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Intriguing thigmonastic (sensitive) stamens in the Plains Prickly Pear *Opuntia polyacantha* (Cactaceae)

J. Hugo Cota-Sánchez[a,*], Odair J.G. Almeida[a,b], Denver J. Falconer[a], Hyeok Jae Choi[c], Lewis Bevan[d]

[a] Department of Biology, University of Saskatchewan, Saskatoon, SK S7N 5E2, Canada
[b] Departamento de Botânica, Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, SP 13509-900, Brazil
[c] Department of Biology, Changwon National University, 20 Changwondae-ro, Uichang-gu, Changwon-si, Gyeongnam 641-773, Republic of Korea
[d] Maple Creek Composite High School, Box 369, Maple Creek, SK S0N 1N0, Canada

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ABSTRACT

The movement of sensitive stamens in flowers of the Plains Prickly Pear (*Opuntia polyacantha*) is described in detail along with the external and internal filament anatomy. The goals of this investigation were: (1) to provide a synthesis of floral phenology and determine whether this rather unique stamen movement is nastic or a tropism and (2) to conduct macro- and micro-morphological analyses of filaments to determine if there are anatomical traits associated with this movement. To better understand the internal and external structure in sensitive filaments of *O. polyacantha*, we performed comparative anatomical analyses in two additional species from the Opuntioideae with stamens lacking such sensitivity. The consistent unidirectional movement of stamens, independent of the area stimulated, indicates a thigmonastic response. This movement serves multiple purposes, from enhancing pollen presentation to facilitating cross-pollination, protecting pollen and preventing insects from robbing pollen. Anatomically, the sensitive and non-sensitive filaments exhibit different tissue organization. Cuticle thickness, presence of capsular structures, two layers of curved cells, and more and larger intercellular spaces are characteristic of sensitive filaments. A thin unicellular epidermal layer is characteristic in sensitive filaments versus 2–3 epidermal layers in non-sensitive filaments. Another striking feature in sensitive filaments is the presence of papillae and capsular structures. We believe that these elements are related to water mobility with subsequent contraction during the thigmonastic response. Capsular structures might have a role in fluid mobility according to the stimulus of the filaments. We hypothesize that the thigmonastic response is controlled by cells with elastic properties, as evidenced by the plasmolyzed curved and contracted cells in the filaments and the fact that the movement is activated by changes in cell turgor followed by contraction as a result of plasmolysis.

Introduction

Plants, like animals, respond to different stimuli with responses triggered by a myriad of intrinsic and extrinsic factors, including genetic makeup as well as physiological and natural parameters. The ability to react to a broad spectrum of stimuli is an adaptation of plants to perceive, from their apparent sessile form, signals from the surrounding environment. Plants sense and respond to mechanical perturbation as part of their natural behavior through still poorly understood signaling machinery (Braam, 2005), and, like in animals, a mechanosensory network accounts for the perception of mechanical signals in plants (Telewski, 2006). Recognition systems to identify insect attack leading to an effective early response, triggering compartmentalized defense mechanisms by protecting the injured area, have been proposed in the cactus *Cipocereus minensis* (Wender.) Ritter (Abreu et al., 2012).

Among plant organs, flowers and leaves are more commonly responsive to external mechanical stimuli (Braam, 2005). Flowers from several plant families have evolved sensitive organs, including the filaments of stamens, the pistil, and petals, which are sensitive to touch. The taxonomic and phylogenetic distribution of thigmoresponses (response to touch) is relatively widespread in higher plants exhibiting distinct life styles and ecological requirements, e.g., legumes, orchids, sundews, and others (reviewed in Jaffe et al., 2002). Thigmonasty (non-directional movement independent of the direction of the stimuli/touch, well known in the Venus Fly Trap), thigmotropism (oriented response, turning/bending or growth of an organ upon direct contact in direction of the stimulus,

* Corresponding author. Tel.: +1 306 966 4405; fax: +1 306 966 4461.
E-mail address: hugo.cota@usask.ca (J.H. Cota-Sánchez).

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e.g., growth and movement of tendrils), and thigmomorphogenesis (unilateral growth inhibition, e.g. in *Monstera* vine) are the main types of movements in plants, in addition to thigmo-adaptations involving secretions, trap movements, and projectile release (Jaffe et al., 2002; Braam, 2005).

Thigmonasty relies on turgor mechanisms and action potential of cell membranes, which in turn are related with growth and cell division (Braam, 2005). According to Jaffe et al. (2002), thigmo-mechanisms are adaptations allowing plants to alter developmental rates and changes in morphology, create tropisms, and avoid barriers in response to extrinsic factors. The thigmo-response of stamens has long been known, and the understanding of this staminal response to touch has been greatly enhanced in the last few decades. Already Linnaeus reported this phenomenon in his 1755 version of the Flora Suecica in flowers of *Berberis vulgaris* L. (reviewed in Harms, R. http://www.biosci.utexas.edu/prc/DigFlora/BERB/early-filstim.html). Other early reports dealing with thigmonasty in stamens include that of Bessey (1873) in *Portulaca grandiflora* Hook. and *P. oleracea* L. In 1877, Meehan confirmed Bessey's findings in *P. oleracea* and later described sensitive stamens in *Talinum patens* (L.) Willd. (Meehan, 1878). Other examples exist in the Asteraceae (Halsted, 1889; Smyth, 1898; Pesacreta et al., 1991; Hasenstein et al., 1993).

Stamen movement is an important floral trait determining the fate of pollen and the accuracy of anther contact and pollen transfer with pollinators (Schlindwein and Wittmann, 1997; Pacini and Hesse, 2004). A recent study (Ren and Tang, 2012) recaps stamen movement in plants distinguishing four types, namely cascade or consecutive (Ren, 2010), explosive (Taylor et al., 2006), simultaneous and slow (Du et al., 2012), and stimulated (Schlindwein and Wittmann, 1997). In the Cactaceae, there are several early records of sensitive stamens, including *Echinocactus whipplei* Engelm. & J.M. Bigelow [= *Sclerocactus whipplei* (Engelm. & J.M. Bigelow) Britton & Rose] and *Opuntia rafinesquei* Engelm. [=*O. humifusa* (Raf.) Raf.] (Meehan, 1883), *O. polyacantha* Haw. (Smyth, 1898), and other species of *Opuntia* Mill. (e.g., Toumey, 1899; Britton and Rose, 1923; Porsch, 1938; Bravo-Hollis, 1978). More recently, pollination studies account of other taxa with sensitive stamens in the family, including *Opuntia* species, namely *O. compressa* J.F. Macbr. [=*O. humifusa*], *O. discata* Griffiths [= *O. engelmannii* Salm-Dyck ex Engelm.], *O. lindheimeri* Engelm. [=*O. engelmannii*], *O. phaeacantha* Engelm. var. *discata* (Griffiths) L.D. Benson & Walk. (Grant et al., 1979), *O. ficus-indica* (L.) Mill. (Rosas and Pimienta, 1986), *O. rastrera* F.A.C. Weber (Mandujano et al., 1996), and *O. brunneogemmia* (Ritter) Schlindwein and *O. viridirubra* (Ritter) Schlindwein (Schlindwein and Wittmann, 1997). This phenomenon seems to characterize the genus *Opuntia*, in which two types of positive thigmotaxis, namely stamens moving toward the place of contact and stamens moving toward the style, regardless of the place of contact, have been summarized (Reyes-Agüero et al., 2006).

