

## Dynamics of pollen release in relation to anther-wall structure among species of *Solanum* (Solanaceae)

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**Abstract.** Dynamics of pollen release in relation to anther-wall structure among species of *Solanum* (sects *Brevantherum*, *Cyphomandropsis* and *Pachyphylla*) were studied. Differences in anther-wall qualities (multi-layered and rigid v. single-layered and flexible) were correlated with shifts between vibratory and pneumatic mechanisms. The species presented different exploitation mechanisms for bees to obtain their pollen. The number of replications (strokes on the bellows and buzzing actions) needed to empty the anthers and the total amount of pollen per flower were significantly different among the species. However, the differences were not correlated with differences in structural anther features. The pollination syndrome was related only to the total amount of pollen per flower, which was larger in the pollen flowers. We propose that the pneumatic mechanisms may have played a role simultaneously with the vibratory mechanism as an unclogging device or as a means of increasing male fitness by the pattern of pollen distribution. The pre-existence of the pneumatic principle in pollen flowers must have opened the possibility of pollen deposition on visitors without vibratory capabilities such as a male bee. Parallel to this change a shift in the reward system must have occurred.

### Introduction

Flowers with poricidal anthers, i.e. tubular anthers opening by apical pores, have evolved various mechanisms of pollen release in response to different modes of pollen extraction by pollinators (King and Buchmann 1995). In the case of bee-pollinated flowers, when pollen is the primary attractant, it may be actively obtained by means of vibrations produced by the bees and transmitted to the anthers. Provided pollen is dry and slides easily, the vibrations put the pollen grains in motion, and collision against anther walls increases their kinesis by resonance (King 1993; King and Buchmann 1996). The pollen grains bouncing inside the anthers are reflected on the wall towards the apical parts of the anthers and are then thrust through the pores. This mechanism is well known for many species of *Solanum* and other functionally convergent plants exhibiting the so-called buzz-pollination mechanism (Buchmann 1983). It is a mechanism present in flowers that attract female bees and reward them with pollen.

Among the nearest relatives of buzz-pollinated solanums, an ecologically and functionally different alternative has evolved, namely the bellows-like mechanism. It has been recently described for species formerly included in *Cyphomandra* and now in the sect. *Pachyphylla* (Bohs and Olmstead (1999) included *Cyphomandra* in *Solanum* and

Nee (1999) placed *Cyphomandra* in sect. *Pachyphylla*). In flowers with this new mechanism, pollen is not the primary attractant since it is passively extracted by perfume-collecting male bees not interested in pollen, although pollen-collecting bees such as *Bombus*, *Augochloropsis* or *Xylocopa* may overlap as pollinators in some of these flowers (Sazima *et al.* 1993). Vibrations are not required since a pneumatic physical principle underlies pollen release. Pollen is also dry but anther walls are flexible since they bend at the slightest pressure applied from outside on the sac-like anther thecae. So, part of the air in the anther is blown out while pollen is expelled by the airflow (Sazima *et al.* 1993).

The bellows mechanism has arisen within *Solanum* apparently from buzz-pollinated ancestors (Bohs and Olmstead 1999), involving shifts of pollen reward and anther anatomy (Sazima *et al.* 1993). Species of *Cyphomandropsis* exhibit *Pachyphylla*-like features at anther level and may be brought to release pollen by a pneumatic mechanism (*Solanum stuckertii* and *S. confusum*) or *Brevantherum*-like features, and pollen is obtained by anther vibration (*S. glaucophyllum*, *S. granulosum leprosum*; Passarelli 1998; Passarelli and Bruzzone 2004). In the mature stage, anthers may be either multi-layered and rigid (*S. glaucophyllum*, *S. granulosum leprosum*) or

single-layered and with flexible thecal walls (*Solanum stuckertii* and *S. confusum*; Passarelli 1998; Passarelli and Bruzzone 2004).

Studying the dynamics of pollen release in species of *Solanum* sects. *Pachyphylla*, *Cyphomandropsis* and *Brevantherum*, we explore the functional transition between the pneumatic mechanism and buzz pollination. It is thus possible to consider how the evolutionary shift between buzz pollination by female bees and euglossine pollination by male bees in *solanums* may have happened.

