Pollen morphology of *Rhynchosia* and *Eriosema* (Fabaceae)

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The closely related genera *Rhynchosia* and *Eriosema* have tricolporate pollen. Twenty-two species of *Rhynchosia* and nine species of *Eriosema* were examined with the light microscope. Most of the species have isopolar-type grains, but 11 species have the hitherto rarely described heteropolar-type pollen, i.e. on a single grain, the ends of the colpi subtend a greater area at one pole than at the opposite pole. Duplicate examination of 13 species indicates that polarity types are constant within those species. While the genera cannot be separated on the basis of pollen morphology alone, there are some pollen characters which embrace most species in any one genus. *Eriosema* species are mostly heteropolar (67%) and *Rhynchosia* are predominantly isopolar (77%). The lumina of the reticulum of *Rhynchosia* tend to be smaller than those of *Eriosema* and, with one exception, the muri of *Eriosema* have acute tops while those of *Rhynchosia* are obtuse. Scanning electron micrographs illustrate pollen morphology.

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Rhynchosia et *Eriosema*, deux genres très voisins, ont un pollen tricolporé. Vingt-deux espèces de *Rhynchosia* et neuf espèces d'*Eriosema* ont été examinées au microscope photonique. La majorité des espèces ont des pollens de type isopolaire, mais 11 espèces présentent le pollen hétéropolaire, rarement décrit, c'est-à-dire que sur chaque grain les extrémités des colpi sous-tendent une surface plus grande à un pôle qu'à l'autre. Des examens répétés de 13 espèces indiquent que les types de polarité sont constants chez ces espèces. Bien que les genres ne peuvent pas être séparés par la seule morphologie pollinique, certains caractères des pollens sont partagés par une majorité d'espèces dans un genre donné. Les espèces d'*Eriosoma* sont surtout hétéropolaires (67%) et celles du genre *Rhynchosia* sont en majorité isopolaires (77%). Les lumens du réticulum de *Rhynchosia* sont généralement plus petits que ceux d'*Eriosoma* et, sauf une exception, les muri d'*Eriosoma* ont des sommets aigus alors que ceux de *Rhynchosia* sont obtus. Des micrographies en microscopie électronique à balayage illustrent la morphologie pollinique.
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Introduction

Rhynchosia Lour., with about 250 species, and *Eriosema* (DC.) G. Don, with about 130 species, are two closely related genera in the Fabaceae (subfamily Lotoideae, tribe Phaseoleae, subtribe Cajaninae). Both genera are essentially pantropical in distribution with the exception of some species of *Rhynchosia* that extend into temperate North America (U.S.), ranging to 38° N latitude in Delaware and Maryland. Our study concerns only representative American taxa.

In the past, the two genera were delimited only by gross morphological characters, which are not always constant or reliable. Grear (1970) found that characters pertaining primarily to the calyx and seed distinguish between the two genera in the New World. In the same study, the pollen of both genera was described as triporate, as triangular in polar view, and as having a reticulate sculpturing of the exine, but no taxonomically significant differences were reported. However, a critical reexamination of the published micrographs indicates that at least some, and probably all, of the pollen have colpae as well as pores, and that the length of the colpae and coarseness of the reticulum appear to differ among species.

A renewed and more intensive palynological study using both the light and scanning electron microscopes reveals pollen differences between the two taxa which help to distinguish them.

Materials and Methods

Pollen from 9 species of *Eriosema* and 22 species of *Rhynchosia* was obtained from sheets deposited in herbaria (TRT, NY, US, F). These are listed below in alphabetical order.

Rhynchosia americana (Miller) Metz
U.S.A.: Texas, *Webster* and *Wilbur 3107* (US 2067889), ROM 754; Texas, Corpus Christi, *J. R. Joor* (US 66290), ROM 752.

