Removal and insertion of pollinia in flowers of *Oxypetalum* (Asclepiadaceae) in southeastern Brazil

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Abstract: Pollinarium removal and pollinium insertion of seven Oxypetalum species (O. alpinum var. alpinum, O. appendiculatum, O. banksii subsp. banksii, O. jacobinae, O. mexiae, O. pachyglossum and O. subriparium) were recorded in Viçosa, Minas Gerais. They presented a tendency of one or two pollinarium removals and one pollinium insertion (single insertion), except O. appendiculatum. In this species, mainly, two pollinia of the same pollinarium were inserted per stigmatic chamber (double insertion), resulting exceptionally in 6-10 inserted pollinia in a flower, an unusual occurrence among the Asclepiadaceae. No association between removal and insertion was found, e.g., O. subriparium and O. banksii subsp. banksii had the highest pollinarium removal (1.78 and 1.45, respectively) and one of the lowest pollinium insertions (0.02 in both species), per flower. Oxypetalum mexiae showed the lowest pollinarium removal and pollinium insertion per flower (0.09 and 0.01, respectively) among the studied species and other Asclepiadaceae. Oxypetalum subriparium, O. banksii subsp. banksii and O. mexiae might be having reproductive limitations. Pollinarium removal and pollinium insertion per flower of the studied species varied from site to site, similarly to what was recorded for other Asclepiadaceae.

Key words: Asclepiadaceae, Oxypetalum, pollinarium removal, pollination, pollinium insertion.

Removal of pollinaria (five per flower, each consisting of a corpusculum, two translator arms and two pollinia) and pollinium insertion into stigmatic chambers (five per flower) characterize Asclepiadaceae pollination (Bookman 1981, Kunze 1991), an activity performed by insects (Ollerton and Liede 1997). The amount of pollinarium removal and pollinium insertion, respectively, allow an estimate of insect activity (Willson and Rathke 1974) and pollination success (Liede and Whitehead 1991). Although these numbers are easily obtainable in Asclepiadaceae, data are available on few species (Table 1).

The objectives of this work were: a) to record the amount of pollinarium removal and pollinium insertion in flowers of seven *Oxypetalum* species in the region of Viçosa, Minas Gerais (20°45' S and 42°51' W); and b) to verify the variation degree of removal and insertion, per flower, among species and among individuals of the same species, in different study sites.

MATERIALS AND METHODS

The studied species were found at ten sites, in different habitats, here named S1 to S10 (Table 2); sites were set apart from one another a minimum of 0.5 km and a maximum of 15 km.

All the studied species have climbing habit, flower along the year, except *O. jacobinae* and *O. subriparium*, which flower between November and July (Vieira and Shepherd 1999a). They were identified by a specialist and voucher specimens were

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TABLE 1 Pollinarium removal and/or pollinium insertion per flower in Asclepiadaceae species

Species	Pollinarium removal	Pollinium insertion	Reference
Asclepias quadrifolia Jacq. ¹ A. solanoana Woods. ¹ A. speciosa Torr. A. syriaca L. A. syriaca L. ¹ Cynanchum foetidum H.B.K.	0.64 - 2.23 2.91 - 4.25 - 3.7 2.08 - 2.48 0.87	0.02 - 1.28 1.23 - 1.73 3.21 - 0.81 - 0.82 0.02	Pleasants and Chaplin (1983), Cabin <i>et al.</i> (1991) Lynch (1977) Bookman (1984) Willson and Rathke (1974) Cabin <i>et al.</i> (1991) Liede (1994)
Funastrum arenarium (Decne. ex Benth.) Liede Gonolobus suberosus (L.) R.Br. Matelea reticulata (Engelm. ex A. Gray) Woods. Sarcostemma viminale R.Br. ¹ S. clausum (Jacq.) Schultes S. pannosum Decne. ¹	2.68 0.31 0.94 0.42 -1.64 2.64 2.43 - 2.48	2.01 0.04 0.19 0.2 2.16 0.57 - 1.52	Liede (1994) Lipow and Wyatt (1998) Liede (1994) Liede and Whitehead (1991) Kunze and Liede (1991) Kunze and Liede (1991)