## Materials and methods

The following species, which show contrasting flower syndromes and anther anatomical features, were studied:

### Sect. *Brevantherum*

*Solanum granuloso-leprosum* Dun., Argentina. Prov. Buenos Aires. Near La Plata city, December 16, 1996, Passarelli 124 (LP).

### Sect. *Cyphomandropsis*

*Solanum confusum* Morton, Argentina. Prov. Tucumán. Quebrada de Los Sosa, January 22, 1995, Km 37, road to Tafi del Valle, between El Nogalar and La Heladera. Passarelli 112 (LP).

*Solanum glaucophyllum* Desf., Argentina. Prov. Buenos Aires. Ensenada, road to Rivadavia, December 4, 1991, Passarelli 4–5 (LP).

*Solanum stuckertii* Bitter, Argentina. Prov. Córdoba. Depto. Calamuchita, Falda de Reartes, route 5, 10 Km from Villa Gral. Belgrano, December 2, 1992. Passarelli 8–9 (LP).

### Sect. *Pachyphylla*

*Solanum betaceum* Cav. Argentina. Prov. Buenos Aires. Cultivated in La Plata University Campus, December 10, 1996, Passarelli 123 (LP).

For manual extractions of pollen, either vibrations of a tuning fork or strokes with a forceps on the anther were applied. The amount of extracted pollen was quantified to the nearest 10 µg with a Mettler microanalytical balance. In first- or second-day flowers that had been excluded from visitations, vibratory replications were applied until no more pollen could be extracted. After that point, the bellows mechanism was applied once and then the vibratory replications were continued until the flower was exhausted again. This procedure was iterated until the anthers were completely emptied. The absence of pollen in the anthers was then checked under a dissecting microscope. To study the conditions of the anther wall structure, free-hand or microtome sections were obtained from fresh or FAA-fixed samples of all species included in the study. For the microtome sections, flowers were dehydrated in an ethanol series, embedded in Paraplast, sectioned to 10 µm and stained with safranin and fast-green.

## Results

### Floral syndromes

Among the species studied, the following two character syndromes are represented (see Table 1): (1) *pollen flowers*, which are characterised by yellow connivent anthers emergent from the stellate or wheel-shaped corolla (*S. betaceum*, *S. glaucophyllum*, *S. granuloso-leprosum* and *S. stuckertii*) and (2) *perfume flowers*, with brown anthers each clearly separated from the others and partially enclosed within the bell-shaped corolla. The latter case is exemplified only by *S. confusum*.

### Anther structure

In the external structure, differences are striking between those species bearing a large and fleshy connective that is visible on the anther's dorsal face (*S. betaceum*, *S. confusum* and *S. stuckertii*) and those where the connective is not visible externally (*S. glaucophyllum* and *S. granuloso-leprosum*). In cross-section, the connective is wedge-shaped in the former species group (Fig. 1A, B, H, I) and nearly circular in the latter species group (Fig. 1E, J). Two conditions were found regarding the anther anatomy at the mature stage. (1) In *S. glaucophyllum* and *S. granuloso-leprosum*, the anther walls were built up by either 2 or 4 cell layers all around the thecae (Fig. 1E, F, G, J, K). In *S. glaucophyllum*, all the epidermal cells bore papillae (Fig. 1G, F), which in fresh samples can be seen containing yellow chromoplasts. Anther walls are rigid in both species.

In *S. confusum*, *S. betaceum* and *S. stuckertii* the anther walls are thick near the connective (up to about 10 cell layers) but have a single cell layer, the epidermis, either at lateral and ventral anther faces (*S. confusum* and *S. betaceum*, see Fig. 1B, C) or only on the ventral anther face (*S. stuckertii*, see Fig. 1I). Epidermal cells of lateral or ventral faces are not papillate (Fig. 1C, I). Where the wall is thin, it is flexible to a slight mechanical pressure.

### Amount of pollen produced

The mean total amount of pollen produced per flower was significantly different among the species studied (see Table 1). The pollination syndrome (pollen flowers *v.* non-pollen flowers) was a meaningful factor for the total amount of pollen obtained (ANOVA for total pollen amount by layers, at  $P = 0.05$ ), being larger in the pollen flowers (see Table 1) than in *S. confusum*.

However, this variation was not correlated with different mechanisms of pollen release since there were no significant differences in the mean total amount of pollen between those species whose anthers at the mature stage had a single cell layer and those with many cell layers in theca walls (ANOVA not significant for total pollen per flower, at  $P = 0.05$ ).