R. balansae Micheli
BOLIVIA: Santa Cruz, *Cardenas 4471* (US 1989917), ROM 761; Robare, *Cardenas 2968* (F 754685), ROM 834. ARGENTINA: Corrientes, *Ibarrola 158* (US 1857205), ROM 656.

R. calycosa Hemsley
PANAMA: *Standley 27699* (US 1217711), ROM 764.

R. corylifolia Mart. ex Benth. var. *corylifolia*
BRAZIL: *Hatschbach 12819* (US 2563642), ROM 654.

R. cytisoides (Bert.) Wilbur
U.S.A.: Florida, *Rugel s.n.* in 1843 (NY), ROM 664.

R. difformis (Elliott) DC
MEXICO: *King and Diboll 3987* (US 2365427), ROM 763.

R. discolor Mart. & Gal.
MEXICO: *Gentry 22045* (US 2545556), ROM 756. GUATEMALA: *A. Molina et al. 15990* (F 1639307), ROM 826.

R. edulis Griseb.
ARGENTINA: *Pedersen 118* (US 2122240), ROM 750.

R. lineata Benth.
BRAZIL: Rio Grande do Sul, *G. O. Malme 1072* (F 691298), ROM 827.

R. longeracemosa Mart. & Gal.
HONDURAS: *A. Molina R. 12941* (NY), ROM 662. MEXICO: *Breedlove 15663* (US 1358544), ROM 769.

R. macrocarpa Benth.
GUATEMALA: *Heyde and Lux 1074* (US 1364264), ROM 760. MEXICO: Durango, *E. Palmer 63* (F 212862), ROM 835.

R. mantaroensis MacBride
PERU: *Tovar 1410* (US 2490539), ROM 771; Andahuaylas, *H. E. Stork and O. B. Turnbull 10669* (F 1053265), ROM 831.

R. minima (L.) DC. var. *minima*
PERU: *Killip and Smith 24050* (US 1358544), ROM 65. WEST INDIES: St. Thomas, *C. Amalia 485* (F 60485), ROM 832.

R. mollissima (Elliott) Watson
U.S.A.: Florida, *Reynolds s.n.* (NY), ROM 660.

R. phaseoloides (S.W.) DC.
BRAZIL: *Irwin and Soderstrom 5887* (TRT 168512), ROM 562. CUBA: Oriente, *J. A. Shafer 3541* (F 286232), ROM 833.

R. pringlei Rose
MEXICO: *Conzatti 4317* (US 1169455), ROM 762.

R. platyphylla Benth.
BRAZIL: *Irwin, Souza, and Reis dos Santos 10822* (TRT 168514), ROM 126.

R. pyramidalis (Lam.) Urban
COSTA RICA: San Jose Prov., *A. F. Skutch 4037* (US 1644576), ROM 836. MEXICO: San Lois Potosi, *R. M. King 4298* (F 1560641), ROM 765.

R. quercetorum Standley
PANAMA: *Maurice 703* (US 1844133), ROM 758.

R. reticulata (Swartz) DC.
PANAMA: *Standley 28449* (US 1218135), ROM 766.

R. schomburgkii Benth.
GUYANA: *Irwin 645* (US 2212656), ROM 770.

R. texana Torrey & Gray
US: New Mexico, *no collector* (NY), ROM 658.

Eriosema brevipes Grear
BRAZIL: *Irwin et al. 32806* (TRT 173475), ROM 829.

E. congestum Benth.
BRAZIL: *Irwin et al. 32003* (TRT 173474), ROM 828.

E. crinitum (HBK) G. Don var. *macrophyllum* Grear
BRAZIL: Cantoni, *Irwin et al. 27206* (TRT 168541), ROM 657; Minas Gerias, *Irwin et al. 28459* (TRT 168545), ROM 838.

E. diffusum (HBK) G. Don
HONDURAS: *von Hagen 1091* (NY), ROM 755.

E. glabrum Mart. ex Benth.
BRAZIL: *Irwin et al. 25257* (TRT 168525), ROM 661; Goias, *Irwin et al. 19392* (TRT 173183), ROM 839.

