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Intra-specific Pollen Morphological Variations in Ten Tropical Species Growing in Sudan

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ABSTRACT. The study re-describes pollen grains of 10 tropical species belonging to 10 families. Significant variation in different pollen morphological characters was encountered in these species which were previously reported to have homo-morphic pollen grains. Pollen samples from various flowers collected from the same plant were analysed using LM and SEM. In each of the species examined, the diagnostic characteristics of the multiple pollen forms were presented. The relative percentages of these pollen forms were designated as common and less common pollen types. The study highlights the importance of the use of many pollen samples encompassing the whole range of variability as pollen reference material in tropical regions.

The phenomenon of pollen morphological variation in angiosperms was first described by Darwin (1892). Since then, various authors have reported its occurrence in various plant species belonging to different genera and families.

The causes of these intra-specific pollen morphological variation have been interpreted by various authors as being associated with internal (intra-floral) or external (inter-floral) factors. Internal factors include: style length (Vuilleumier 1967), stamen length (El-Gazali 1993b), position of flower on the inflorescence (Schoch-Bodmer 1940), stigma surface (Lee 1961), cytology (Martin and Valsaladevi 1989), number of nuclei in the grain (Fisher and Wells 1962), pseudomeiotic sporogenesis (Morzenti 1962) and timing mechanism or length of flowering period prior to the time at which the pollen sample is taken or both (Wells

1971).

External factors effecting pollen variation include: environmental conditions (Jones and Newell 1942), geographical location (Birks 1978), nutritional conditions (Bell 1959), temperature (Mikkelsen 1949), reaction of the grain to stains (Horner and Street 1978), percentage of germination in sugar (Dnyansagar and Sudhakaran 1972) and moisture content of the soil (Kurtz and Liverman 1958).

Pollen reference material and identification keys are generally constructed on the bases or assumption that they adequately encompass the intra-specific pollen morphological variation throughout the ecological and geographical range of the species concerned. In spite of this fact, few attempts have been made to assess this variation in a comprehensive way.

The aims of the present work are to highlight the presence of a considerable number of pollen morphological variations in tropical plants, to evaluate the range of the pollen morphological variability, and to contribute positively to precise species identification and consequently accurate taxonomic conclusions.

Materials and Methods

The material chosen for the present study was selected after an extensive pollen morphological survey of about 350 species growing in Sudan. For each of the 10 species selected as exhibiting well marked variation, pollen samples were collected from various flowers (at least five) belonging to one plant. To avoid pollen contamination and to ensure that the pollen sacs are not empty, flowers were collected as far as possible at the bud stage or just at the point of their opening.

The material was treated according to the standard acetolysis method (Erdtman 1960). The bulk of the samples prepared after chemical treatment and staining with safranin, were stored in small stock vials. These pollen samples were then used partly to make voucher slides for light microscopic (LM) examination, and partly for preparation of aluminum stubs for scanning electron microscopic (SEM) examination.

The voucher slides were mounted in glycerine-j elly and sealed with Entellan. LM examination was done with an Olympus microscope using X 100 magnification objectives and X 10 magnification eye-piece. SEM examination was done with a Jeol JSM-200 microscope with 25 KV accelerating voltage and 8 mm working

distance. Voucher specimens are deposited at the Medicinal and Aromatic Plants Research Institute, National Centre for Research, Khartoum, and at Osaka City University, Osaka, Japan.

In pollen description, the families are arranged alphabetically. For each species described, a total number of 100-150 pollen grains were counted to calculate the relative percentage of the common and the less common pollen types. Since most of the species have been previously described by various authors, only diagnostic characters are used in description. The descriptive terminology used is based on Kuyle *et al.* (1955), Erdtman (1971), Faegri and Iversen (1989), El Ghazali and Krzywinski (1989), El Ghazali (1990) and Punt *et al.* (1994).

