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EARLY FLORAL DEVELOPMENT OF *HELICONIA LATISPATHA* (HELICONIACEAE), A KEY TAXON FOR UNDERSTANDING THE EVOLUTION OF FLOWER DEVELOPMENT IN THE ZINGIBERALES[1]

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We present new comparative data on early floral development of *Heliconia latispatha*, an ecologically and horticulturally important tropical plant within the order Zingiberales. Modification of the six members of two androecial whorls is characteristic of Zingiberales, with a reduction in number of fertile stamen from five or six in the banana families (Musaceae, Strelitziaceae, Lowiaceae, and Heliconiaceae) to one in Costaceae and Zingiberaceae and one-half in Marantaceae and Cannaceae. The remaining five infertile stamens in these later four families (the ginger families) are petaloid, and in Costaceae and Zingiberaceae fuse together to form a novel structure, the labellum. Within this developmental sequence, Heliconiaceae share with the ginger families the possession of an antisepalous staminode, a synapomorphy that has been used to place Heliconiaceae as sister to the ginger family clade. Here, we use epi-illumination light microscopy and reconstruction of serial sections to investigate the ontogeny of the *Heliconia* flower with emphasis on the ontogeny of the staminode. We compare floral development in *Heliconia* with that previously described for other species of Zingiberales. A comparison of floral structure and development across Zingiberales is presented to better understand the evolution of the flower in this charismatic group of tropical plants.

Key words: floral development; floral evolution; *Heliconia latispatha*; heterochrony; morphology; nectaries; oblique zygomorphy; Zingiberales.

The Zingiberales are a morphologically diverse and species-rich order particularly suited for studying the evolution of floral diversity. The eight families recognized within the order are often divided into two groups: the monophyletic ginger families (Marantaceae, Cannaceae, Zingiberaceae, and Costaceae) and the basal, paraphyletic banana families (Heliconiaceae, Strelitziaceae, Musaceae, and Lowiaceae) (Tomlinson, 1962; Kirchoff, 1991; Kress, 1991; Kress et al., 2001) (see Fig. 1).

Most members of the ginger and banana families possess large, showy flowers, many of which demonstrate specialized relationships with pollinators (Kirchoff, 1983a; Kress, 1985; Kress and Stone, 1993; Kress et al., 1994; Pedersen and Kress, 1999; Sakai and Inoue, 1999; Liu et al., 2002a, b). Comparative morphology across the order reveals a trend in floral evolution, with flowers of the ginger families having higher degrees of organ fusion and specialization. Flowers of the banana families typically have five (occasionally six) fertile stamens, produced as trimerous inner and outer stamen whorls (Fig. 1) (Schumann, 1900; Andersson, 1981, 1985; Kronestedt and Walles, 1986; Kirchoff, 1991). Petals and stamens are often fused at the base to form a floral tube, but there is no reported fusion solely within the stamen whorls and, with the exception of Heliconiaceae, there are no staminodes (sterile, petaloid androecial members) in the mature flower. Flowers of the ginger families are characterized by highly modified androecial whorls containing four or five staminodes, one or fewer fertile stamens, and complex patterns of fusion among floral organs including the staminodes (Fig. 1) (Petersen, 1889; Schumann, 1902, 1904; Dahlgren and Rasmussen, 1983; Kress, 1990; Kirchoff, 1991). Flowers of Heliconiaceae are typically considered to be allied with those of the banana families due to the possession of five or six fertile stamens, a character state that is considered plesiomorphic within the order. However, the presence of an anti-sepalous staminode in the mature flowers of *Heliconia* provides an underlying synapomorphy for a clade containing the Heliconiaceae plus the ginger families (Fig. 1). The staminodes of the ginger families, however, tend to be showy, while the staminode in *Heliconia* is the most inconspicuous organ of the mature flower. Nonetheless, the evolution of a persistent staminode in the common ancestor of Heliconiaceae and the ginger family clade may have been an important morphological innovation that was exploited in the radiation of the ginger families (Specht, 2005, 2006).