Staminal movement is an adaptation normally correlated with cross-pollination in plants (Ren and Tang, 2012). It aids in diverse mechanisms of pollen presentation, such as fast and explosive pollen release (Taylor et al., 2006), and both assists (Nagy et al., 1999), and prevents self-pollination (Braam, 2005). It could be argued that stamen sensitivity is a relatively common phenomenon in flowers of several lineages of the Cactaceae, but to date the characterization of these unique sensitive plant structures has been insufficient. Similarly, the role of stamen movement in pollination is not entirely clear. Several assertions have been made in regards to receptive stamens in the cactus family, from the lack of functional role (Troll, 1922) to their involvement in promoting contact with the stigma (Porsch, 1938), facilitating pollen placement onto the insect's body (Rauh, 1979), encouraging self-pollination (Grant and Hurd, 1979; Negrón-Ortiz, 1998), and promoting outcrossing (Vogel, 1983). This movement has also been interpreted as a mechanism to present pollen in the upper anthers while protecting the innermost pollen-rich anthers, a reward available only to the most efficient pollinators able to reach the lower layers of the filaments (Schlindwein and Wittmann, 1997).

Given the controversial role of sensitive stamens in flowers of various cacti and despite the fact that this event has been documented in *Opuntia polyacantha* in the state of Kansas in the U.S. (Smyth, 1898), to our knowledge the occurrence of this phenomenon has remained unnoticed in populations in the northernmost geographic areas of the U.S. and those of southern Canada. This floral aspect is not specifically mentioned by Osborn et al. (1988) in a pollination study of *O. polyacantha* in Colorado. Similarly, Parfitt and Gibson (2003) make no allusion of this event in the cactus flora of North America, nor did Harms (1983) or Cota-Sánchez (2002) refer to its incidence in the province of Saskatchewan; the same seems to be the case for the rest of the Canadian populations. Here, we give an account of the occurrence of sensitive stamens in flowers of the Plains Prickly Pear (*O. polyacantha*) in southern Saskatchewan. Our general objective is to build basic knowledge about this rather unique staminal movement in plants and its putative role in pollination, specifically (1) to provide a synthesis regarding the floral phenology and determine whether the nature of stamen movement in this species is nastic or a tropism and (2) conduct macro- and micromorphological analyses of the filaments to determine whether any anatomical traits are associated with this movement. In order to better understand the internal and external structure of the sensitive filaments in *O. polyacantha* flowers, we performed comparative anatomical analyses of stamen filaments in two additional species from the Opuntioideae, namely *Opuntia cochenillifera* (L.) Mill. (=*Nopalea cochenillifera* (L.) Salm-Dyck) and *Brasiliopuntia brasiliensis* (Wild.) A. Berger, *sensu* Anderson (2001), two taxa with stamens lacking sensitivity. A brief description of flower visitors in relation to their potential role in pollination and triggering the stamen reaction is also provided.

Materials and methods

Study sites

Our descriptions are based on observations of flowers from numerous individuals of *Opuntia polyacantha* made during the summers of 2010–2012. This species is one of the three Saskatchewan native cacti and common in southern areas of central and western Canada (Harms, 1983; Cota-Sánchez, 2002), the northernmost distribution of the Cactaceae. The study sites are found south of the city of Saskatoon, Saskatchewan, Canada, where three populations were selected at coordinates 51°55′57″ N and 106°44′11″ W, 51°46′48″ N and 106°43′18″ W, and 51°57′11″ N and 106°43′21″ W. The populations are found in prairie habitats in exposed areas and slopes with southern and southwestern exposure. This cactus prefers dry alkaline and sandy soils and banks of coulees (Cota-Sánchez, 2002). One individual per population was collected and prepared as representative permanent voucher (SASK 180694, SASK 180695, and SASK 180696) and deposited in the collection of the W.P. Fraser Herbarium (SASK) of the University of Saskatchewan (UofS). The flowers of *Brasiliopuntia brasiliensis* and *O. cochenillifera* were obtained from the Ingá Park, a conservation area inside the city of Maringá, Paraná, Brazil (23°55′29″ S and 51°55′54″ W) in December 2011. Vouchers were deposited at Herbário da Universidade Estadual de Maringá (HUEM 25141 and HUEM 25142), respectively.

Floral attributes of Opuntia polyacantha

The number of *Opuntia polyacantha* flowers inspected in the field and laboratory varied according to seasonal fluctuations and plant

phenology. Whenever possible, at least 30 flowers per population were inspected for stamen sensitivity and brought to the laboratory to further investigate the floral traits. Symmetry, length, diameter of perianth, number of perianth parts, and number of ovules were estimated for each flower with a digital caliper (0.01 mm precision) and recorded immediately after collection. The flower length was taken from the base to the top (including pericarpel, tube and perianth). The terminology to describe the floral morphology was adapted from that of Reyes-Agüero et al. (2006) for *Opuntia*. High-resolution digital pictures were taken and chosen for inclusion in figures. The images and plates were labeled and assembled using the programs Adobe Photoshop CS3 and Corel Photo-Paint X3, version 13.

Measurement of nectar sugar concentration in Opuntia polyacantha flowers

Nectar collection took place after a preliminary exploration of the flowers to locate the nectary and nectar. The nectar collection was scheduled at different times of the day (from 09:00 to 16:00). In view of the small nectar volume produced and in order to prevent nectar concentration changes due to the atmospheric humidity and temperature conditions prevailing in the field, we opted to collect the flowers and place them in plastic containers in a cooler for further analyses in the laboratory, where nectar was collected by gently touching the floral nectary with a micropipette of known volume (1.0–5.0 μL), always in virgin flowers, following Almeida et al. (2012). The nectar was then expelled onto the prismatic surface of a hand refractometer (0–50% and 40–85%; Bellingham and Stanley, Tunbridge Wells, Kent) to determine solute concentrations, measured as percent nectar concentration by weight (% NCW). Nectar was difficult to obtain from most flowers. Readings were performed only in eight flowers, and the mean was then calculated.