Pollen description

1- ACANTHACEAE: *Peristrophe bicalyculata* (Retzius) Nees. -Sudan: Nuba Mountains, El Ghazali G11/83 (Khartoum University Herbarium - KHU, University of Bergen Herbarium - BG). Previous Work: Raj (1961), Maley (1970).

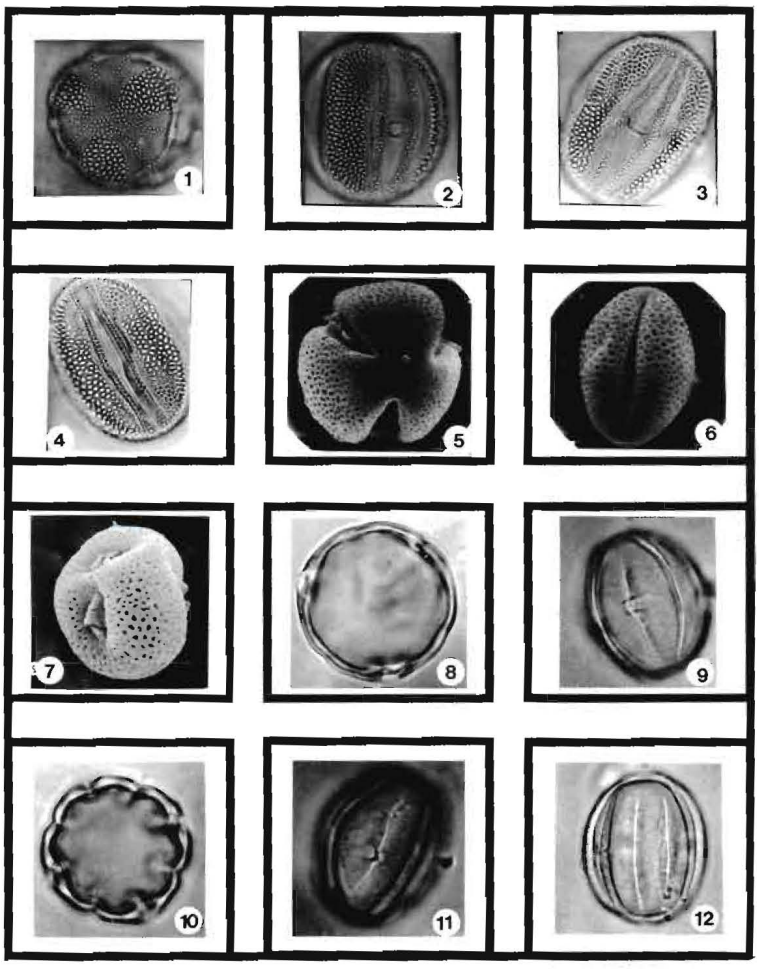
Common pollen type (95 %) (Figs. 1, 2). P = 52.1 µm (45.4-51.4), E = 34.5 µm (24.2-42.4), P/E = 1.51. Grains heterocolpate. Apertures 9; every composite aperture alternates with 2 simple ones. Endoapertures distinct, ± circular, with closed costae. Sculpturing supra-reticulate, hetero-brochate.

Less common pollen types (5 %) (Figs. 3, 4). Endoapertures distinct or indistinct, transversely elongated or irregular.

2- BIGNONIACEAE: *Kigelia africana* (Lamprey) Bentham. - Sudan: Khartoum Province, El Ghazali G68/87 (KHU). Previous Work : Maley (1970).

Common pollen type (98 %) (Figs. 5, 6). P = 74.4 µm (69.6-75.6), E = 54.4 µm (48.4-60.5), P/E = 1.37. Grains tricolporate. Ectocolpi tapering to both ends, endoapertures represented by distinct or indistinct endo-cracks. Sculpturing per-reticulate, lumina much wider than the corresponding muri, hetero-brochate; brochi decreasing towards the polar area.

Less common pollen type (2 %) (Fig. 7). Ectocolpi with equatorial bridge, polar edges rounded. Sculpturing per-reticulate, lumina width ± equal to that of the corresponding muri.