1 Variation in different studied populations.

TABLE 2 Studied species of Oxypetalum, their habitats and sites of occurrence

Species	Habitats	Study sites ¹	
O. alpinum (Vell.) Font. and Schw. var. alpinum	Marsh	S1	
O. appendiculatum Mart.	Pasture and road margins	S2, S3, S4, S5	
O. banksii Roem. and Schult. subsp. banksii	Pasture and road margins	S2, S3, S6	
O. jacobinae Decne.	Pasture and road margins	S7, S8	
O. mexiae Malme	Forest margins, along water stream	S9	
O. pachyglossum Decne.	Marsh	S1	
O. subriparium Malme	Pasture and margins of road and forest	S2, S8, S10	

1 S1 = pasture and neighboring marsh; S2 = Botanical Gardens of the Federal University of Viçosa; S3, S4, S5, S6, S7, S8 = pasture near brushwood located at road margins; S9 and S10 = forest margins (biological reserves).

deposited in the VIC herbarium (M.F. Vieira - 700, 701, 705, 711, 785, 786, 789).

Flowers of each species were collected from the natural population at the senescence stage, throughout 1996-1997 and preserved in 70% alcohol. Removed pollinaria and inserted pollinia were counted under a dissecting microscope.

RESULTS

The *Oxypetalum* species had flowers with one or two pollinaria removed (Table 3); these flowers correspond to about 55% (*O. subripar*- *ium*) to 100% (*O. mexiae*) of those with removed pollinaria.

After removal, the pollinia change their original position due to a rotation of, approximately, 45° (*O. alpinum* var. *alpinum* and *O. pachyglossum*) to 90° of the translator arms (the other species). They are thus brought into a position to be inserted through the anther slits and into the stigmatic chamber.

The species had flowers with, mainly, one inserted pollinium, except *O. appendiculatum* (Table 4); these flowers correspond to about 65% (*O. pachyglossum*) to 100% (*O. mexiae*) of the flowers with inserted pollinia. In these

			5	5 51	1			
Species (no. of individuals)	Flowers examined (no.)		Pollinaria removed (no. of flowers / % of flowers)					Pollinaria removed per flower
		0	1	2	3	4	5	
O. alpinum var. alpinum ¹ (10)	507	317/ 62.52	134/ 26.43	51/ 10.06	4/ 0.79	1/ 0.20	0/ 0.00	0.50
O. appendiculatum (7)	503	189/ 37.57	143/28.43	95/18.89	55/ 10.93	21/4.17	0/ 0.00	1.41^{2}
O. banksii subsp. banksii (7)	510	131/25.69	151/29.61	133/26.10	72/14.12	21/4.12	2/0.39	1.45^{2}
O. jacobinae (3)	533	146/27.40	194/36.40	121/22.70	54/10.13	14/ 2.63	4/ 0.75	1.40^{2}
$O. mexiae^1(3)$	540	491/90.92	48/ 8.89	1/0.18	0/ 0.00	0/ 0.00	0/ 0.00	0.09
O. pachyglossum ¹ (10)	507	174/34.32	143/28.21	106/20.91	48/ 9.47	29/ 5.72	7/ 1.38	1.28
O. subriparium (3)	541	115/ 21.26	127/ 23.47	106/ 19.59	74/ 13.68	62/11.46	57/ 10.54	1.78^{2}