Optic and scanning electron microscopy (SEM) of filaments

Macro- and micromorphological observations were made on both fresh and fixed samples of the apical, middle, and basal parts of the filament obtained from flowers of *Opuntia polyacantha*, *O. cochenillifera*, and *Brasiliopuntia brasiliensis*. For anatomical scrutiny with compound microscope, plant material was prepared using a modified version of the protocol in Davis et al. (1998). The fresh stamens were dissected in small sections and then fixed in formaldehyde-acetic acid-alcohol (FAA50), Johansen (1940), for 48 h followed by dehydration using an increasing ethanol-*n*-butanol series (Jensen, 1962), and then embedded in Paraplast® wax in an incubator (60 °C). Tissue sectioning was made with a rotary microtome at 7–12 μm width, and sections were heat-mounted on glass slides, stained with 0.05% toluidine blue O in 20 mmol L^{-1} sodium benzoate buffer, pH4 (O'Brien and McCully, 1981), and then submerged in xylene to remove wax from tissue. Slides were then covered with glass cover slips, sealed with Permount, and analyzed using a Nikon Alphashot 2 YS2 compound microscope.

For SEM analysis the filaments were dissected in small (longitudinal and transversal) sections, fixed in 2.5% glutaraldehyde in buffer solution (0.05 M sodium phosphate, pH 7.2) for 48 h, dehydrated in a graded acetone series to 100%, critical-point dried with liquid CO_2 (Polaron Instruments E3000), and affixed on aluminum stubs following Almeida et al. (2012). After gold coating (Edwards Sputter Coater S150B), different portions of the filaments were examined with a Philips SEM 505 at 30 kV, and micrographs were taken using Polaroid 665 positive/negative film and the Animator DV (image capture) program. The structures were observed in filaments of three different flowers to verify the external characters of the filament epidermis.

Floral visitors

Floral visitors were collected for identification. Their activities, frequency of visits, and behavior were observed during two field seasons. Insect visitors to flowers were captured using standard entomological nets and plastic aspirators and then placed in a killing vial containing a sponge with a few drops of ethyl acetate. The specimens were sent to our local expert (see "Acknowledgements") for identification.

Results

Flower morphology and phenology

Opuntia polyacantha plants are creeping or prostrate with spines, weakly to strongly barbed. The stem segments are round to broadly obovate, mostly over 5.0 cm long, 3.0 cm wide or bigger (Fig. 1), not readily separable; the areoles are 8–10 mm apart; glochids yellow, inconspicuous; spines 6–10, needle-like, 1.0–2.5 cm long. The flowers are yellow to orange-yellow, 2.4–7.0 cm long ($\bar{x} = 4.4$) and 2.1–7.3 cm in diameter ($\bar{x} = 4.4$), with an average of 19 perianth parts, a wide range in stamen number varying from 110 to 361 ($\bar{x} = 222.6$): Table 1. The cream-colored to reddish filaments (Figs. 2 and 3) are arranged in several concentric series (Fig. 4); the outermost series of filaments are larger and gradually decrease in length in proximity to the center of the flower. The ovary length and width ranges from 1.3 to 3.2 cm ($\bar{x} = 2.19$) and 1.4–2.5 cm ($\bar{x} = 1.91$), respectively. The fruit is dry at maturity, spiny, ovoid, tan or brown, producing 5–55 seeds ($\bar{x} = 26$) (Table 1).

The flowers are protandrous, with the stigma lobes remaining closed, indicating the lack of receptivity. On warmer days anther dehiscence can take place concurrently with anthesis, whereas cooler days can delay anther dehiscence until the flower has completely opened. Field observations during the last three summers indicate that, depending on weather fluctuations, particularly hot and sunny and cloudy and cool conditions have an effect on floral phenology and can shift the blooming window one week earlier or later. In general floral buds begin forming the second week of June, and the first flowers open before the end of that month and continue to bloom until the middle of July. That is, there is a maximum of three weeks blooming period. The number of flowers per cladode varies from 1 to 6, with the number depending on the size and maturity of the stem. Flowers sharing the same cladode do not necessarily bloom at the same time, but it can occur. Flower anthesis is short-lived, with flowers opening midmorning (ca. 10:30 am) and remaining open all day, closing late in the evening. Flowers that do not open until later in the afternoon contract slightly overnight and remain open for a portion of the following day and are fully closed by mid-afternoon. The length of this period is also greatly affected by daily fluctuations in temperature, humidity, and cloud cover. In total, anthesis can take place in as little as 12 h (a full summer day) and, in extreme cases, as long as 30 h. Early in anthesis the stamens are clustered around the base of the style, and as the flower opens the stamens do so as well, relaxing into an open state around the style column.

A nectary at the base of the floral tube is characteristic of this species. The nectar is typically transparent and odorless (to human olfactory sense), produced in very small amounts (1.0–2.0 μL) and has a sugar concentration (as percent nectar concentration by weight – % NCW) varying from 25.6–29.5 brix ($\bar{x} = 26.9$): Table 1.

Stamen movement

In order to determine whether the stamen reaction in *Opuntia polyacantha* is a tropism or a nasty, the movement was repeatedly

