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Floral Ontogeny of *Pelargonium ×domesticum*

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ABSTRACT. **Floral ontogeny of two cultivars of *Pelargonium ×domesticum* L.H. Bailey, (regal pelargonium) 'Duchess' and 'Jennifer', was examined. Plants of both cultivars were grown together in a growth chamber at 15.5 °C with a photosynthetic photon flux of 10 mol·m^{-2}·d^{-1}. Meristems were examined at 5-day intervals over an experimental period of 170 days. The initial vegetative meristem was convex with leaf primordia initiated on either side in an alternate pattern. Early floral initiation was characterized by formation of two clefts on either side of the meristem. Between the clefts new meristems developed. Proliferation of meristems continued until numerous meristems were organized in a cluster arrangement at the apex of the shoot. New meristems lacked leaf primordia and would develop into flowers. Floral organ primordia on a floral meristem were initiated in a succession of four whorls: sepals, petals, androecia, and gynoecium.**

Pelargonium ×domesticum (regal pelargonium) is a day-neutral plant (Post, 1949). Previous studies have demonstrated that development of visible flower buds in regal pelargonium is controlled by both temperature and irradiance (Hackett and Kister, 1974; Nilsen, 1975; Post, 1949). However, development of visible flower buds is a relatively late stage in the transition of a vegetative meristem to an inflorescence and the subsequent development of floral organs. Preceding this development, molecular changes occur in the plant which signal changes in the meristem (induction) (Bernier, 1986; Bernier et al., 1993). Molecular and cellular changes occur in the meristem in response to the inductive signal (evocation). As cells respond to molecular signals, cellular reorganization occurs in the meristem. The result is a change in morphogenesis of the meristem and commencement of floral initiation (Huala and Sussex, 1993; Sattler, 1973; Steeves and Sussex, 1994). The sequence of events in a plant as the meristem changes from indeterminate (vegetative) to determinate (floral) is vegetative → induction → evocation → initiation → organogenesis → floral organ development.

Since both temperature and irradiance affect development of visible flower buds in regal geranium (Hackett and Kister, 1974; Nilsen, 1975; Post, 1949), it is possible that different environmental factors are important at different stages of floral ontogeny. Therefore, it is desirable to examine each stage of floral ontogeny under a controlled set of environmental factors. In the future it will be possible to alter environmental factors and examine the effects, if any, on floral ontogeny compared to the set of environmental factors used in this study.

Descriptions of the floral ontogeny of *Pelargonium inquinans* L. (Payer, 1857) and *P. ×hortorum* L.H. Bailey (bedding geranium) (this species is also known in the horticultural literature as *Pelargonium zonale*) are available (Sattler, 1973; Wetzstein and Armitage, 1983). However, there is no published description of the floral ontogeny of *Pelargonium ×domesticum.* Therefore, the objective of this study was to examine and describe the floral ontogeny of regal pelargonium meristems under a controlled set of environmental conditions.

Materials and Methods

PLANT MATERIALS AND CULTURE. Six-week-old cuttings of two cultivars of *P. ×domesticum,* 'Duchess' and 'Jennifer', were obtained from Oglevee, Ltd. (Connellsville, Pa.), on 19 Dec. 1996. The rooted cuttings were planted into Rubber Dirt plugs (Oglevee, Ltd.), in a styrofoam Pod Pack (Oglevee Ltd.). Rubber Dirt is composed of peat moss, water, and a hydrophilic polymer. Each of four pod packs contained 26 plants, for a total of 104 cuttings of each cultivar. The two cultivars had been chosen based on several criteria: 1) they are modern commercial cultivars; 2) they were developed under different environmental conditions ('Duchess' was developed in England by Ernest Walters and 'Jennifer' was developed in California by David Lemon of Oglevee, Ltd.); 3) they were developed from different germplasm sources; and 4) they have different total cumulative irradiance requirements for floral initiation: 'Duchess' cuttings require ≈200 mol and 'Jennifer' cuttings require ≈300 mol (Loehrlein, 1997).