 TABLE 3

 Pollinarium removal in flowers of Oxypetalum species

1 All flowers were collected on individuals of the same site (Table 2).

2 Average of removal per flower on individuals of different study sites (Table 5).

TABLE 4 Pollinium insertion in flowers of Oxypetalum species

Species (no. of individuals)	Flowers examined (no.)	Pollinia inserted (no. of flowers / % of flowers)			Pollinia inserted per flower				
		0	1	2	3	4	5	•	
O. alpinum var. alpinum ¹ (10)	507	408/ 80.47	80/ 15.78	15/ 2.96	4/ 0.79	0/ 0.00	0/ 0.00	0.24	
O. appendiculatum ² (7)	503	239/ 47.51	61/12.13	123/24.45	21/4.17	38/ 7.55	11/ 2.19	1.49 ³	
O. banksii subsp. banksii (7)	510	491/96.27	18/ 3.53	1/ 0.20	0/ 0.00	0/ 0.00	0/ 0.00	0.02^{3}	
<i>O. jacobinae</i> (3)	533	193/ 36.21	227/ 42.59	88/ 16.51	20/ 3.75	5/ 0.94	0/ 0.00	0.82 ³	
$O. mexiae^1(3)$	540	533/ 98.70	7/ 1.30	0/ 0.00	0/ 0.00	0/ 0.00	0/ 0.00	0.01	
$O. pachyglossum^1$ (10)	507	270/ 53.25	155/ 30.57	63/ 12.43	14/ 2.76	5/ 0.99	0/ 0.00	0.68	
<i>O. subriparium</i> (3)	541	532/ 98.34	8/ 1.48	1/ 0.18	0/ 0.00	0/ 0.00	0/ 0.00	0.02^{3}	

1 All flowers were collected on individuals of the same site (Table 2).

2 Flowers with 6, 8 and 10 inserted pollinia correspond to 1.59% (eight flowers), 0.20% (one flower) and 0.20% (one flower) of the total, respectively.

3 Average of insertion per flower on individuals of different study sites (Table 6).

species, except *O. appendiculatum*, the number of inserted pollinia in the flowers corresponded to the number of pollinated stigmatic chambers, *i.e.*, one pollinium was inserted per chamber (single insertion) with more than one pollinium per chamber being uncommon, as observed in few flowers of *O. alpinum* var. *alpinum* and *O. pachyglossum*. These flowers had two inserted pollinia per chamber (double insertion), probably belonging to different pollinaria, since they were isolated.

In *O. appendiculatum*, insertions of two pollinia were observed in 123 flowers (Table 4), corresponding to about 47% of the pollinat-

ed ones, 95% of them (= 117 flowers) having two pollinia, from the same pollinarium, inserted together in a stigmatic chamber (double insertions). In this case, the entire pollinarium was left in the stigmatic chamber. Thus, this species, similarly to the others, had mainly one pollinated stigmatic chamber per flower. Flowers of *O. appendiculatum* with three, four and five pollinia inserted (Table 4) also showed mostly double insertions (respectively, 86% with a double insertion + one single insertion; 95% with two doubles; 91% with two doubles + one single). Thus, flowers with 6-10 inserted pollinia were observed (Table 4). In *O. appendiculatum* double insertions, "pollinaria were inserted" in such a way that the pollinia remained almost entirely outside the stigmatic chamber, just below the anther slits. Only the pollinia section near the translator arms and half of the translator arms were inside the chamber; the remaining pollinaria was outside the chamber. In single insertions, the pollinia remained covered inside the chamber and, in some cases, only their terminal part was visible, just below the anther slits (*e.g.* in *O. banksii* subsp. *banksii* and *O. mexiae*).

After insertion, the translator arm is broken, except in *O. appendiculatum*. Sometimes, the whole pollinarium is left unbroken on the flower, but only one pollinium is inserted with the other, along with the translator arm and the corpusculum, remaining outside the stigmatic chamber (*e.g.* in *O. alpinum* var. *alpinum* and *O. pachyglossum*).

Pollinarium removal per flower was similar, except in *O. mexiae* and *O. alpinum* var. *alpinum* (Table 3). Pollinium insertion per flower varied greatly among the species (Table 4). Pollinarium removal per flower tends to present higher numbers than pollinium insertion, except in *O. appendiculatum* (Tables 3, 4). This species, as explained before, is pollinated with double pollinia in comparison to the other species, and explains in part the results obtained. *Oxypetalum subriparium* and *O. banksii* subsp. *banksii* had the highest number of pollinium removal per flower (Table 3) and one of the lowest pollinium insertions per flower (Table 4). *Oxypetalum mexiae* had the lowest number of removal and insertion of pollinia per flower (Tables 3, 4).

Pollinarium removal and pollinium insertion per flower of *Oxypetalum* varied from site to site (Tables 5, 6). *Oxypetalum subriparium* showed the highest removal variation (Table 5) and *O. appendiculatum*, the highest insertions (Table 6).