E. glaziovii Harms
BRAZIL: Fed. Dist., *Irwin et al. 26352* (TRT 168529), ROM 561; Goias, *Irwin et al. 18817* (TRT 173189), ROM 837.

E. heterophyllum Benth.
BRAZIL: *Irwin et al. 25222* (TRT 168521), ROM 659.

E. longifolium Benth.
BRAZIL: *Irwin et al. 15345* (NY), ROM 759.

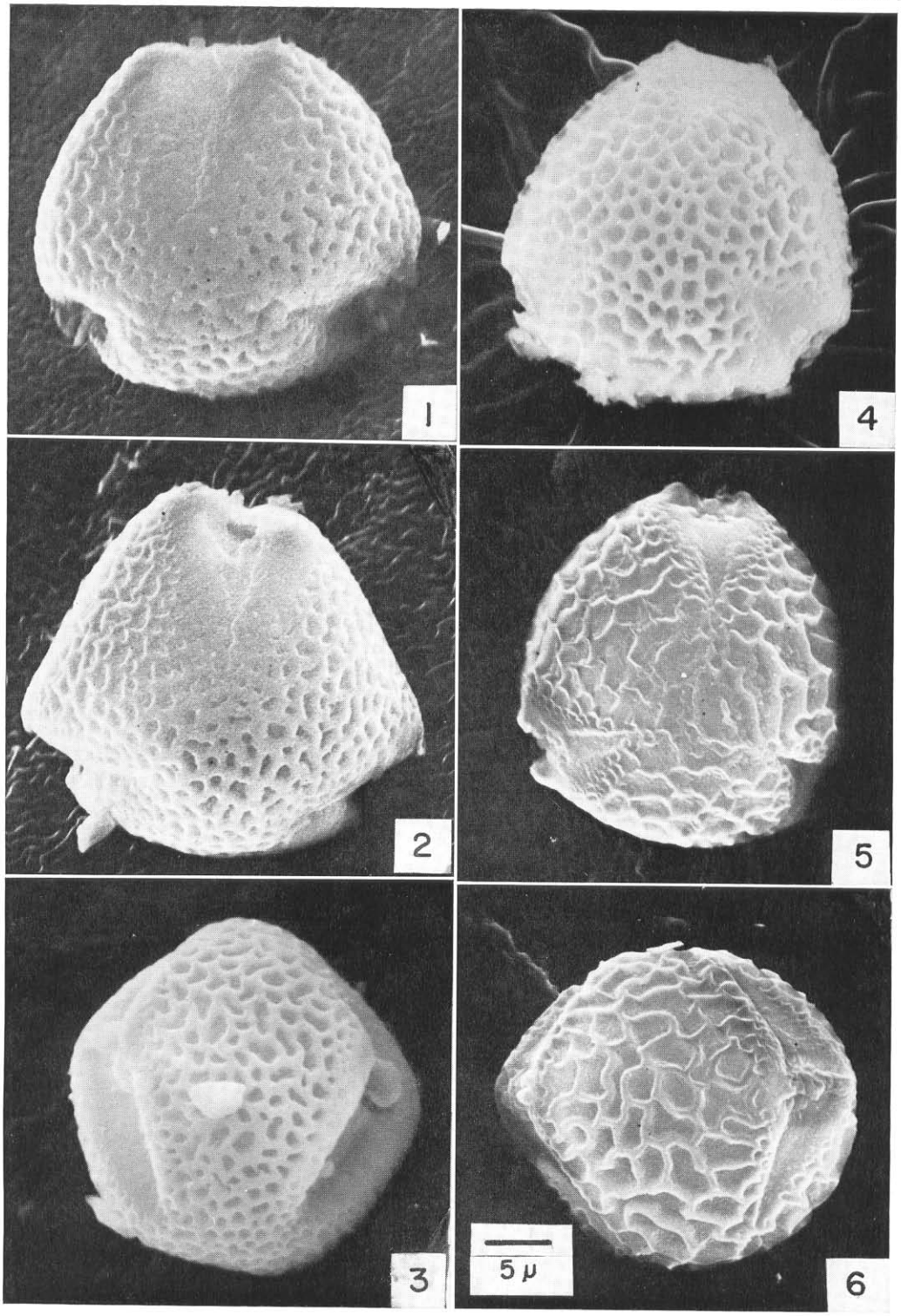
E. rufum (HBK) G. Don var. *rufum*
BRAZIL: *Irwin et al. 27068* (TRT 168533), ROM 665; Goias: *Irwin et al. 21766* (TRT 173195), ROM 825.