Figs. 1-4. *Peristrophe bicalyculata* (Retzius) Nees. (LM X 465) 1. Polar view showing 9 apertures. 2. Equatorial view. Endoapertures distinct, circular 3. Equatorial view. Endoapertures distinct, transversely elongated. 4. Equatorial view. Endoapertures indistinct.

Figs. 5-7. *Kigelia africana* (Lamprey) Bentham (SEM X 698, bar lines = 5μm). 5. Polar view. Grains tricolporate. 6. Equatorial view. Endoapertures with endocracks. 7. Equatorial view. Endoapertures with eqautorial bridge.

Figs. 8-12. *Heliotropium supinum* L. (LM X 465). 8. Polar view. Grains heterocolpate with 6 apertures. 9. Equatorial view. Endoapertures with parallel costae. 10. Polar view. Grains heterocolpate with 8 apertures. 11. Equatorial view. Endoapertures with conspicuous edges. 12. Equatorial view. Endoapertures represented by a rupture in costae.

3- BORAGINACEAE: *Heliotropium supinum* L. - Sudan: Khartoum Province, El Ghazali G7/92 (KHU).

Common pollen type (93 %) (Figs. 8, 9). P = 40.2 µm (38.6-43.1), E = 32.4 µm (30.7-35.3), P/E = 1.24. Grains heterocolpate. Apertures 6. Endoapertures distinct, with parallel costae, meridional edges lacking. Sculpturing psilate.

Less common pollen types (7 %) (Figs. 10, 11, 12). Apertures 8. Endoapertures distinct, edges conspicuous or sometimes represented by a rupture in the costae.

4- CAESALPINACEAE: *Parkinsonia aculeata* L. - Sudan: Khartoum Province, El Ghazali G6/86 (KHU, BG). Previous work: Nair and Sharma (1962), Tsukada (1963), Smith (1964), Martin and Drew (1970), Sowunmi (1973), Markgraf and D'Antoni (1978), Lieux (1982).

Common pollen type (92 %) (Figs. 13,14,15). P = 35.8 µm (34.4-39.6), E = 34.4 µm (32.7-37.9), P/E = 1.04. Grains prolate in equatorial view. Endoapertures indistinct. Sculpturing per-reticulate, hetero-brochate; brochi decreasing towards colpi and polar area.

Less common pollen types (8 %) (Figs. 16, 17, 18, 19). Grains oblate or circular in equatorial view. Ectocolpi tapering to both ends; endoapertures distinct, ± circular or elongated; costae distinct, closed, converging or tattered.