DISCUSSION

The results obtained from pollinarium removal in this study are similar to those shown by Liede and Whitehead (1991), with flowers of *Sarcostemma viminale* (L.) R.Br., and by Liede (1994), with flowers of *Matelea reticulata* and *Cynanchum foetidum*. On the other hand, Liede (1994) found that around

Species	Site1 (no. of individuals)	Flowers examined		Pollinaria	
			Total	Removed (%)	Removed per flower
O. appendiculatum	S2 (1)	15	75	26 (34.67)	1.73
	S3 (2)	92	460	145 (31.52)	1.58
	S4 (2)	131	655	205 (31.30)	1.56
	S5 (2)	265	1325	206 (15.55)	0.78
O. banksii subsp. ban	uksii S6 (1)	38	190	49 (25.79)	1.29
	S3 (3)	139	695	245 (35.25)	1.76
	S2 (3)	333	1665	433 (26.01)	1.30
O. jacobinae	S7 (2)	162	810	282 (34.81)	1.74
	S8 (1)	371	1855	392 (21.13)	1.06
O. subriparium	S8 (1)	80	400	48 (12.00)	0.60
-	S10(1)	182	910	526 (57.80)	2.89
	S2 (1)	279	1395	520 (37.27)	1.86

 TABLE 5

 Removal of pollinaria per flower of Oxypetalum species, in individuals of different sites

1 Table 2.

Species	Site1 (no. of individuals)	Flowers examined		Pollinaria	
			Total	Inserted (%)	Inserted per flower
O. appendiculatum	S2 (1)	15	150	19 (12.67)	1.27
	S3 (2)	92	920	192 (20.87)	2.09
	S4 (2)	131	1310	250 (19.10)	1.91
	S5 (2)	265	2650	182 (6.87)	0.69
O. banksii subsp. ban	<i>ksii</i> S6 (1)	38	380	0 (0.00)	0.00
	S3 (3)	139	1390	1 (0.07)	0.01
	S2 (3)	333	3330	19 (0.57)	0.06
O. jacobinae	S7 (2)	162	1620	100 (6.17)	0.62
	S8 (1)	371	3710	383 (10.32)	1.03
O. subriparium	S8 (1)	80	800	1 (0.12)	0.01
	S10(1)	182	1820	1 (0.05)	0.01
	S2 (1)	279	2790	8 (0.29)	0.03

TABLE 6
Pollinium insertion per flower of Oxypetalum species, in individuals of different sites

1 Table 2.

81% of the flowers of *Funastrum arenarium* had three to five removals.

Twisting of the translator arms, after removal of the pollinarium, as observed in the studied species, is commonly seen in Asclepiadaceae species (Bookman 1981, Kunze 1991, Kunze and Liede 1991), although in some of them the pollinia retain their original position (Kunze 1991).

The results obtained from pollinium insertion in this study are similar to those shown by Sparrow and Pearson (1948) and Liede and Whitehead (1991) in flowers of *Asclepias syriaca* and *Sarcostemma viminale*, respectively. The results of the former authors contrast with those of Moore (1947), who found out that *A. syriaca* has mostly from three to five pollinated stigmatic chambers per flower. Liede (1994) found out that two or three pollinated stigmatic chambers per flower were the most frequent condition in *Funastrum arenarium*.

Since the flowers of all the studied species usually had one pollinated stigmatic chamber per flower, only one follicle was produced per flower (Vieira 1998), as observed in other Asclepiadaceae (Sage *et al.* 1990, Liede and Whitehead 1991). However, in *O. banksii* subsp. *banksii*, one inserted pollinium per flower produced two follicles (Vieira and Shepherd unpublished).