Pollen used for light microscopy was acetolyzed and mounted in silicone oil (Faegri and Iversen 1964); pollen for scanning electron microscope observations was prepared by the acetolysis and critical-point drying method (Adams and Morton 1972). Microscope slides used for measurements are deposited in the Geology Department of the Royal Ontario Museum with duplicates in the University of Toronto herbarium.

Light microscopy was with a Leitz Ortholux microscope, equipped with apochromatic objectives × 95 and × 40. Scanning electron micrographs were taken with a Cambridge Stereoscan Mark II.

Size measurements through the light microscope were made with an ocular micrometer having a 2-micron (μ) interval when combined with the × 40 objective.

On each of 10 grains of a slide three measurements were made: the equatorial diameter; and, on each pole, the distance between the ends of two colpae. For each pole, the polar area index (PAI), the ratio of the distance between the ends of adjacent colpae to the equatorial diameter (Faegri and Iversen 1964), was calculated and the difference between the two indices was determined. Thus the mean difference of polar area index (dif. PAI) is the average difference in the polar area indices of 10 pollen grains from an herbarium collection.



FIGS. 1–6. Scanning electron micrographs of pollen grains of *Rhynchosia* and *Eriosema*: (1) *R. phaseoloides* polar view; (2) *R. cytisoides* polar view; (3) *R. cytisoides* equatorial view; (4) *E. glabrum* polar view; (5) *E. glaziovii* polar view; (6) *E. glaziovii* equatorial view.

Results

The pollen of both genera (Figs. 1–6) are spheroidal monads which are semiangular (Huang 1972) in polar view, tricolporate, and angulaperturate. Pores are situated within the colpi and located on the equator of the grain. However, in some species, the length of colpus on either side of the equator is unequal. The grains range in mean equatorial diameter from 20.0 μ to 37.4 μ (Tables 1, 2; Fig. 7). The exine is reticulate, with lumina of variable shape and size; the lumina are finer adjacent to the colpus, forming a margo.

Figure 7 shows the mean difference in polar area indices plotted against mean equatorial diameter for 45 pollen collections, including 14 replications of 13 species. There are two clusters of indices. The cluster above 0.10 is regarded as heteropolar, while the cluster between 0 and 0.07 represents isopolar collections with positive values as a result of measurement of errors rather than heteropolarity in the grains. Isopolar and heteropolar species occur in both genera, with heteropolarity recorded in 5 of 22 *Rhynchosia* species and in 6 of 9 *Eriosema* species. Replication of index measurements of 13 species are consistent. The diameter of heteropolar species tends to be larger than isopolar species.