ENVIRONMENTAL CONDITIONS. Upon arrival, rooted cuttings were transferred to a growth chamber (model GC15; Environmental Growth Chamber, Chagrin Falls, Ohio) with temperature and humidity set at 15.5 °C and 30%, respectively. A relatively low humidity was desired to prevent infection by fungal pathogens. Irradiance was provided at a photosynthetic photon flux (*PPF*) of 175 µmol·m^{-2}·s^{-1} for a 16-h photoperiod, with a daily light integral of 10 mol·m^{-2}. Irradiance was provided by 16 cool-white fluorescent (F72T12CWHO; Kloninklijke Philips Electronics, N.V., Eindhoven, The Netherlands), high output 160-W lamps. Ten percent of the input wattage was provided by 60-W incandescent bulbs (Philips 60 W, 130 v). Irradiance was measured using a light meter fitted with a quantum sensor (LI-185A; LI-COR, Lincoln, Neb.) to measure *PPF*. Readings were made at six locations within the chamber, and an overall average was then calculated. At the center of the chamber irradiance was 200 µmol·m^{-2}·s^{-1}, while near the outer edges, irradiance was 150 µmol·m^{-2}·s^{-1} *PPF*. To provide all experimental units with uniform exposure to irradiance, pod packs were rotated daily.

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Plants were checked daily and fertigated when media began to dry with 1.33 g·L^{-1} Excel 15–2.2–12.5 Cal-Mag water-soluble fertilizer (Scotts-Sierra Horticultural Products, Marysville, Ohio). Plants were irrigated once a week with tap water to reduce salt accumulation.

Experimental design and sampling. Plants were arranged in a completely randomized design. Three meristem samples were removed from each cultivar at 17 collection times over an 85-d period. Samples were taken at the following times: the day the plants arrived at the University Park campus, 10 d later (plants had been exposed to 100 mol of cumulative irradiance) and every 5 d [at 50 mol photosynthetic photons (*PP*) intervals] thereafter.

Meristems were taken by removing 5 to 10 mm of tissue from the apical shoot tip. They were preserved in FAA (37.5% formaldehyde: 70% ethanol: 100% glacial acetic acid, 1:8:1 by vol.) fixative before examination with a stereo microscope using 25× oculars and a 10× objective. Photographs were taken using scanning electron microscopy.

Results

Floral morphology. Florets of *Pelargonium ×domesticum* were five-merous, perfect and complete. They were borne in a compound umbel. Petiolate florets formed secondary umbels, which were subtended by bracts that formed an involucel and held by a peduncle. Primary umbels consisted of two or more peduncles, which together were subtended by an involucre. Floret number on an inflorescence ranged from one to 10. The first inflorescence initiated on plants of 'Duchess' had an average of nine florets, while subsequent inflorescences on a plant had an average of five florets. The average number of florets in the first inflorescences initiated on 'Jennifer' was seven. Subsequent inflorescences had not developed fully in 'Jennifer' at the termination of the experiment.

Floral initiation. The vegetative meristem was convex with leaf primordia developing in a spiral around the axis of the stem (Fig. 1A). At the onset of floral initiation, clefts became visible