In O. appendiculatum, the common double insertions (with pollinia of the same pollinarium and the entire pollinarium left in the flower) and the amount of inserted pollinia found in this work are unusual in Asclepiadaceae and were recorded for the first time. Double insertion seems to be a reproductive strategy, preventing the accumulation of pollinarium remains on the pollinator body (Vieira and Shepherd 1999a); without accumulation, the chances for the insects to correctly catch the pollinarium and to insert the pollinia into the stigmatic chamber might increase. This strategy seems to be favorable for the reproduction of O. appendiculatum, since this species had the highest number of flowers with inserted pollinia. Furthermore, O. appendiculatum is self-compatible with 5.6% of fruit set on individuals of natural population being recorded (Vieira and Shepherd 1999b), a value superior to that commonly found in Asclepias species (average of 1-5%, according to Wyatt and Broyles 1994).

Translator arm breaking after insertion, as observed in the studied species (except in *O. appendiculatum*) is also found in other Asclepiadaceae species (Macior 1965, Bookman 1981, Kunze 1991). In *O. alpinum* var. *alpinum* and *O. pachyglossum*, the remaining pollinarium attached to the pollinator may provide it with an additional appendage, often catching other corpuscula, so that chains of removed pollinaria are formed (Vieira and Shepherd 1999a).

The differences found between number of removal and insertion of pollina per flower, as mainly observed in O. subriparium and O. banksii subsp. banksii, indicate lack of association. The greatest number of floral visitors able to carry pollinaria was recorded on these species (Vieira and Shepherd 1999a), a factor that may have contributed to the removal. However, the visitors seem to be able to remove pollinaria and, sometimes, to insert pollinia, similarly to what was found by Liede (1994) for floral visitors of C. foetidum (Table 1). The pollinium insertion per flower in O. banksii subsp. banksii and O. subriparium was similar to that observed among other Asclepiadaceae (Table 1): Asclepias quadrifolia (Cabin et al. 1991) and C. foetidum (Liede 1994). According to Cabin et al. (1991), A. quadrifolia seems to have reproductive limitations. Results obtained with O. banksii subsp. banksii and O. subriparium also seem to suggest reproductive limitations. Vieira and Shepherd (1999b), using natural populations of O. banksii subsp. banksii, found a 0.8% of fruit set, lower than the lowest mean fruit set found in Asclepias species (Wyatt and Broyles 1994).

Oxypetalum mexiae had the lowest number of removal and insertion of pollinia per flower among the studied species of Asclepiadaceae (Tables 1, 3, 4). This species is endemic to the region of Viçosa (J. Fontella-Pereira pers. comm.) and our results confirm those of Vieira and Shepherd (1999a), *i.e.*, visits to flowers that could result in pollination, are rare. Oxypetalum mexiae also seems to present reproductive limitations. The variation, from site to site, of removal and insertion of pollinia per flower, observed in this study was also recorded for other Asclepiadaceae species (Table 1). These differences may be explained by the greater insect activity at a particular study site (Sparrow and Pearson 1948) and also by temporal changes of the effective pollinators (Fishbein and Venable 1996).

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RESUMEN

Se registró la remoción de polinarios y la inserción de polinios de siete especies de Oxypetalum (O. alpinum var. alpinum, O. appendiculatum, O. banksii subsp. banksii, O. jacobinae, O. mexiae, O. pachyglossum y O. subriparium) en Viçosa, Minas Gerais. Estas presentaron una tendencia de una o dos remociones de polinarios y una inserción de polinios (inserción única), excepto O. appendiculatum. En esta especie, principalmente, fueron insertados dos polinios del mismo polinario por cámara estigmática (inserción doble), resultando excepcionalmente en 6-10 polinios insertados en una flor, una ocurrencia inusual en Asclepiadaceae. No se encontró asociación entre remoción en inserción, e.g., O. subriparium y O. banksii subsp. banksii tuvieron la remoción de polinarios más alta (1.78 y 1.45, respectivamente) y una de las inserciones de polinios más baja (0.02 en ambas especies), por flor. Oxypetalum mexiae mostró la remoción de polinarios y la inserción de polinios por flor más bajas (0.09 y 0.01, respectivamente) entre las especies estudiadas y otras Asclepiadaceae. Oxypetalum subriparium, O. banksii subsp. banksii y O. mexiae pueden estar teniendo limitaciones reproductivas. La remoción de polinarios y la inserción de polinios por flor de las especies estudiadas varió de sitio a sitio, similarmente a lo registrado para otras Asclepiadaceae.

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