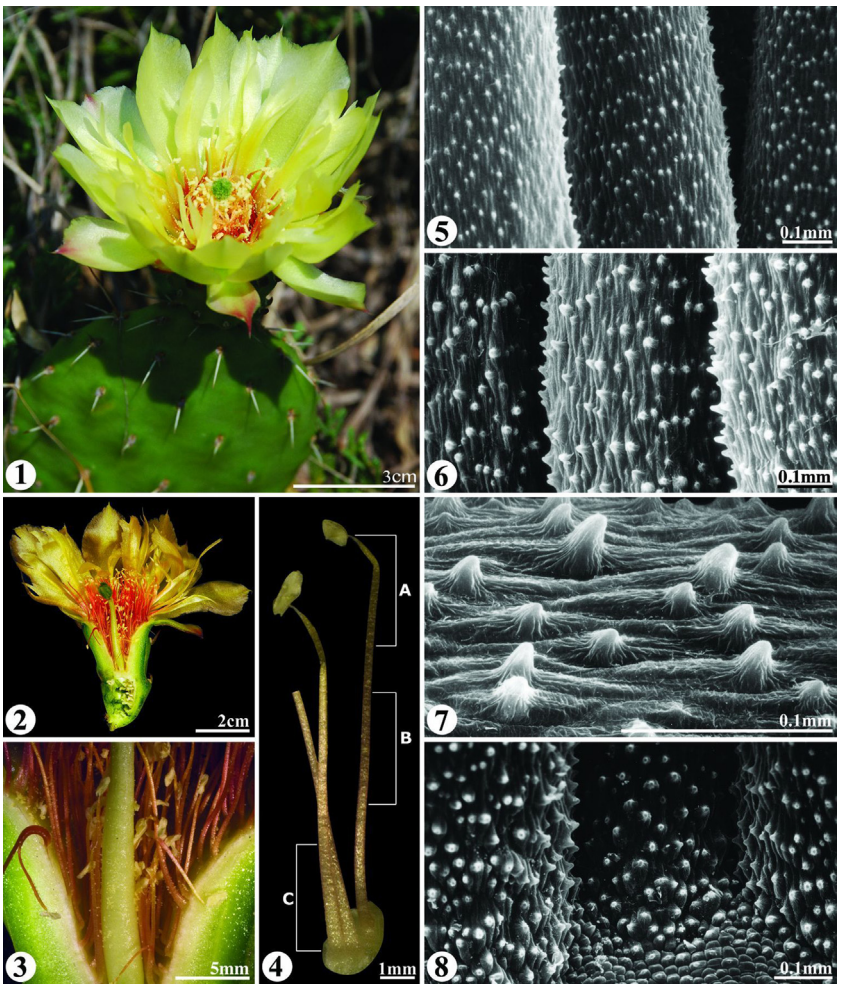

Plate I Figs. 1–8. Overview of floral features in *Opuntia polyacantha* and macro- and micromorphology of staminal filaments. (1) General flower morphology. (2) Longitudinal section of flower. (3) Close-up of base of floral cup showing the basal potion of the style and inner filaments. (4) Stamens removed from flowers. (5) SEM micromorphological view of the upper portion of filament with small papillae (region A in Fig. 4). (6) SEM micromorphological view of the middle portion of the filament with larger size papillae (region B in Fig. 4). (7) Close-up of papillae on filament (region B in Fig. 4). (8) SEM micromorphological view of papillae of the basal region of the filament (region C in Fig. 4).

observed in the same flower when potential pollinators visited and also when the same flowers were manually stimulated. The behavior of the stamens and visiting insects was further recorded with video camera. Four short video clips showing stamen movement in *O. polyacantha* flowers are available at http://www.usask.ca/biology/cota-sanchez/lab/sec/video.html. We used these video sequences to further examine and repeatedly verify the reaction of the stamen filaments in the flowers described here. Newly opened, virgin flowers were also mechanically stimulated to compare the action of the stamens (see video series 1–4).

The effects of various devices were used to test the response of the filaments. When the stamens of newly opened, intact flowers were mechanically stimulated by objects such as a small twig (see video series 1, 2), pencil, or with a finger, either in the inner side of the filament facing the base of the style or on the outer side of filament (away from the style), the response was always in the same direction, i.e., inwards and toward the central part of the flower (style). This consistent unidirectional movement, independent of the area stimulated, led us to classify the response in the stamens of *O. polyacantha* as thigmonastic. In general and when the stimulus was applied in the inner part of the androecium, the staminal response varied among different flowers from 8 to 10 up to 20 s but always occurred within 1 s after the stimulus, and the longer the elapsed time for the filaments to cluster, the older the flower was. The stamens continued the movement inwards, and at the end of the movement the filaments and anthers densely grouped around

Table 1
Range of variation and mean values of floral attributes in three populations of *Opuntia polyacantha*. Number of flowers/population: $n = 30$; number of fruits/population: $n = 10$.

	Flower length (mm)	Flower diameter (mm)	No. of perianth parts	No. of ovules per flower	No. of seeds per fruit*	No. of stamens	Nectar sugar concentration
Range Pop. 1	(33.9–57.3)	(27.1–59.6)	(14–34)	(0–74)	(16–45)	(110–307)	26.3 brix 0.2898 mg/μL
Mean value	45.0	47.2	19.4	37.4	27.9	213	$n = 3$
Range Pop. 2	(34.2–70.1)	(21.4–72.5)	(16–26)	(19–57)	(10–50)	(120–361)	29.5 brix 0.3306 mg/μL
Mean value	44.8	44.3	19.2	40.5	26.5	236.9	$n = 2$
Range Pop. 3	(24.2–56.7)	(22.3–66.8)	(15–22)	(0–58)	(5–55)	(126–298)	25.6 brix 0.2837 mg/μL
Mean value	42.0	41.0	18.3	33.4	24.4	217.9	$n = 3$
Grand average (three pops.)	43.9	44.2	18.9	37.1	26.3	222.6	26.9 brix 0.2977 mg/μL

the base of the style. During this response, the tip of the outermost stamens bent toward the center of the flower, just below the stigma lobes covering the anthers of the lower stamens, which are shorter than the outer ones. The stamens remained clustered at the base of the style column and began relaxing about 6–8 min after the stimulus; the filaments returned to normal, relaxed position within approximately 20 min. When a second stimulus was applied to the same flower, the movement was equally fast in relation to the first stimulus. However, as anthesis proceeded the filaments moved more slowly to the extent that movement ceased, and finally when all the anthers had dehisced, the anthers rested in a clustered position, marking the end of anthesis. A similar pattern of thigmonasty occurs in *Opuntia microdasys* (Lehm.) Pfeiff., a close relative of *O. polyacantha*, but stamen movement in the former is slower, with the time elapsed in the filament contraction ranging from 30 to 36 s (Cota-Sánchez and Choi, pers. obs.). Normally, stamen movement is more active in virgin and recently opened flowers and seems to be correlated with sunlight and temperature; that is, filaments move relatively faster in sunny, warm conditions as opposed to cloudy, cold and rainy days, in which the response is delayed.