### Relation of the vibratile and bellows mechanism with anther-wall structure and pollination syndrome

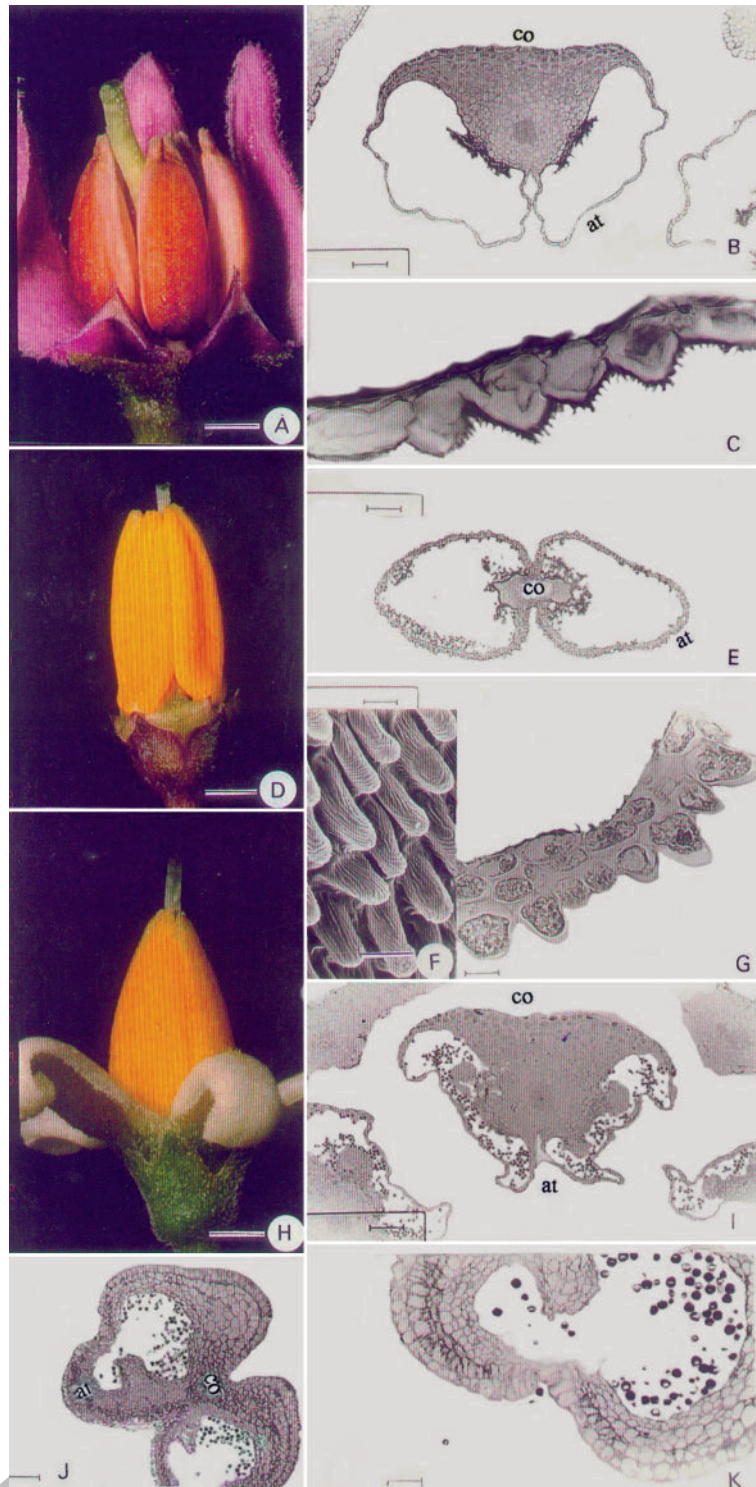
The number of replications (strokes on the bellows and buzzing actions combined) needed to empty the anthers was significantly different among the species studied (see Table 1) and this was correlated with the different structural features of anthers between *Solanum betaceum* and *S. stuckertii*. (ANOVA not significant for replications by cell layers as factor, at  $P = 0.05$ ). However, there were significant differences in the mean number of strokes on the bellows needed to empty the anthers between species with single- and multi-layered thecae (ANOVA significant for number of

