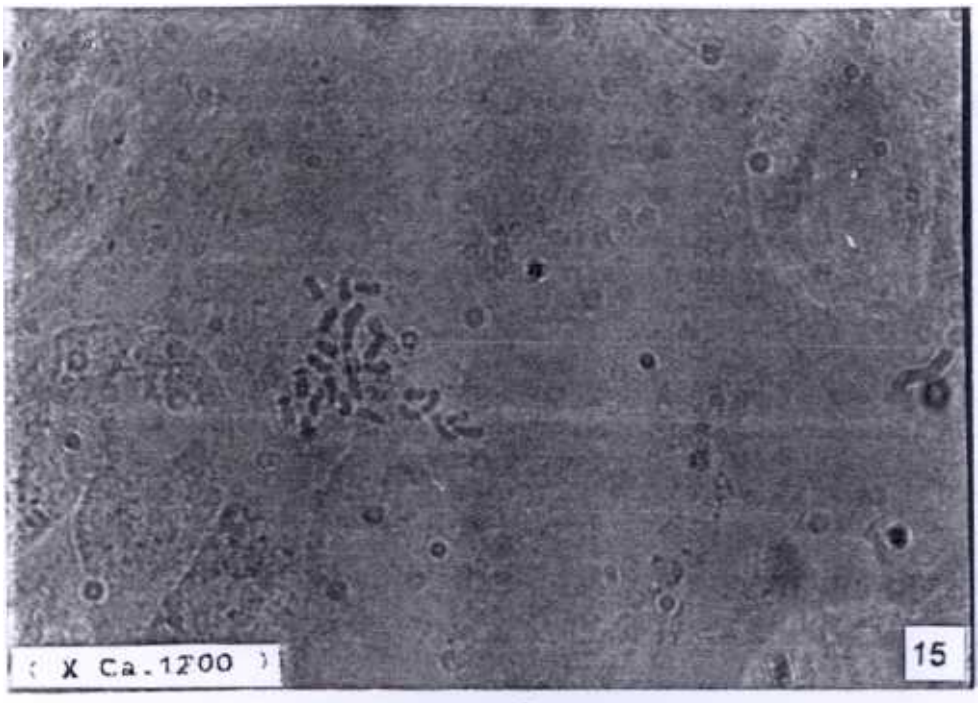
Calamus andamanicus

Fig. 15. A cell with 26 chromosomes.

Fig. 16. A cell with 20 chromosomes.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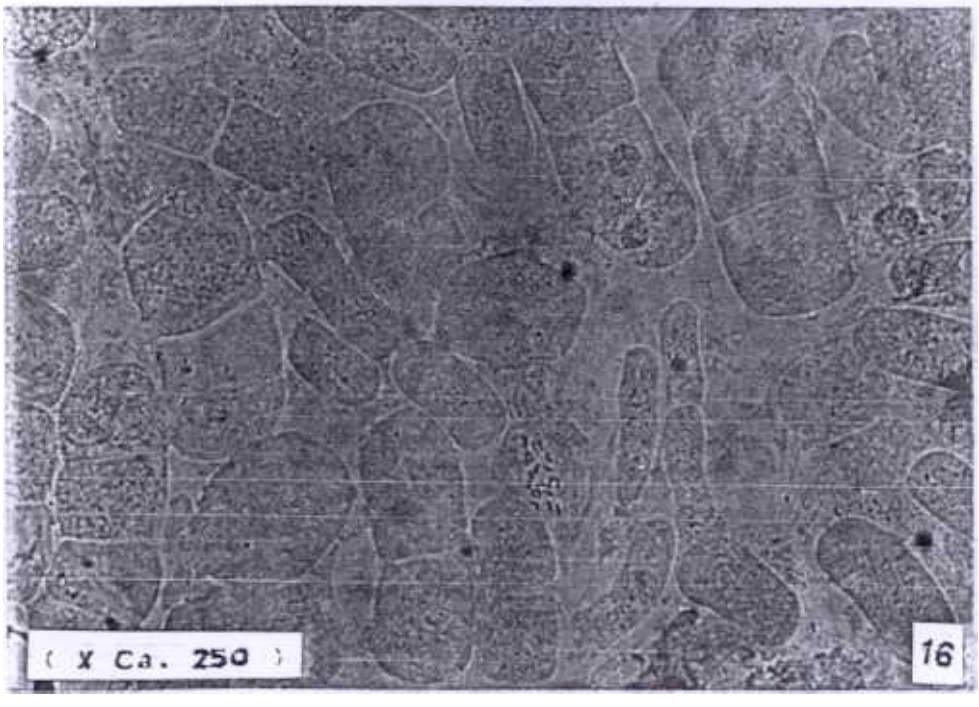
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