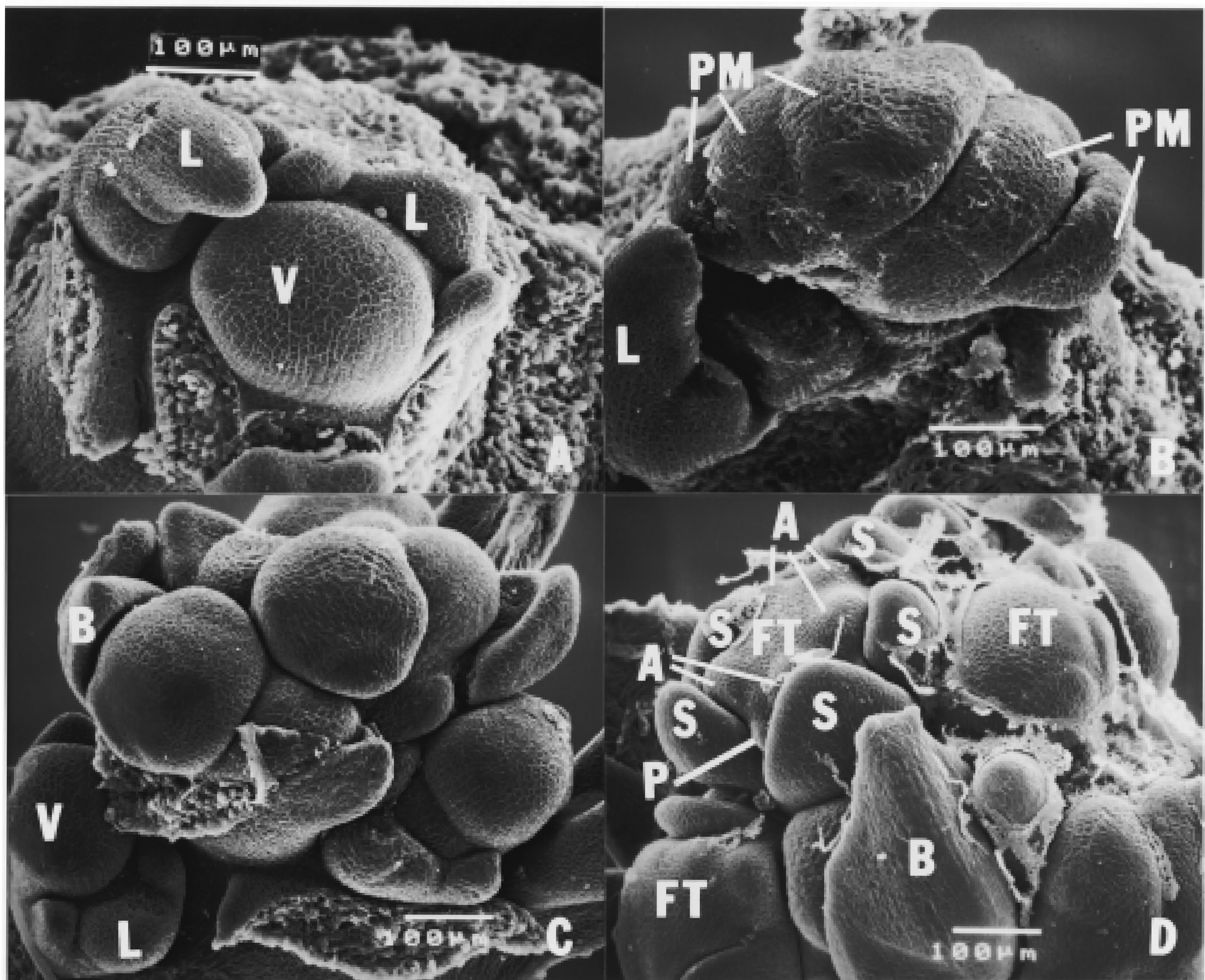

Fig. 1. Scanning electron micrographs showing *Pelargonium ×domesticum* apical meristem as it changes from the vegetative to the reproductive stage. (**A**) Dome shaped vegetative meristem (V) with two visible leaf (L) primordia. (**B**) Transitional meristem with five primordial meristems (PM) separated by clefts. A leaf primoridum (L) is visible in the lower left. (**C**) Shoot apex in which different meristem types are visible. The lower meristem is subtended by a leaf primordium (L), indicating a vegetative meristem (V). Other meristems lack leaf primordia, but are organized into inflorescences. These are subtended by bracts (B). (**D**) Three floret meristems (FT) are visible. On the floret meristem on the upper left sepals (S) have formed; anthers (A) and petals (P) are beginning to develop. Floret meristems on the upper right and lower left exhibit a dome shape with sepal primordia commencing development.

towards either side of the meristem, as multiple primordial meristems formed (Fig. 1B). Each of the primordial meristems developed into either a floret or inflorescence meristem. While vegetative meristems had subtending leaf primordia, these were notably absent on the floret and inflorescence meristems (Fig. 1C). With 150 mol cumulative *PP*, many inflorescence and floret meristems had developed at the shoot apex (Fig. 1C and D) where they were arranged in a cluster.

Floral development. Floral organ primordia were initiated in a succession of four whorls: sepals, petals, androecium, and gynoecium (Figs. 1D and 2A and B). Petals and androecium appeared to develop simultaneously soon after sepal primordia were visible (Fig. 1D). When sepals were fully developed, they were detached easily from the floral meristem, thus revealing fully formed anthers with petal primordia anterior to and alternating with stamen filaments (Figs. 1D and 2A). During anther development, the gynoecium flattened (Fig. 2A), then became concave as the gynoecial ridge developed (Fig. 2B and C). As the ridge developed, divisions appeared which developed into stigmatic lobes and carpels at the base (Fig. 2C). As the gynoecium developed further, stigmatic lobes became much elongated (Fig. 2D). In 'Duchess', two florets opened on one experimental unit at 850 mol *PP*. No 'Jennifer' florets had opened by the time the experiment was terminated.