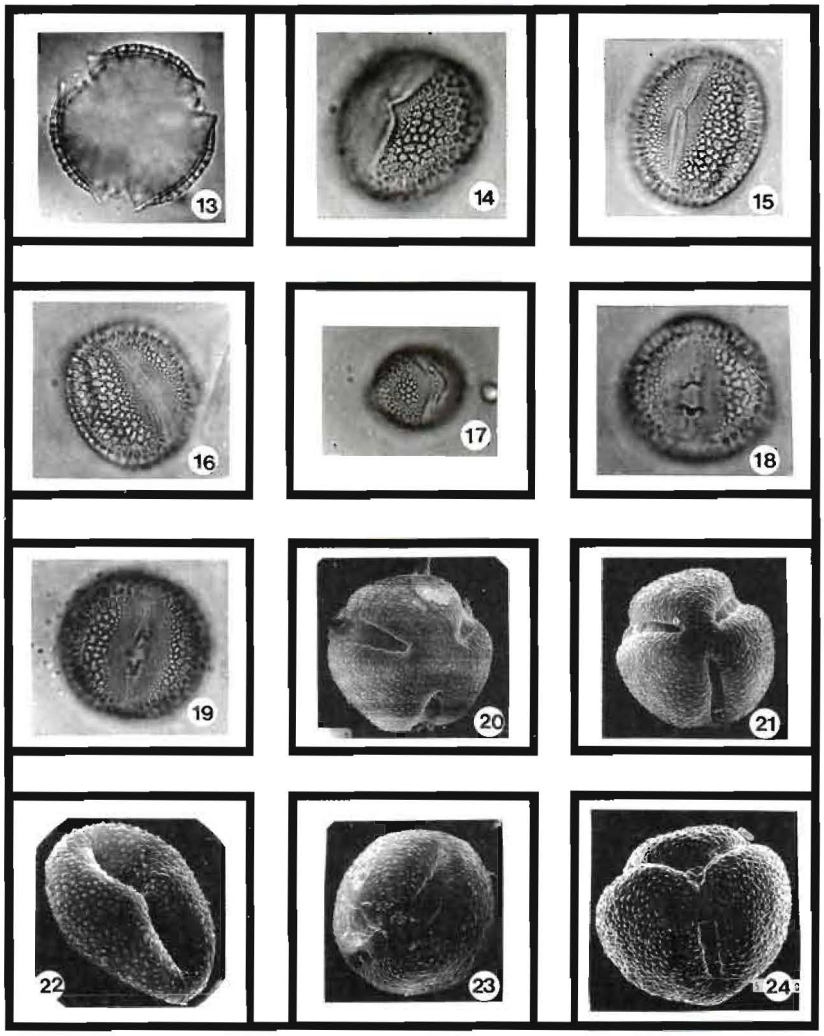
5- CAPPARIDACEAE: *Boscia senegalensis* (Persoon) Lamprey ex Poiret. - Sudan: Nuba Mountains, El Ghazali G105/83 (KHU). Previous Work: Maley (1970), Mitra (1975).

Common pollen type (97 %) (Figs. 20, 21, 22). P = 35.6 µm (31.3-39.1), E = 28.8 µm (25.1-34.4), P/E = 1.24. Grains tricolporate. Ectocolpi with equatorial constriction; endoapertures indistinct.

Less common pollen types (3 %) (Figs. 23, 24). Grains syncolpate, colpi fused totally or partially at the polar area, endoapertures distinct, or indistinct.

6- CUCURBITACEAE: *Luffa echinata* Roxburgh. - Sudan: Dinder, El-Ghazali G69/87 (KHU). Previous Work: Maley (1970), Bonnefille and Riollet (1980).

Common pollen type (89 %) (Fig. 25). P = 80 µm (59.5-87.6), E = 62.5 µm (50.1-81.3), P/E = 1.28. Grains circular in polar view, sub-prolate in equatorial view.



Figs. 13-19. *Parkinsonia aculeata* L. (LM X 505). 13. Polar view. Grains tricolporate. 14. Equatorial view. Ectocolpi with equatorial constriction. 15-16. Equatorial views. Ectocolpi with equatorial bridges. 17. Equatroial view. Grains oblate. 18. Equatorial view. Grains circular, endoapertures distinct and with coverging costae. 19. Equatorial view. Grains circular, endoapertures with elongated and tattered costae.

Figs. 20-24. *Boscia senegalensis* (Persoon) Lamprey (SEM X 505, bar lines = 5μm). 20-21, Polar view. Grains tricolporate with slight variation in polar areas. 22. Equatorial view. Ectocolpi with equatorial bridge. 23. Equatorial view. Endoapertures distinct, with tattared edges. 24. Polar view. Grains syncolpate, colpi fused at polar area.

Tricolporate. Ectocolpi tapering to both ends, endoapertures indistinct. Sculpturing per-reticulate, hetero-brochate; brochi decreasing towards colpi and polar area.

Less common pollen types (11%) (Figs. 26, 27, 28, 29). Grains circular or semi-angular. Tricolporate or syncolpate. Ectocolpi partially or totally fused at the polar area; endoapertures distinct, ± circular, without costae.

This species was also noticed to exhibit a pronounced variation in size (Fig. 30).