Macro- and micromorphology of stamen filaments

Since the stamen filaments in the flowers of *Opuntia cochenillifera* and *Brasiliopuntia brasiliensis* are not sensitive, comparative observations were relatively straightforward. We expected to find structure differences in the filaments of stamens in flowers having the thigmotactic response versus the filaments of anthers in flowers lacking the ability to respond to touch. Foremost, analyses of the external surface of the sensitive filaments of *O. polyacantha* reveal that the filament has a continuous and thick cuticular layer with numerous papillae distributed from the base to the top of the filament (Fig. 4). Larger size papillae are present from the middle to the lowermost basal portion of the filament (Figs. 5–8). The area around an individual papilla exhibits some longitudinal folding, which increases the filament surface area (Figs. 6–8). An equivalent pattern in the presence, size and distribution of papillae exists in sensitive filaments of *O. microdasys* (Cota-Sánchez and Choi, pers. obs.).

Internal anatomical analyses of the *Opuntia polyacantha* filaments indicate that the papillae are unicellular and a thick cuticular layer covers the external periclinal cellular wall of the filament (Figs. 9–12). There are several multicellular extensions forming capsule-like structures made of epidermal cells located on the outside or periphery of the filament cylinder (Figs. 9 and 11). These capsular structures are lined by cells with a thin, single-layer epidermis and are filled with extracellular compounds (Figs. 10 and 12). The filament's parenchyma consists of four to five layers of large cells containing calcium oxalate crystals, similar to those observed at the base of the papillae, and large intercellular spaces (Figs. 11 and 12). In the second and third layers the cells are evidently curved and/or contracted and appear in the microscopic sections plasmolyzed (arrows in Fig. 9). This latter may be a result of water loss and could indicate that the filament was under tension. It should be noted that the filament experienced a contractile reaction when the flower was collected, sectioned, and fixed, and that the non-stimulated condition is difficult to obtain because touching immediately provokes the reaction. In *Opuntia polyacantha* each filament is traversed by a single amphicribral vascular bundle, i.e., phloem surrounding xylem (Fig. 11).

The epidermis of the insensitive filaments of *O. cochenillifera* and *Brasiliopuntia brasiliensis* is made of one layer of elongate cells with smaller single papillae and a thin cuticle layer (Figs. 13 and 14). The parenchyma in these two species is more compact. That is, the cells are smaller with compacted tissue and reduced intercellular spaces (Figs. 13 and 14) than those of *O. polyacantha*. Concisely, the sensitive filament tissue of *O. polyacantha* is loosely organized with thin-walled capsule-like structures on the outside, while the non-sensitive filaments of *O. cochenillifera* (Fig. 13) and *B. brasiliensis* (Fig. 14) flowers have dense parenchyma and lack capsular structures in the external layer.

Insect visitors

We also asked whether the staminal movement was, perhaps, a response to visiting insects. Visitors to *Opuntia polyacantha* flowers include a relative broad diversity of insects (Figs. 15–23), such as bees, including honeybees, bumblebees (Apidae), sweat bees (Halictidae), mining bees (Andrenidae), and bee flies (Bombyllidae), in addition to small beetles and ants. The Halictidae bees include *Agapostemon* sp., a striking metallic green bee (Figs. 15–17) and *Lasioglossum* sp., a medium-size sweat bee (Figs. 18–20; see also video series 3). These two insects collect both nectar and pollen, the latter in small baskets located in the tibia of the hind legs (Figs. 17, 20). The bee flies consist of *Systoechus vulgaris* (Bombyliidae), a species with a characteristic "stinger" sticking out the front (Fig. 21), bumblebees (*Bombus* sp.), and honeybees (*Apis* sp.), species (see video series 4), widely distributed in North America. A receptive response occurred only when medium- to larger-sized insects (>5 mm), with larger body mass, such a Halictidae and honeybees, visited the flower (discussed in next section).

Beetles were the most common insects visiting *O. polyacantha* flowers. These animals quickly move and converge at the base of the floral cup but never make contact with the stigma. Among these are the flower-feeding Cerambycidae beetles (*Batyle* aff. *suturalis*, Fig. 22), Nitidulidae sap beetles (*Carpophilus* aff. *pallipennis*), and the Mordellidae tumbling flower beetles. The ants, in turn, patrol the external parts of the perianth, accidentally enter the flowers, and move around the tepals but rarely make contact with the anthers. Ants are attracted to these flowers by minute drops of nectar secreted by the bracts of the pericarpel, which latter hence are acting as extranuptial nectaries. Other unusual visits included two unidentified species of small butterflies and the clearwinged grasshopper (*Cannulla* sp. – Fig. 23 left), which did not spend much time patrolling the flowers.

Discussion

Two types of stamen movements (positive and negative) exist in flowers of *Opuntia* (Reyes-Agüero et al., 2006). Several ideas have been proposed explaining the staminal reaction in the Cactaceae. For example, the movement toward the area stimulated promotes contact with and pollen transfer to the insect body (Toumey, 1895), and this contact prompts insect to visit another flower, landing directly on the stigma rather than on the stamens (Grant and Hurd, 1979). Further, the movement and clustering of stamens toward the style discourages pollen-robbing insects from foraging on lower anthers (which are richer in pollen and covered by the upper anthers) because the shorter stamens remain covered by the anthers of the outer series of longer stamens (Schlindwein and Wittmann, 1997). As indicated, the stamens of *Opuntia polyacantha* exhibit thigmonasty because they always move in the same direction regardless of the area stimulated. Based on our observations, we believe that the aggregation of stamens around the style in flowers of this cactus is part of the mechanism for pollen presentation and an adaptation to force insects to land on perianth parts from which they then crawl to the base of the floral cup to either feed on pollen or collect nectar from the floral nectary, in addition to preventing robbing insects from feeding on pollen. In our view, the fundamental role of this reaction is cross-pollination, with some degree of pollen transfer