Calyx. Five sepal primordia were initiated in a single whorl (Figs. 1D and 2A). The second and third sepal primordia were initiated nearly simultaneously opposite the first one. The fourth and fifth sepals were then initiated on either side of the first, again, nearly simultaneously. When anthers were fully formed, trichomes began to develop on the sepals (Fig. 2A). Mature sepals were imbricate (overlapping) and covered with trichomes (Fig. 2A).

Corolla and androecia. Anthers initiated almost simultaneously with petals. In order to view developing organs, sepals were removed when they were long enough to allow their removal without damaging meristematic tissue. Beneath sepals, a whorl of

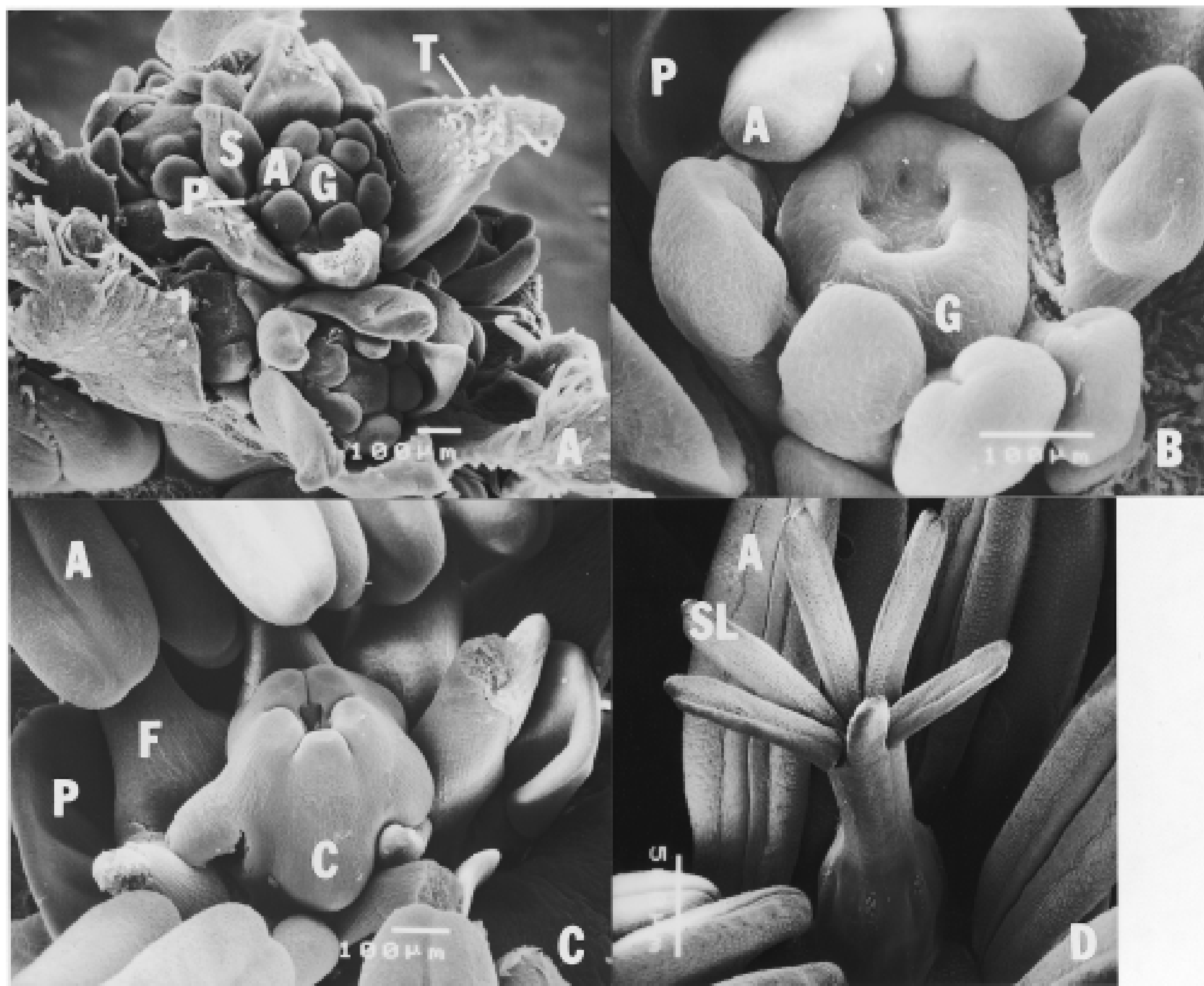

Fig. 2. Scanning electron micrographs showing development of *Pelargonium ×domesticum* floral organs. (**A**) Individual floret meristems in varying stages of development. Floral organ primordia have appeared. In the earliest developing meristem, sepals (S) and trichomes (T) are identifiable; anthers (A) are larger than petals (P), and the gynoecium (G) is beginning to develop a concave shape in the center. (**B**) Floret meristem with anthers (A) and petals (P) visible. Gynoecial ridge is apparent on the gynoecium (G). (**C**) Floret meristem with well-developed anthers (A) on nonelongated filaments (F). Gynoecium is beginning to elongate. Notice petals (P) about the same length as gynoecium. Carpels (C) are visible at the base of the gynoecium. (**D**) Late floral meristem showing stigmatic lobes (SL) and elongated anther (A). Bar = 500 µm.

Table 1. Total cumulative irradiance (of photosynthetic photons in mol) required for floral initiation and subsequent development of floral organs in two cultivars of *Pelargonium ×domesticum.* Number of meristems observed in parentheses (out of three).

Cultivar	Vegetative meristem	Transition meristem	Inflorescence meristem	Sepals visible	Petals and androecium formed	Gynoecium visible	Anthesis
Duchess	0–150 (3)	150 (2)	150 (2)	200 (2)	200 (1)	350 (2)	850
Jennifer	0–150 (3)	150 (3)	200 (3)	200 (2)	250 (1)	400 (3)	---

five petal primordia was visible. Alternating with petal primordia were five to eight clearly visible anther primordia. Petal primordia remained small while the anthers continued to grow (Figs. 1D and 2A). Petal and gynoecium elongation occurred synchronously (Fig. 2C). Then, when the length of the pistil exceeded that of the anthers, petal length was greater than both (not shown). Petals were fully pigmented at this time.