7- MIMOSACEAE: *Acacia seyal* Delile, var. *seyal*. - Sudan: Khartoum Province, El - Zubeir 4-86 (KHU). Previous Work: Maley (1970), El Amin (1982), Sowunmi (1973), Bonnefille and Riollet (1980).

Common pollen type (86 %) (Figs. 31, 32). D = 71.9 µm (68.0-74.5). Grains 16-celled polyads, characteristically differentiated into square central part and a peripheral part composed of 8 cells. Central cells with 3 pores and 3-arm-shaped colpi, peripheral cells with 4 pores and H-shaped colpi. Sculpturing psilate -foveolate.

Less common pollen types (14 %) (Figs. 33, 34, 35, 36). Grains 13- or 15-celled polyads; without clear differentiation into central and peripheral parts.

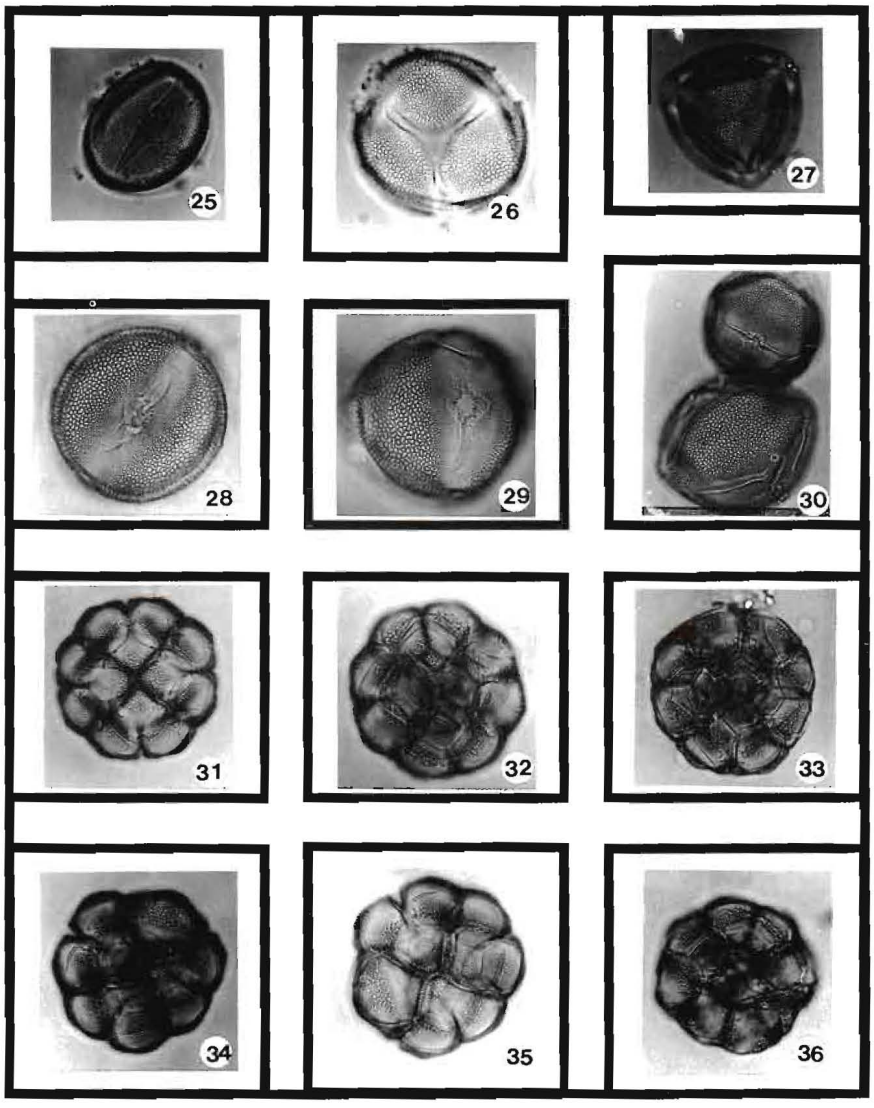
8- PEDALIACEAE: *Rogeria adenophylla* Gay ex Delile. - Sudan: El-Jabalien, El Ghazali G10 /93 (KHU).