The grains of all species examined in both genera are heterobrochate. The species can then be put into two groups according to the arrangement of the lumina. In some species, e.g. *R. phaseoloides*, the lumina increase in size from pole to equator of the grains, while in others, no such gradation is observed (ungraded condition). The condition of distinct lumina size differences

TABLE 1

Pollen morphological characteristics of *Rhynchosia*

Species name	No.	ROM slide No.	$\bar{x}$ difference PAI	Equatorial diam., μ		Reticulum pattern				Lumina shape	
				Mean	Range	Graded	Ungraded	Homogeneous	Heterogeneous	Elongated	Polygonal
R. quercetorum	1	758	0.007	25.0	22–28		x		x	x	
R. cytisoides	2	664	0.008	26.4	26–28	x			x	x	
R. pyramidalis	3	765	0.020	20.2	20–22		x	x			x
		836	0.056	21.6	20–22	?	?	?	?		x
R. difformis	4	763	0.028	21.4	20–22		x		x		x
R. corylifolia var. *corylifolia*	5	654	0.031	28.0	26–32	x			x	x	
R. longeracemosa	6	769	0.030	20.0	18–22		x	x			x
		662	0.053	19.0	18–20		x	x			x
R. minima	7	65	0.031	20.0	18–24	x			x		x
var. *minima*		832	0.040	20.0	18–22	x			x		x
R. americana	8	754	0.032	26.4	24–28		x	x			x
		752	0.032	24.6	22–26		x	x			x
R. mollissima	9	660	0.032	25.2	24–28	x			x		x
R. texana	10	658	0.037	21.8	20–24	x			x		x
R. calycosa	11	764	0.050	24.6	22–26	x			x		x
R. platyphylla	12	126	0.050	29.6	28–32	x			x		x
R. pringlei	13	762	0.052	28.8	26–32	x			x		x
R. schomburgkii	14	770	0.052	26.4	24–28	x			x		x
R. balansae	15	761	0.037	22.0	20–24	x			x	x	
		656	0.052	22.6	20–26	x			x	x	
		834	0.056	24.6	22–26	x			x	x	
R. edulis	16	750	0.057	28.6	26–30	x			x	x	
R. reticulata	17	766	0.058	24.4	24–26	x			x		x
R. mantaroensis	18	831	0.115	27.6	24–32	x			x		x
		771	0.130	26.6	24–28	x			x		x
R. discolor	19	826	0.186	37.4	34–40	x			x		x
		756	0.189	31.8	30–36	x			x		x
R. macrocarpa	20	760	0.208	36.8	34–40	x			x	x	
		835	0.234	32.2	26–36	x			x	x	
R. lineata	21	827	0.221	25.4	24–26	x			x		x
R. phaseoloides	22	833	0.313	24.8	22–26	x			x		x
		562	0.315	26.6	26–28	x			x		x

on various parts of the grain is called the heterogenous condition; the homogenous condition is a random arrangement of both larger and smaller lumina and shows no pattern.

The reticulate sculpturing pattern varies among species. In some species the reticulum, consisting of muri (ridges) and lumina (space contained by surrounding muri), is finer toward the poles (graded condition, Fig. 1), while in others the reticulum is ungraded (Fig. 5). The graded condition is common in *Rhynchosia* (17 of 22 species), but relatively uncommon in *Eriosema* (2 of 9 species). Distinction between adjacent lumina of differing size and shape is either sharp (heterogeneous condition, Fig. 1) or slight (homogeneous condition, Fig. 4). The homogeneous condition is present in three species of *Rhynchosia* but is absent in *Eriosema*. Species of the two genera, except for *Eriosema glabrum*, are separable by the shape of the muri crests, which are obtuse in *Rhynchosia* (Figs. 1, 2), and acute in *Eriosema* (Figs. 5, 6). In addition, the lumina of *Rhynchosia* (Figs. 1–3) are generally smaller than those of comparable regions of *Eriosema* (Figs. 4–6).

