Growth of anthers in *Lilium longiflorum*

A kinematic analysis

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**Abstract.** The post-initiation growth of 64 anthers (1.1–17.4 mm long) in *Lilium longiflorum* Thunb. was examined by time-lapse marking experiments in combination with serial sections and the scanning electron microscope. Each anther was characterized by spatial and temporal variation in growth rate. Larger anthers had two, and occasionally three, series of peaks and troughs in local growth rate. Regions of negative growth rate were frequently encountered. When observed over several days, the growth maxima and minima were found to move basipetally as a waveform down the length of the anther. The wavelength was longer in taller anthers; amplitude and frequency were variable, and anthers of the same size were not always synchronous. Distribution patterns of cell division (and elongation, once division has ceased) recapitulate the growth data. Anther growth is a non-steady system, therefore, with growth centers constantly shifting. Implications for future studies in organ growth patterns are discussed.

**Key words:** Anther development – Growth analysis – *Lilium* – Mitosis.

**Introduction**

There are several hundred accounts, spanning more than a century, on the initiation and histogenesis of anthers (e.g. Coulter and Chamberlain 1903; Maheshwari 1950; Davis 1966; Sattler 1973); however, none attempts to provide a complete picture of the three-dimensional growth of an anther from initiation to maturity. Most have relied on transverse or longitudinal sections, in combination with direct observation or scanning-electron-microscope (SEM) images, of anther primordia. For example, Boke (1949) examined the density of cells in median longitudinal sections through stamen primordia of *Vinca rosea*. He concluded that until it is 200 µm tall, the stamen grows predominantly through the activity of apical and sub-apical cell initials. Thereafter, and up to its mature length of 2.5 mm, growth is intercalary. Boke considered his results supportive of the theory that stamens are homologues of leaves.

The exclusive use of a limited number of sections to obtain information on the sites of meristematic activity has been criticized (Poethig 1984). Such an approach (i) confines attention to limited regions, and thereby emphasizes the role of localized meristems; (ii) restricts observations to one or two planes; and (iii) should be used only for indeterminate organs having a regular pattern of cell division, such as roots (Poethig 1984). For example, Avery (1933) studied the early development of the tobacco leaf through transverse section. He proposed that apical growth is superseded by intercalary growth before the leaf reaches 1% of its final length, and that the lamina arises from discrete marginal and sub-marginal meristems. These contentions have been refuted by many workers using an assortment of alternative techniques, including surface-marking experiments (Richards and Kavanagh 1943; Hara 1957; Maksymowycz and Erickson 1960; Dubuc-Lebreux and Sattler 1980; Poethig and Sussex 1985). Until now, no surface-marking techniques have been employed to study floral organ growth.

We describe here the results of a surface-growth analysis of anthers of the Easter lily, *Lilium longiflorum* Thunb. Surface-marking experiments have been shown to provide an accurate description of both the spatial and material aspects of plant de-
development (Silk 1984), and for several reasons lily anthers are particularly useful for this kind of study: (i) They are exceptionally long (at least 25 mm at maturity) and are therefore accessible to superficial marking. (ii) Bud length and anther length are tightly correlated (Erickson 1948). The length of an anther can therefore be predicted prior to opening up the bud. (iii) The length of the anther serves as an accurate index of its developmental stage. Erickson (1948) showed that the cytological events leading to microspore production occur at predictable anther lengths. This characteristic has already been utilized in physiological studies (Hotta and Stern 1961, 1963). (iv) The ontogeny and histology of floral organs has been well documented for the Liliaceae (Guerin 1927; Pfeiffer 1935; Emsweller and Pryor 1943; Kosugi 1952; Davis 1966; Einert et al. 1970; Cremer et al. 1974; De Hertogh et al. 1976).

We present a concept of anther growth that could not have been demonstrated using classical microtechnique, autoradiography, or any other procedure which presupposes a steady distribution of meristems.

Material and methods

Bulbs of Lilium longiflorum cv. Nellie White (Dahlstram & Watt Bulb Farms, Smith River, Cal. USA) were planted in pots and grown in a greenhouse in Riverside, Calif., until floral buds were formed. The time to flowering varied with the duration of cold storage prior to planting (Emsweller and Pryor 1943). At least two weeks before harvesting, pots were transferred to a growth chamber at 25 °C with a photon fluence rate of 1 mmol (photons) m⁻² s⁻¹ and an 18 h photoperiod.

Growth in situ. The method of Erickson (1948) was used to estimate the natural growth rate of anthers in undamaged buds. buds were harvested and dissected, and the lengths of tepals and anthers determined with a ruler. Tepal length was plotted against the time elapsed after the buds were removed with a scalpel to expose the dorsal locules of one anther (Erickson 1948). At least two weeks before harvesting, pots were transferred to a growth chamber at 25 °C with a photon fluence rate of 1 mmol (photons) m⁻² s⁻¹ and an 18 h photoperiod.

Sections. The six anthers in each of three control buds (anther lengths 0.65, 1.1, and 2.1 mm) were fixed in 2.5% glutaraldehyde buffered in potassium phosphate at pH 7.0 (Glauert 1975). They were dehydrated with 2,2-dimethoxypropane, infiltrated with and embedded in glycol methacrylate, serial-sectioned at 4 μm, and stained with 1% toluidine blue in 0.1% sodium borate (O'Brien and McCully 1981).

Five of the anthers per bud were serial-sectioned transversely. Sections were scored for nuclei undergoing mitosis by making a camera-lucida drawing of the anther outline and the dividing cells. Because of the relative rarity of metaphases, cells at any stage from mid-prophase to late telophase were included. Coordinates were given to each dividing cell by placing a grid over the camera-lucida outlines. By this method successive sections through the same dividing cell could be located, so that each cell was scored for mitosis only once. Every section through anthers of the smallest bud was examined; for anthers of the two larger buds, every fifth section was examined.

The sixth anther per bud, and also larger anthers processed by an identical microtechnique, were sectioned longitudinally. From approximately median longitudinal sections, the stage at which cell division ceased, and lengths of cells in different tissues of the anther were determined.

Results

Anther growth in situ. Elongation of the tepals is exponential in Lilium (Fig. 1a); the relative rate of elongation, \( r = 0.103 \cdot d^{-1} \). The relationship between tepal and anther lengths is biphasic (Fig. 1b), yielding allometric constants \( k = 1.145 \) at the earlier phase, and \( k = 0.258 \) beyond the 35-mm tepal (20-mm anther) stage. From these...