Common pollen type (90 %) (Figs. 37, 38). D = 35.7 µm (32.1-40.3). Grains circular or elliptic in polar view. Stephanocolpate; colpi 8, with costae along the whole colpi. Sculpturing gemmate.

Less common pollen types (10 %) (Figs. 39, 40). Grains stephanocolpate; colpi 7 or 9.

9- SCROPHULARIACEAE : *Striga hermonthica* (Delile) Bentham. - Sudan, Khartoum province, El Ghazali G13-87 (KHU). Previous Work: Bonnefille (1970), Bonnefille and Riollet (1980).

Common pollen types (96 %) (Figs. 41, 42). P = 15.1 µm (11.6-17.4), E= 18.0 µm (14.4-20.3), P/E = 0.84. Grains stephanocolpate; colpi 4, with distinct costae along the colpi. Sculpturing scabrate-verrucate.



Figs. 25-30. *Luffa echinata* Roxburgh (LM X 535). 25. Equatorial view. Grains subprolate, ectocolpi tapering to both ends, endoapertures indistinct. 26. Polar view. Grains circular, syncolpate. 27. Polar view. Grains semi-angular. 28-29. Equatorial views. Grains circular, endoapertures with tattared edges. 30. Equatorial view of two grains showing variation in size.

Figs. 31-36. *Acacia seyal* Delile (LM X 535). 31-32 Polyads with 16 cells, regular. 33-35. Polyads with 15 cells, irregular. 36. Polyad with 13 cells, irregular.

Less common pollen types (4 %) (Fig. 43). Grains tricolpate. This species was also noticed to exhibit a pronounced variation in size (Fig. 44).

10- SOLANACEAE: *Lycium persicum* Miers. - Sudan: Erkowit, El Ghazali G2/92 (KHU). Previous Work: El Ghazali (1993a).

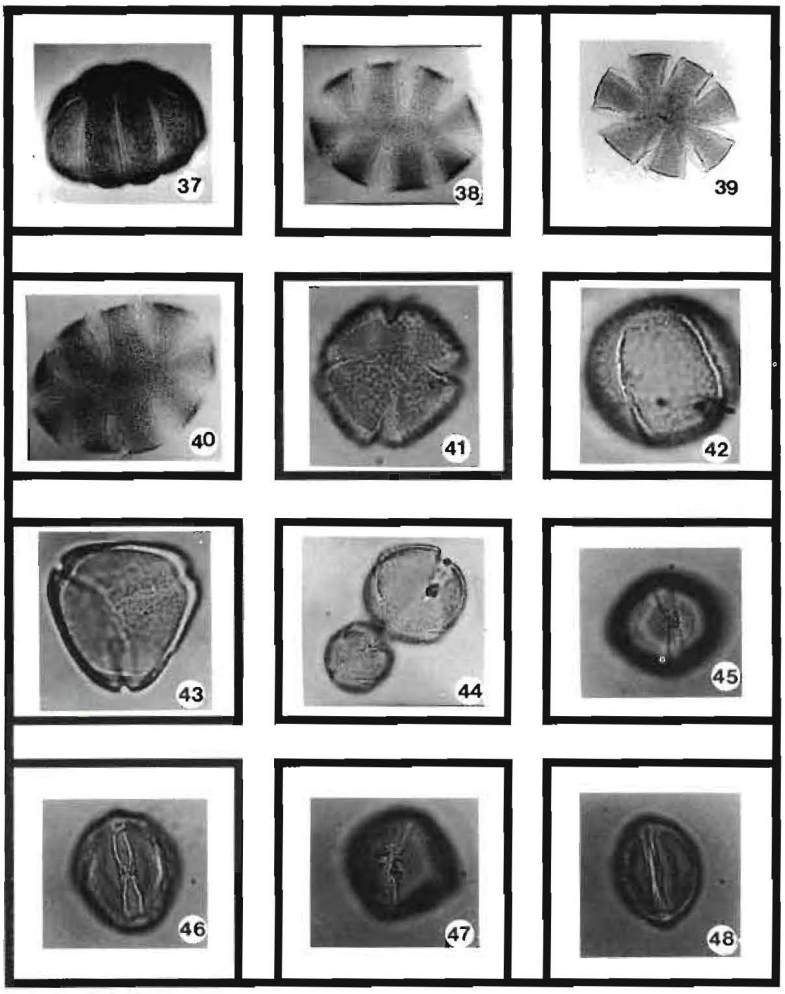
Common pollen type (88 %) (Fig. 45). P = 33.5 μm (32.9-35.9), E = 28.7 μm (26.9 - 29.9), P/E = 1.17. Grains oblate in equatorial view. Tricolporate. Ectocolpi with distinct costae, from pole to pole, equatorially constricted endoapertures indistinct, with isolated ektexinous elements, Sculpturing striate, vallae faint.