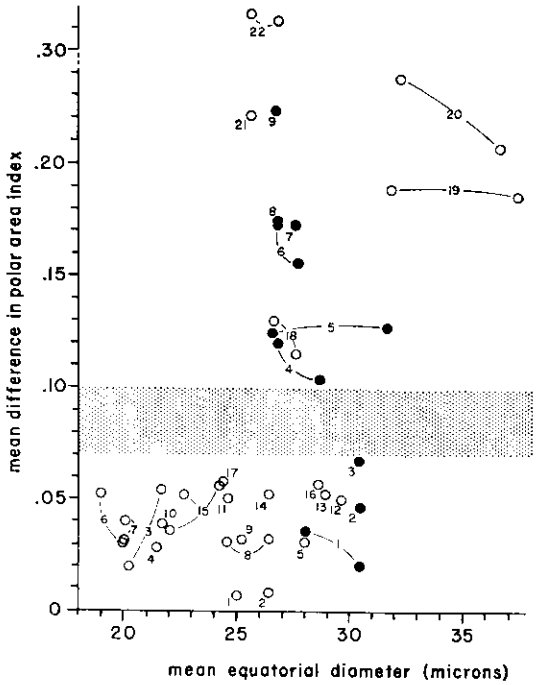


FIG. 7. Scatter diagram of mean difference in polar area index vs. mean equatorial diameter for pollen of *Rhynchosia* (○) and *Eriosema* (●). Species numbers are identified in Tables 1 and 2.

Discussion

While the heteropolar condition in pollen grains has been used as a taxonomic character, heteropolarity in tricolporate pollen has neither been described before nor used as a taxonomic tool, although Faegri and Iversen (1964) and Huynh (1970) describe and use the condition of heteropolarity in tricolpate grains for taxonomic purposes in *Gratiola* (Scrophulariaceae) and in *Anemone* (Ranunculaceae). The taxonomic usefulness of polar indices in this study is that it

TABLE 2

Pollen morphological characteristics of *Eriosema*

Species name	No.	ROM slide No.	$\bar{x}$ difference PAI	Equatorial diam., μ		Reticulum pattern				Lumina shape	
				Mean	Range	Graded	Ungraded	Homogeneous	Heterogeneous	Elongated	Polygonal
E. glaziovii	1	837	0.020	30.4	28–32		x		x	x	
		561	0.036	28.0	26–30		x		x	x	
E. congestum	2	828	0.046	30.4	26–32		x		x		x
E. brevipes	3	829	0.066	30.4	28–32		x		x		x
E. glabrum	4	839	0.104	28.6	26–32		x		x	x	
		661	0.120	26.8	26–28		x		x	x	
E. crinitum var. macrophyllum	5	657	0.125	26.6	26–28		x		x		x
		838	0.128	31.6	26–34		x		x		x
E. rufum var. *rufum*	6	825	0.155	27.6	24–32	x			x		x
		665	0.173	26.6	24–30	x			x		x
E. diffusum	7	755	0.173	27.6	24–30		x		x		x
E. heterophyllum	8	659	0.174	26.8	24–32	x			x		x
E. longifolium	9	759	0.224	26.6	24–32		x		x	x	

provides critical supporting data for species delimitation when other morphological characters are not distinct or are few in number.

In two genera which show a great deal of character overlap and intergradation, it is not surprising to also find some overlap of pollen characters. The two clusters observed in the scatter diagram (Fig. 7) can most probably be explained as parallel evolution within the two genera. The ancestral populations from which the two genera arose were probably eurypalynous (having considerable pollen variation) with respect to polarity, and this condition has been retained in certain species of both genera. Another explanation for the existence of isopolar and heteropolar grains in both genera could be due to occasional intergeneric hybridization. Both genera occupy similar habitats and are sympatric over most of their ranges in Central America, the Caribbean, and South America. Phyletic relationships are difficult to assess because of intricate patterns of phylogeny in both genera. However, knowledge of the pollen polarity of species might eventually help elucidate relationships, both intergeneric and intrageneric.

If the occurrence of both isopolar and heteropolar grains in both genera is due to parallel evolution, this should not detract from the usefulness of pollen in classification, any more so than other characters used in taxonomic studies which are subject to parallelism.

Thus, such information obtained from pollen morphology, when combined with other morphological characters, can be used in delimiting *Rhynchosia* and *Eriosema*.

Acknowledgments

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