In 'Duchess', casual observations suggested that usually five (sometimes six or seven) anthers and two filaform staminodia (antherless stamens) developed. In 'Jennifer', usually seven (sometimes six or eight) anthers, and two or three (sometimes four or five) filaform staminodia developed. Five fertile anthers developed first, and were opposite the sepals. Any additional fertile anthers that developed were positioned opposite petals, as were staminodia that developed. Anthers were introrse (facing inward) and fused at the base.

Gynoecium. Following development of anthers, the final floral organ to develop was the gynoecium. The gynoecial ridge was visible when anthers and petals were fully formed (Fig. 2B). The stigmatic terminus of the pistil developed into five individual stigmatic lobes (Figs. 2B and 2C). These lobes were visible very early in gynoecial development before stylar elongation (Fig. 2C), and became more prominent during stylar elongation (Fig. 2D). The pistil had fused styles and carpels. Carpels were visible before style elongation (Fig. 2C). Usually five carpels developed in both cultivars, although 'Jennifer' gynoecia sometimes had six carpels. At anthesis, stigmatic lobes were closed, and remained so until two or more days after pollen had dehisced.

Genotypic effect. The sequence of floral ontogeny was similar for 'Duchess' and 'Jennifer'; however, the timing of organogenetic events differed (Table 1). All samples were vegetative in both cultivars at plant arrival and after the accumulation of 100 mol *PP*. Inflorescence meristems had developed in both cultivars at 150 mol cumulative *PP*. However, anthers were developing in 'Duchess' as early as 200 mol cumulative *PP* and fully formed anthers were visible at 300 mol cumulative *PP*. Early development of anthers was visible in 'Jennifer' meristems at 250 to 300 mol cumulative *PP*, and the first fully formed anthers were visible at 350 mol cumulative *PP*.

Gynoecia developed in 'Duchess' between 350 and 450 mol cumulative *PP*, and well-formed stigmatic lobes were visible at ≈500 mol cumulative *PP*. Gynoecia developed in 'Jennifer' at 400 to 700 mol cumulative *PP*, while well formed stigmatic lobes were visible at ≈750 mol cumulative *PP*.

At 850 mol cumulative *PP*, anthesis had occurred in two florets of an inflorescence of one experimental unit of 'Duchess', while anthesis did not occur in 'Jennifer' before termination of this experiment.