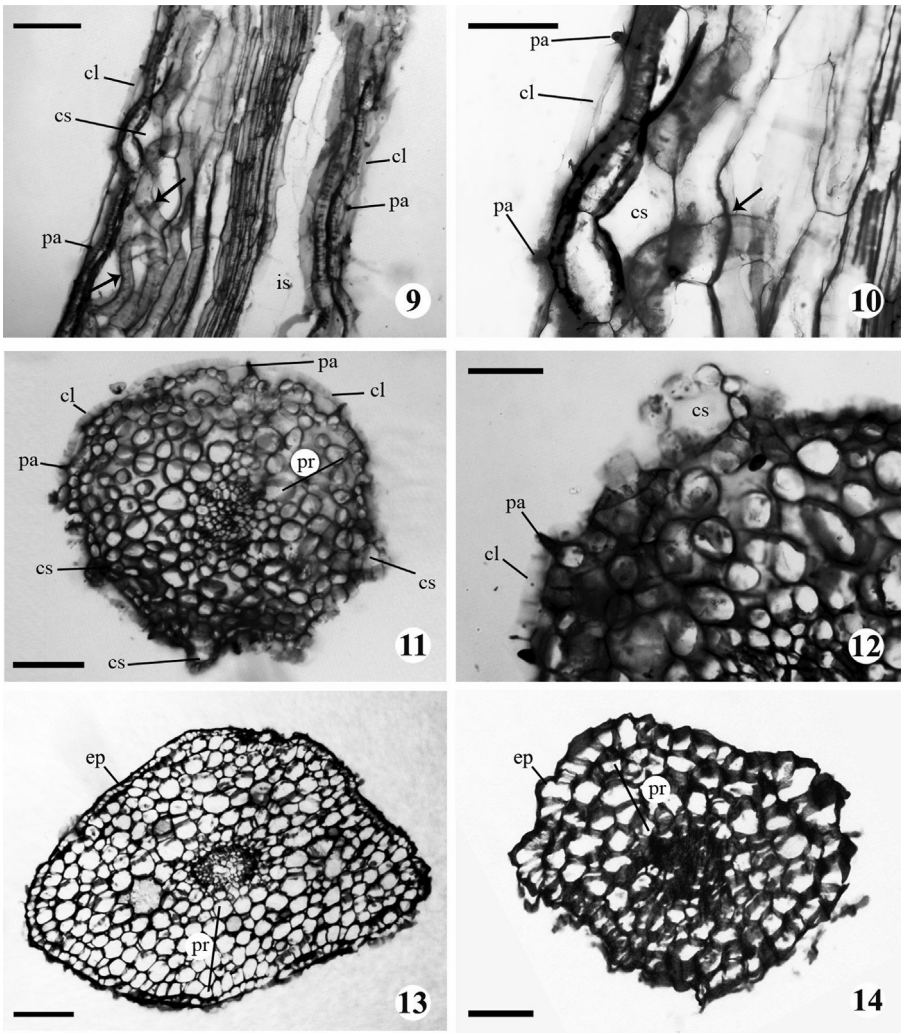

Plate II Figs. 9–14. Anatomy of the filament of *Opuntia polyacantha*, *O. cochenillifera*, and *Brasiliopuntia brasiliensis* in cross and longitudinal sections. (9–12) Sensitive filament of *O. polyacantha*. (9) Longitudinal section of filament (arrows indicate plasmolyzed cells). (10) Detail of epidermis region in longitudinal section (arrow indicates plasmolyzed cells). (11) Cross section of staminal filament. (12) Detail with capsule-like structures and papillae in cross section. (13) Cross section of non-sensitive filament in *cochenillifera*. (14) Cross section of non-receptive filament in *B. brasiliensis*. cl = cuticular layer; cs = capsule-like structure; is = intercellular space; pa = papillae; pr = parenchyma. Scale bars: 100 mm (Figs. 9–11, 13); 50 mm (Figs. 12 and 14).

to conspecific stigmas. This conclusion was reached based on the behavior of the main pollinators as described next.

Another remarkable characteristic of the staminal filaments of *Opuntia polyacantha* is that they are not susceptible to contact by insects less than 5 mgin weight, in this case, the small cerambycid (Fig. 22) and nitidulid beetles, common flower visitors with an average weight of 1 mg. Hence, the lack of staminal response to minute visitors is apparently a function of insect body mass. A nonexistent response to small visiting insects may be another adaptive feature of this flower.

The magnitude of and sensitivity to the thigmo-stimulus vary significantly among plant groups. For instance, epidermal cells with thin cell walls in pea tendrils are more susceptible to perturbation (Jaffe et al., 2002). Also, thigmomorphogenesis has been documented in the development of woody habit in *Arabidopsis*, in which application of weight on the stem induced secondary growth in this herbaceous plant, suggesting that self-weight plays a significant role in the development of the woody growth habit (Ko et al., 2004, reviewed in Telewski, 2006).

Our observations indicate that insects triggering stamen movement in *O. polyacantha* had a body size >5 mm and larger weight, e.g., bumblebees and halictid bees (Figs. 16, 17, 19 and 20), than the most common visitors, the small beetles (Fig. 22). These bees tend to land on the tepals and, after patrolling the perianth, move to the anthers and then push deep into the base of the filaments, quite likely feeding mainly on pollen and the scanty nectar. This continuous bee activity stimulates and triggers the movement until all the stamens cluster around the style. The thigmonastic response occurs when they plunge and start exploring the inner and basal portion of the androecium (filaments). The bees prepare to leave the flowers, moving around the anthers first, and then make direct contact with the stigma lobes, which occurs only when they take off (see video series 3). This insect behavior has been repeatedly observed in various flowers. High pollen loads are common in hind legs of bee foragers (Figs. 17 and 20). Thus, we suggest that these small- to medium-sized halictid bees, a cosmopolitan family consisting of insects varying from 4 to 8 mm and pollinating a wide range of flowering plants (Michener et al., 1994), are the major pollinators in flowers of *O. polyacantha* in these populations. In general, the thigmo-movement forces insects to walk around the mass of anthers and then to penetrate to the base of the floral cup to collect nectar/pollen. As they leave the flowers, they make contact with the stigma, thereby contributing to pollination (see video series 3 and 4). Medium- to large-sized bees are attracted by