**Table 1. Number of replications (strokes on the bellows and buzzing actions combined) needed to empty the anthers**  
*n* = Number of flowers; different letters indicate significant differences (at *P* = 0.05, ANOVA or Kruskal–Wallis): plain letters, *P* < 0.05; bold letters, *P* < 0.01

Species	<i>n</i>	No. of wall layers	Syndrome	No. of replications to empty anthers <sup>A</sup>				100%	No. of bellows	Amount per extraction (µg) <sup>B</sup>	Total amount per flower (µg) <sup>A</sup>
				50%	75%	95%	100%				
<i>S. adelphum</i>	4	One	Perfume	3.50 ± 1.29a	6.25 ± 2.50a	9.00 ± 3.46a	9.50 ± 3.70 a	2.75 ± 1.26	51.07 ± 40.00a	420.00 ± 215.85a	
<i>S. betaceum</i>	1	One	Pollen	7.00b	13.00b	18.00a,b	20.00 b	2.00	44.00 ± 28.73a	880.00a,b	
<i>S. glaucophyllum</i>	6	Many	Pollen	3.33 ± 1.03a	5.00 ± 2.45a	8.00 ± 3.03a	9.67 ± 0.17 a	0.17 ± 0.41	158.97 ± 205.41b	1536.67 ± 818.85a,b	
<i>S. granuloso-leprosum</i>	2	Many	Pollen	1.50 ± 0.71a	4.50 ± 2.12a	10.00 ± 5.66a	11.00 ± 0.70 a	1.00 ± 0.00	76.82 ± 146.46a	845.00 ± 636.4a,b	
<i>S. stueckertii</i>	3	One	Pollen	9.33 ± 1.51b	13.67 ± 1.53b	23.33 ± 4.93b	25.33 ± 4.93 b	2.67 ± 1.53	97.24 ± 85.22b	2456.67 ± 247.05b	

<sup>A</sup> ANOVA.

<sup>B</sup> Kruskal–Wallis test.



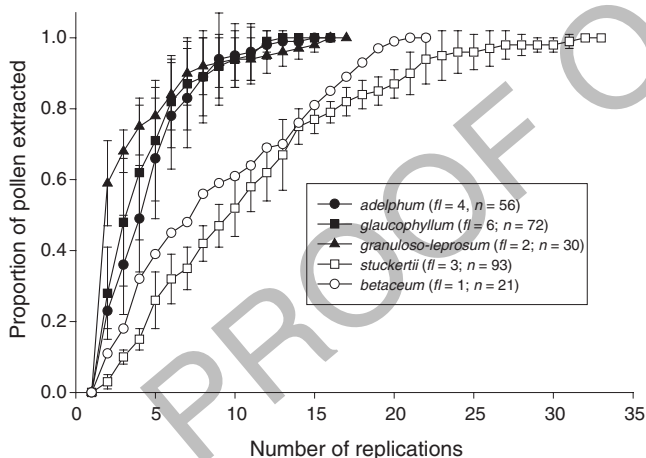
**Fig. 1.** Anther structure. (A–C) *Solanum confusum*. (A) Outer view of the anthers in a fresh flower; (B) mature anther cross-section; (C) epidermal cells of the thecae. (D–G) *S. glaucophyllum*. (D) Outer view of the anthers in a fresh flower; (E) mature anther cross-section; (G) epidermal cells of the thecae; (F) superficial view of the epidermis of thecae, with SEM, papillae. (H, I) *S. stuckertii*. (H) Outer view of the anthers in a fresh flower; (I) mature anther cross-section. (J, K) *S. granulosum leprosum*, mature anther cross-section. Scale bars: 2 mm (A, D, H); 200  $\mu$ m (B, E, I, J); 10  $\mu$ m (C); 50  $\mu$ m (F); 25  $\mu$ m (G); 400  $\mu$ m (K); at = anther thecae; co = connective.

strokes on the bellows and layer as factor, at  $P=0.05$ ). In the species with single-layered thecae (*Solanum confusum*, *S. betaceum* and *S. stuckertii*), the anther pores were blocked after a certain number of replications. However, anthers were not completely empty since extractions could be resumed after this point when a pressure was applied on the thecae, thus putting the bellows-like mechanism in action. The sequence vibratory replication continued by unblocked strokes could be iterated a number of times (1–4 times) until complete exhaustion of the anther.