Discussion

Floral initiation in rooted stem cuttings of 'Duchess' and 'Jennifer' regal pelargonium is a response to total cumulative irradiance rather than chronological time (Loehrlein, 1997). Therefore, as daily irradiance increases, floral initiation occurs in a shorter period of time. 'Duchess' and 'Jennifer' were selected for this study because of their different irradiance requirements for floral initiation (Loehrlein, 1997). However, observations in this study revealed that 'Jennifer' floral meristem development required only 50 additional mol (5 d) *PP* compared to that of 'Duchess' rather than 100 mol as reported previously (Loehrlein, 1997). This may have been due to the 10-d lapse between collection of the first sample at time of arrival and the second sample, at 100 mol *PP*. Some 'Duchess' meristems may have been reproductive at 50 mol, but were not observed. 'Jennifer' floral organ development continued to lag behind 'Duchess' by ≈50 mol (5 d) throughout floral ontogeny from floral initiation to development of a visible gynoecium.

Floral ontogeny of the *Pelargonium ×domesticum* cultivars differed in several respects from that reported for *Pelargonium ×hortorum* (Sattler, 1973; Wetzstein and Armitage, 1983). The first difference occurred during early inflorescence development. In *Pelargonium ×hortorum* two clefts developed and divided the apical meristem into three meristems. Of these three, the central meristem elongated into an inflorescence-bearing peduncle, while the other two were quiescent initially, then developed into either vegetative or additional inflorescence meristems. In *Pelargonium ×domesticum*, multiple meristems formed at the apex of the shoot. Individual meristems developed into either a floral meristem or into an inflorescence.

Two differences were observed in floral organogenesis of *Pelargonium ×domesticum* as compared to *P. ×hortorum* (Sattler, 1973). First, Sattler described sepal and petal initiation as nearly simultaneous, whereas in *P. ×domesticum* sepals were initiated first, then petals and stamens were initiated nearly simultaneously. Secondly, Sattler (1973) described stamen initiation in *Pelargonium ×hortorum* as being obdiplostemonous, having two whorls of five stamens, with one whorl opposite the sepals and the other opposite the petals. In the latter whorl, usually two or three stamens are sterile filaform staminodia. However, in our study only a single whorl of usually seven fused anthers developed in *Pelargonium ×domesticum.* Nevertheless, a close similarity existed between the two species, with respect to stamen size and sterile filaform staminodia. In P. *×domesticum,* as in *P. ×hortorum,* five larger anthers were initiated opposite the sepals, and usually two additional smaller ones were initiated opposite the petals, with two to three filaform staminodia initiated opposite the remaining petals (Sattler, 1973; Wetzstein and Armitage, 1983). This pattern was also reported in *P. inquinans* (Payer, 1857).

The pattern of sepal primordia initiation followed the pattern described by Sattler (1973) of *Pelargonium zonale* (*P. ×hortorum*), in that the second and third sepal primordia were initiated nearly simultaneously apposite the first one. The fourth and fifth sepals were then initiated on either side of the first, again, nearly simultaneously. Petals and anthers initiated nearly simultaneously.

However, petals remained small while the anthers continued to grow. This concurs with Sattler's (1973) observation of *Pelargonium zonale* (*P.* ×*hortorum*).

Varying numbers of stamens and staminodia were observed in both 'Duchess' and 'Jennifer'. This is contrary to floral ontogeny patterns described by Endress (1990), in which he described two conditions for instability in floral organ position. First, when the organ primordia were very small compared to the entire floral primordium, as is often the case in flowers with numerous organs; second, when a perianth was reduced or lacking. Neither of these cases was true for *P.* ×*domesticum.* In addition, synorganization of both androecium and gynoecium is expected to result in fixed symmetry as a rule (Endress, 1990).

While plasticity was observed more often in androecia in both 'Duchess' and 'Jennifer', it was also observed in gynoecia of 'Jennifer'. Plasticity in carpel number has not been reported in other *Pelargonium* L'Herit. ex Ait. species, or in other genera of the Geraniaceae (Payer, 1857). The reasons for plasticity in androecia and carpels may be explained by the interspecific nature of the plants and resultant cytogenetic irregularities. Although cytogenetic studies have not been conducted for 'Jennifer' or 'Duchess', aneuploidy and polyploidy have been reported in other regal cultivars (Knicely, 1964). Further study of carpel and ovule development in *P.* ×*domesticum* would be necessary to confirm the presence of this trait in other cultivars of the species. The plasticity exhibited in stamen number may be an evolutionary development in which reduction of organ numbers is favored. This is supported by observations in related species and genera (Glicenstein, 1986; Payer, 1857; Sattler, 1973).

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