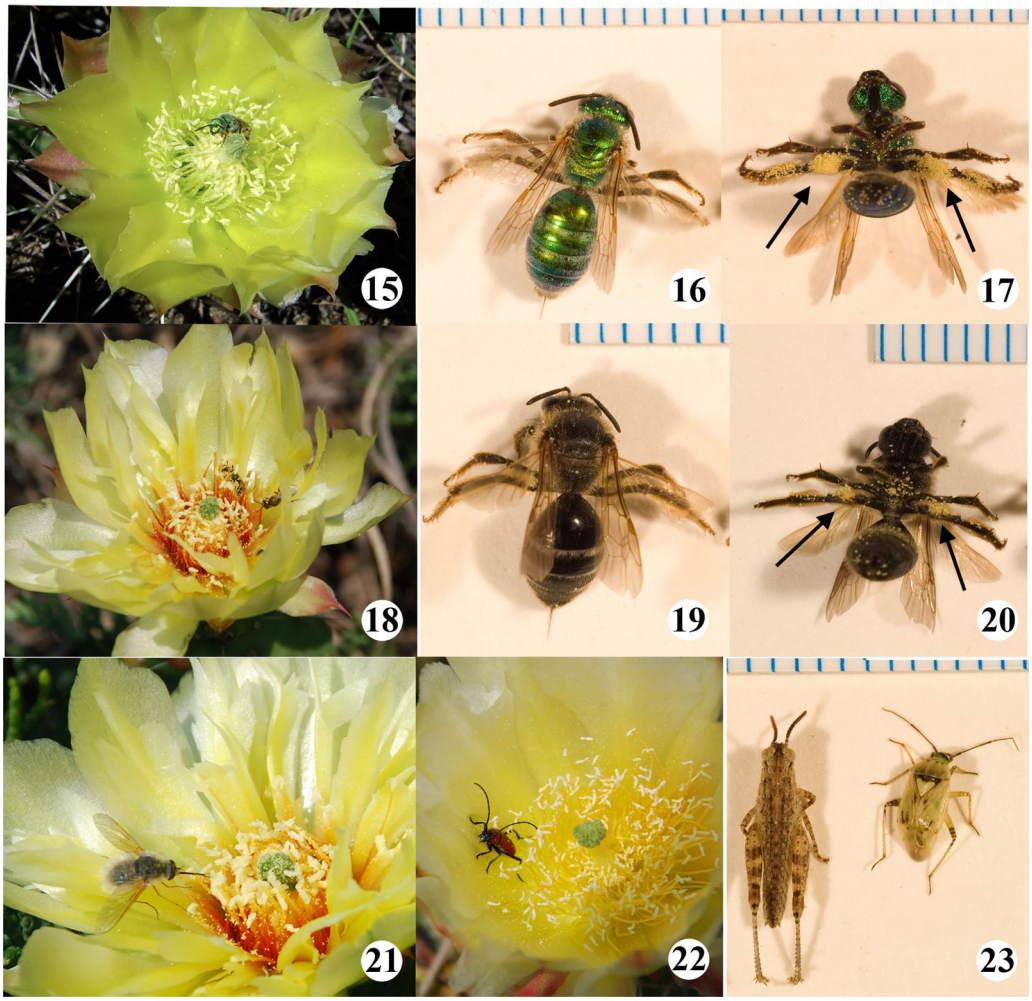

Plate III Figs. 15–23. Major visiting insects in flowers of *Opuntia polyacantha*. (15–17) *Agapostemon* sp., a sweat bee. (15) *Agapostemon* sp. visiting flower. (16) Dorsal view of *Agapostemon* sp. (17) Ventral view of *Agapostemon* sp. (arrows indicate pollen baskets). (18–20) *Lasioglossum* sp., a sweat bee. (18) *Lasioglossum* visiting a flower. (19) Dorsal view of *Lasioglossum* sp. (20) Ventral view of *Lasioglossum* sp. (arrows indicate pollen baskets). (21) A bee fly (*Bombylius* sp.) visiting flower. (22) A flower-feeding cerambycid beetle, *Batyle* aff. *suturalis*, patrolling the flower. (23) A clearwinged grasshopper (*Cannulla* sp.) and an unidentified floral visitor.

large flowers (Grant and Hurd, 1979) and are also considered the effective pollinators of *O. polyacantha* in southern Colorado (Osborn et al., 1988) and in two other species of *Opuntia*. Medium- to relatively large-sized oligolectic bees are also capable of pollinating the flower and exploiting the lower pollen-rich stamens (Schlindwein and Wittmann, 1997). Lastly, the beetles, though much more common than any other insect visiting *O. polyacantha* flowers in the three populations investigated, seem not to play a significant role in pollination. These insects crawl into the flower and plunge directly into the androecium, moving around filaments and anthers while they feed/rob pollen but never make contact with the stigma. Similar beetle behavior was documented in *O. rastrera* (Mandujano et al., 1996). Grant and Connell (1979) referred to this as a one-sided beneficial relationship between these insects and the cactus flowers because such insect visits do not result in pollination (Grant and Hurd, 1979; Grant et al., 1979). Ants, in turn, sporadically visit the inner part of the floral cup and go directly to the floral nectary, but since nectar production is limited, they move to the scales of the flower pericarpel with the extranuptial nectaries. Small nectar amounts were also recorded in populations of *O. polyacantha* in Colorado (Osborn et al., 1988).

In general, our study provides insight into the potential pollen and nectar reward in connection with the insects visiting the innermost and central part of the flower, where both the nectar and anthers with richer pollen are located. The retractable movement of stamens functions as a response to protect pollen from non-pollinating agents and robber insects and, at the same time, provides an important incentive for successful pollinators. The stamen movement in *Opuntia polyacantha* described here is equivalent to that mentioned by Schlindwein and Wittmann (1997) for *O. brunneogemmia* and *O. viridirubra*. We believe that this response has an adaptive significance related to cross-pollination in *O. polyacantha*. To explain this idea, it is necessary to consider the flower phenology of this species. Foremost, even though insects take off from the stigma and potentially deposit pollen from the same flower, putatively favoring selfing, autogamy may not necessarily be operating in this species because the flowers are protandrous. Protandry, characteristic of *O. polyacantha* flowers, is a potential mechanism to avoid selfing, as the stigma lobes remain closed in an apparently non-receptive phase during the stamen dehiscence. The early anther dehiscence coinciding with closed, non-receptive stigma lobes suggests that the lack of synchrony in mature reproductive structures is an adaptation to prevent autogamy. In addition, preliminary pollination experiments in the investigated three populations show that seed set in artificially cross- and self-pollinated bagged flowers is higher in the former than in the latter flowers. Average seed set in all fruits was much lower than in control flowers that were not subjected

to bagging. Hand cross-pollinated flowers show the largest average seed set at 26 seeds/fruit and also had the fewest number of fruits that were unable to produce any seeds. Self-pollinated and un-pollinated bagged flowers produced on average less than one seed per fruit, which was corroborated by the very low frequency at which seed set occurred, with three and one, respectively (Falconer and Cota-Sánchez, unpub. data). Succinctly, flowers greatly benefited from increased and diverse pollen deposition, leading to a larger number of seeds produced. This leads to the idea that outcrossing is integral in seed production in *O. polyacantha*, and as such, the pollination process provides an important service to both plants and pollinators. Populations of *O. polyacantha* in Colorado also exhibit an out-crossing breeding system, a correspondence in number of stamens (average of 265), and relatively large floral characters (Osborn et al., 1988) to those observed in the Saskatchewan populations investigated here, further supporting the notion that larger floral parts and numerous stamens and pollen are correlated with allogamous flowers (Cruden, 1977). Based on all this information, it makes sense to consider that the movement in filaments of *O. polyacantha* (and perhaps other species with sensitive stamens) serves multiple purposes, from enhancing pollen presentation, to greatly facilitating cross-pollination (and possibly some selfing), to protecting pollen and preventing insects from robbing pollen.