The anthers of *S. granuloso-leprosum* (which are multi-layered) were temporarily obstructed when the flowers had been emptied about 80%. After strokes were applied they released only a small amount of pollen (10–50  $\mu\text{g}$ ).

In *Solanum glaucophyllum*, the anthers (which are multi-layered) could be totally emptied by vibratory replications and no more pollen could be obtained when strokes were applied on the thecae. However, the pneumatic principle does apply since a certain amount of pollen could be discharged if the bellows was worked before all the pollen had been extracted.

In *S. confusum*, *S. glaucophyllum* and *S. granuloso-leprosum*, the number of extractions (vibratory replications and strokes) needed to completely exhaust the flowers was significantly lower than those in *S. betaceum* and *S. stuckertii*. The same relation held for the number of replications needed to extract 50, 75 and 95% of the available pollen. The rate with which the flowers can be emptied is made visible by plotting the cumulative proportion of pollen extracted per vibratory or pneumatic replication against the number of replications (see Fig. 2). The two species with single-layered walls (*S. betaceum* and *S. stuckertii*) showed patterns distinctive from the other species except for *S. confusum*, also



**Fig. 2.** Cumulative amount of pollen extracted per replication (vibratory and pneumatic), in all the studied species. *fl* = number of flowers, *n* = number of replications.

a species with single-layered thecae, but with similar patterns to those of the many-layered species.

A more detailed picture was evident when curves of exponential decay were fitted for successive amounts of pollen extracted in each replication (Fig. 3A, E). This showed that *S. granuloso-leprosum* had the ‘fastest’ pollen-flow pattern (exponent  $-2.14$ , Fig. 3D), followed by *S. glaucophyllum* (exponent  $-0.36$ , Fig. 3C) and *S. confusum* (exponent  $-0.36$ , Fig. 3A). The slowest to be emptied were *S. betaceum* (exponent  $-0.09$ , Fig. 3B) and *S. stuckertii* ( $-0.05$ , Fig. 3E). Thus, the species with single-layered thecae and flexible anthers tended to be emptied more slowly than species with multi-layered thecae.

The amount of pollen extracted in each replication varied. As a general pattern, most of the replications extracted little pollen, with only very few extracting pollen in larger amounts (generally the first ones). The frequency of pollen release decreased exponentially (Fig. 3F). The mean amount of pollen extracted per replication was significantly larger (Kruskal–Wallis test) in *S. glaucophyllum* and *S. stuckertii* than in other species. These species also exhibited (see Table 1) mechanisms of pollen release different from those of the other species.