Less common pollen types (12 %) (Figs. 46, 47, 48). Grains circular or prolate in equatorial view. Ectocolpi with distinct costae along the whole colpi or interrupted equatorially or forming an equatorial bridge; endoapertures distinct or indistinct, edges conspicuous.

Discussion and Conclusions

This study describes the pollen grains of 10 species exhibiting intra-specific pollen morphological variation in Sudan. A sound variation in different pollen morphological characters were reported in these species. Since unopen flowers have been used in this study, the possibility of pollen contamination from the neighbouring flowers was excluded. Intra-specific pollen morphological variation was obsreved in shape in polar view as in *Luffa echinata*, in shape in equatorial view as in *Parkinsonia aculeata*, in shape of colpi as in *Kigelia africana*, in the number of apertures as in *Striga hermonthica,* in number of cells in compound pollen grain as in *Acacia seyal,* and in shape of endoapertures as in *Peristrophe bicalyculata.*

Like other bilogical characters, pollen morphology may show considerable variability or remarkable uniformity. Pollen morphological variability has been reported by a number of workers in different taxa (*e.g.* El Ghazali 1993b, Mathew and Valsaladevi 1989, Saggo and Bir 1983), to be associated with various internal or external factors. These factors do not always exhibit consistent mode of action throughout. Macro-morphological variation (an internal factor) within the plant may be reflected in pollen morphology (Ferguson 1980), or may not be reflected (Ferguson 1972). Also, geographical variability (an external factor) of the same plant may be reflected in pollen morphology (Nilsson 1967) or may not be related (King and Robinson 1967).



Figs. 37-40. *Rogeria adenolphylla* Gay ex Delile (LM X480). 37. Equatorial view. Grains with 8 colpi. 38. Polar view. Grains with 8 colpi. 39. Polar view. Grains with 7 colpi. 40. Polar view. Grains with 9 colpi.

Figs. 41-44. *Striga hermonthica* (Delile) Bentham (LM X 480). 41. Polar view. Grains with 4 colpi. 42. Equatorial view. Colpi with distinct costae. 43. Polar view. Grains with 5 colpi. 44. Polar views of two grains showing variation in size.

Figs. 45-48. *Lycium persicum* Miers (LM X 480). 45. Equatorial view. Ectocolpi equatorially constricted. 46. Equatorial view. Ectocolpi with equatorial bridge. 47. Equatorial view. Endoapertures with conspicuous edges. 48. Equatorial view. Endoapertures indistinct.