Anatomically, the sensitive and non-sensitive filaments exhibit different patterns in the organization and tissue arrangement. Cuticle thickness, presence of capsular structures, two layers of cells curved, and more and larger intercellular spaces are characteristic of sensitive filaments. The density of the cells in the parenchyma and the epidermis of *Opuntia polycantha* is greatly reduced (Figs. 9–12) compared to that of *O. cochenillifera* (Fig. 13) and *Brasiliopuntia brasilienis* (Fig. 14). This increase in intercellular space among cells of the filaments coupled with the larger rounded cell structure produces an opportune anatomical arrangement to support rapid osmotic flow and pressure among filament tissues. As noted in Jaffe et al. (1977), the rapid efflux of water is crucial in the directionally independent movement in filaments of *Portulaca grandiflora*. The slow recovery time and unresponsiveness of triggered filaments in *O. polycantha* is indicative of osmotic potential recovery within the cells. We observed this reloading time becoming more prolonged as the flower came to the end of anthesis, which was concomitant with the flower dehydrating. Water facilitates the movement of K^+ ions in and out of the vascular bundle, which are required for extension of filaments of *Lilium* (Heslop-Harrison et al., 1987). This leads us to believe that the movement witnessed in *O. polycantha* depends on a similar mechanism. Thin cell walls and/or only one single layer of cells have been noted in epidermis and hypodermis in sensitive areas of reproductive structures of various plants (Uphof, 1923), and changes in cell shape are characteristic of motor cells with thin cells walls in the extensor structure of sensitive pulvini (Fleurat-Lessard, 1988). Within this is conform that a unicellular epidermal thin layer is characteristic of the sensitive filament on *O. polyacantha*, as opposed to the 2–3 epidermal layers of non-sensitive filaments of *O. cochenillifera* and *B. brasiliensis*. The different anatomy of sensitive and non-sensitive filaments suggests a strong importance of the presence of such thin-layered structures for the thigmonastic response in filaments of *Opuntia*.

The presence of papillae and capsular structures throughout the stamen filament is another interesting feature in *Opuntia polyacantha* flowers, and there are clear structural differences compared with *Brasiliopuntia brasiliensis* and *O. cochenillifera*. The density of papillae throughout the epidermis, presence of capsular structures, layers of bended cells, and loose arrangement of the parenchyma are anatomical features of *O. polyacantha* filaments but not in those of *B. brasiliensis* and *O. cochenillifera*. We suppose that these elements are related to water mobility, with subsequent contraction during the thigmonastic response, and that the capsular structures might have a role in the fluid mobility, i.e., storing and releasing it according to the stimulus of the filaments. In order to explain this, we compared the anatomical profile of the filament of *O. polyacantha* in longitudinal section with that of a thistle (*Cirsium horridulum* Michx.), another species with thigmonastic stamen movement (Pesacreta et al., 1991) and one of the few taxa for which anatomical studies of sensitive filaments have been made. We found remarkable similarities in the arrangement of cortical cells in the filaments of both species. According to Pesacreta et al. (1991, page 177), some of the cortical cells undergo plasmolysis and decrease cell turgor with the contraction of the filament, as seen in Fig. 4b there (page 177). Comparable elongated contracted cells are evident in the filament of *O. polyacantha* (Figs. 9 and 10 arrows). The shape change may be associated with the movement. By analogy, we can assume that the mechanisms of the movement in sensitive stamens of *O. polyacantha* are related to those recorded for this thistle. Hence, we hypothesize that the thigmonastic response in *O. polyacantha* stamens is controlled by cells with elastic properties and that the movement is activated by changes in cell turgor followed by contraction as a result of a kind of plasmolysis response. The epidermal cells of the cactus filament are also very thin; however, the low thickness of the epidermal cell walls is not localized on a particular side of the filament, even though these structures experience bending in a single direction, always toward the center of the flower. Similarly, the organization of the parenchyma tissue in the filament does not display differential organization in different sides (internal/external). The mechano-reception hypothesis (Telewski, 2006) would be most effective in this case when referring to the cascade signaling between adjacent cells within the filament. In our case, the response is identical regardless of position of the stimuli. It is only the strength of the initial contact that initiates the response in the filament. This signal would need to be broadcast along the entire length of the filament to facilitate the movement, which would require a contraction of the adaxial epidermis and an extension of the abaxial epidermis, and the anatomy of these filaments as well as the contraction of internal cells (Figs. 9 and 10) seems to be congruent with this method of movement in *O. polyacantha*.

The occurrence of thigmo-events in phylogenetically distant plant groups conspires against the full understanding of these unique responses. Although this topic is beyond the objectives of this paper, we briefly will address some issues concerning thigmonasty in the stamens of *Opuntia.* According to various studies, the physiological bases of thigmo- and tropic responses in the movement of stamen filaments in flowers involve action potentials (Bünning, 1959; Sinyukhin and Britikov, 1967; Simons, 1981; Braam, 2005), a cuticular elastic mechanism (Pesacreta et al., 1991), and elastic, reversible changes (Fleurat-Lessard, 1988; Hasenstein et al., 1993). Physiological processes controlling stamen movement involve ATP in *Portulaca grandiflora* (Jaffe et al., 1977) and different ion concentrations and exchange at the cellular level in *Berberis vulgaris* (Lechowski and Bialczyk, 1992). Further, plant thigmomechanisms require integrin-like proteins, a possible formation of Hechtian strands, and appropriate cytoskeletal structures as potential signal transduction components (Jaffe et al., 2002), and a mechanosensory network will be responsible for the perception of a wide array of mechanical signals (Telewski, 2006). It is unknown whether, and how in detail, any of these processes are involved in the sensitivity of *O. polyacantha* filaments. At this time we can only speculate that the stamen movement in this species is the result of an orchestrated combination of changes in turgor pressure, as evidenced by volume changes and contractions of cells in the filaments. There must be involved changes in the polarity of cell membranes, coupled with ion exchange, as part of the complex mechanoperception system. We propose the characteristic thin cell walls as the sensitive areas of the filaments. Detailed ultra-structural, developmental, and physiological studies in

representative *Opuntia* species are needed to unveil the mechanism in detail based on which stamen movements occur in these cacti.

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