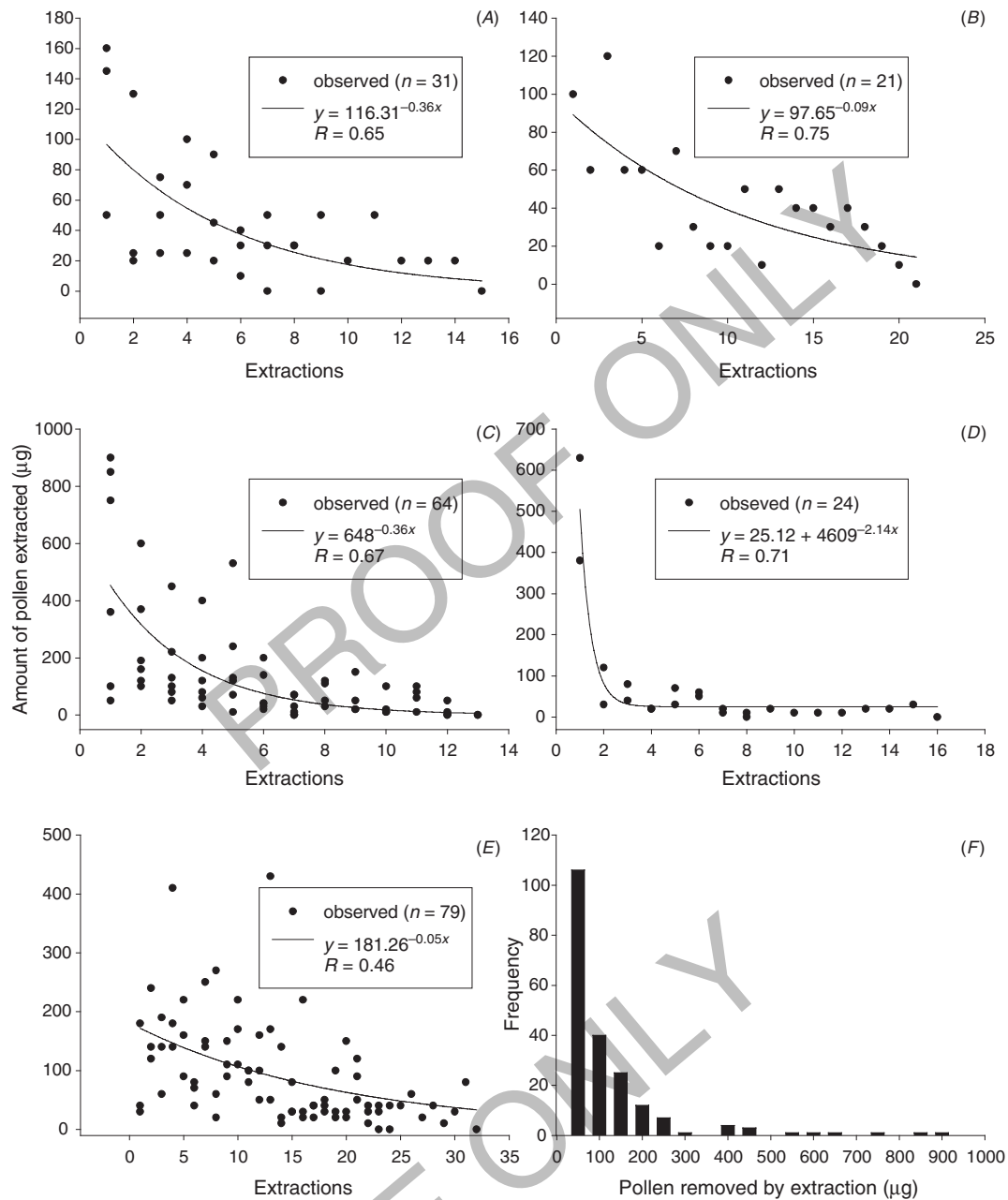
## Discussion

The different mechanisms of pollen release (vibratory *v.* pneumatic) are not linked to pollination syndromes, a fact that was already evident from field observations (Sazima *et al.* 1993). Rather, these mechanisms appear to be related to the dynamics of pollen release. Species with many-layered and rigid anther walls release pollen more readily, *i.e.* a greater proportion of the pollen available in the flower can be obtained in fewer extractions. This points to differences in the mechanical qualities of the two kinds of anther structures. It is possible that the many-layered thecae allow a more energetic bouncing of the pollen against the walls when it is vibrated.

Another important fact is that anthers with single-layered thecae become temporarily clogged and that strokes on the bellows are needed to allow the extractions to be resumed. Thus, in species with single-layer anther thecae that are pollinated by pollen-collecting bees both the pneumatic and vibratory mechanisms may need to be employed.

The mechanical qualities of the anthers also entail consequences in the rate at which pollen is released to pollinators. In this way, pollen could be distributed over more visits. Such a strategy may increase male fitness by maximising pollen dispersal. Pollen available in a single flower is apportioned among many pollinators (see Harder and Wilson 1994).

These differences in mechanical anther functionality can help interpret the major evolutionary shift that took place from buzz-pollinated pollen flowers to purely pneumatically pollinated perfume flowers. In view of the present results,



**Fig. 3.** (A–E) Pollen flow patterns. Curves of exponential decay for the pollen amount extracted in each replication. (A) *Solanum confusum*, (B) *Solanum betaceum*, (C) *S. glaucophyllum*, (D) *S. granuloso-leprosum*, (E) *S. stuckertii*, (F) amount of pollen extracted in each replication and including all the studied species.

we suggest that the pneumatic mechanisms may have played a role simultaneously with the vibratory mechanism as an unclogging device or as a means of increasing male fitness by the pattern of pollen distribution. The acquisition of the pneumatic principle in buzz-pollinated solanums could have opened the possibility of passive pollen deposition on visitors without vibratory capabilities. This in turn would have made possible a shift in reward system from pollen to perfume,

such as is the case in euglossine-pollinated species of the sect. *Pachyphylla*.

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