Percentages of pollen fertility and cytology are other factors which are frequently associated with pollen morphological variation. These factors although might lead to potentially interesting results, were not examined in the present study since only acetolysed pollen grains have been used. Most important, however, is the fact that most of the publications encountered to document pollen morphological variations were done on tropical or subtropical taxa. Examples of these taxa include: *Mapouria* (Bremekamp 1963), *Stevia* (King and Robinson 1967), *Urena lobata* (Gupta and Roy 1970), Alangiaceae (Reitsma 1970), *Turnera aurantaca* (Ong and Rao 1973), Acanthaceae (Mathew and Valsaladevi 1989), *Saliva leucantha* (Gupta and Sharma 1990) *etc*.

The different pollen forms identified in each species of the present study were designated as common and less common pollen types. Terms like atypical, aberrant, irregular and abnormal as used by Erdtman (1969), Wells (1971), Clarke (1976) and Ferguson (1980) respectively, were rejected. The reason is threefold. Firstly; biological variation is an inherent phenomenon of nature and all species normally seems to exhibit variation. Secondly; the pollen types present in each species bear some sort of similarity to each other, and most importantly they are recurrent. Thirdly; these pollen types were identified on frequency bases only, and consequently there is no point in describing the less common or rare multiple forms as atypical, aberrant, irregular or abnormal.

Most of the species examined in this study were previously described by various authors, but no reference was encountered to document the existence of intra-specific pollen variation in them. This fact clearly highlighted the peculiarity of pollen morphological studies in tropical and sub-tropical regions. Another possible explanation is probably the use of limited sampling not encompassing the whole range of intra-specific variability present in each species.

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الاختلافات البينية في أشكال حبوب اللقاح لعشرة أنواع استوائية تنمو في السودان

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تهدف الدراسة إلى تأكيد وجود عدد مقدر من النباتات الاستوائية التي لها اختلافات بينية في أشكال حبوب لقاحها وتقييم المدى الكلي لهذه الاختلافات وبالتالي الاسهام الفعَّال في التعريف التصنيفي الدقيق لمختلف أنواع النباتات بواسطة حبوب اللقاح .

هذه الدراسة عبارة عن اعادة وصف حبوب اللقاح لعشرة انواع استوائية تنتمي إلى عشر فصائل مختلفة لنباتات مزهرة سبق وصفها بانها متماثلة الشكل الخارجي في العديد من المراجع العالمية .

تم جمع حبوب لقاح هذه النباتات من أزهار مختلفة لنفس النبات . هذه النباتات تم اختيارها بعد دراسات مستفيضة لحوالي ٣٥٠ نوع تنمو طبيعاً في السودان وتمتاز بالتباين في أشكال حبوب لقاح النبات الواحد . هذه الانواع تم فحصها بواسطة المجهر الضوئي المركب والمجهر الالكتروني الماسح . في كل واحد من هذه الانواع تم تحليل وعرض الصفات الخارجية المميزة فقط لاشكال حبوب اللقاح المتعددة .

سميت هذه الاشكال بانواع سائدة وانواع أقل سيادة حسب النسبة المئوية

لكل شكل بعكس الدراسات العلمية السابقة التي تعرَّضت لمصطلحات عديدة لا توضح بدقة مدى التباين في الاشكال منها على سبيل المثال الاشكال غير القياسية وغير المنظمة والشاذة والمنحرفة .

وقد تم تحديد الاختلافات البينية لأشكال حبوب اللقاح على ضوء عدد من الصفات منها الشكل الخارجي للمنظر القطبي والمنظر الاستوائي ، شكل الاخاديد ، عدد التجاويف وعدد الخلايا المكونة لحبوب اللقاح المركبة . ويرجع سبب هذه الاختلافات البينية إلى عدة عوامل داخلية (كاختلاف أطوال المياسم والاسدية في الزهرة الواحدة) وأخرى خارجية (كاختلاف البيئة والتوزيع الجغرافي) .

القت الدراسة الضوء على أهمية استعمال أكبر عدد من عينات حبوب لقاح النبات الواحد بحيث تغطى المدى الكلي للاختلافات البينية مما يكسب صفة حبوب اللقاح وزن أكبر كمادة مقارنة في العينات المرجعية وخاصة في المناطق الاستوائية .