Heliconia, the single genus within the family Heliconiaceae, has a primarily neotropical distribution with approximately 215 species native to Central and South America and six species native to the South Pacific. *Heliconia* species are herbaceous

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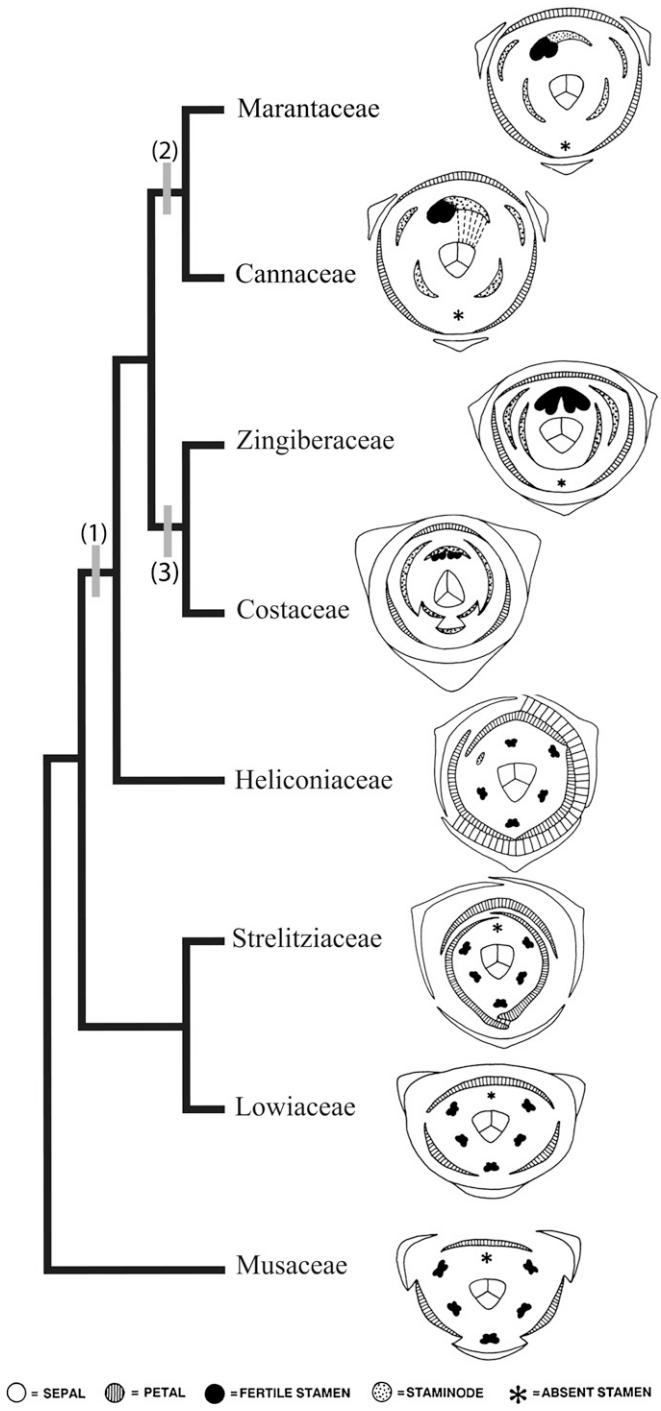


Fig. 1. Phylogeny of Zingiberales (Kress et al., 2001) with representative floral diagrams for each family. Flower orientation places homologous organs in equivalent positions. Location of major changes in floral evolution indicated along branches: (1) Fertile stamen suppression shifts from an anti-petalous position to an anti-sepalous position and from a member of the inner androecial whorl to a member of the outer whorl; staminode persists to maturity. (2) Shift from zygomorphy to asymmetry with the development of a 1/2 fertile stamen. (3) Fusion of petaloid staminodes to form the labellum.

monocots characterized by banana-like leaves, a pseudostem, and inflorescences comprised of thick, coriaceous bracts that subtend partial florescences of cincinni (Schumann, 1900; Weberling, 1982). In neotropical species, the bracts on the main axis of the inflorescence are brightly colored and form part of a bird pollination syndrome. Flowers of New World taxa are morphologically specialized for hummingbird pollination (Stiles, 1975; Gill, 1987; Taylor and White, 2007), while bat and honeyeater pollination have been reported in the Old World species (Kress, 1985; Pedersen and Kress, 1999). All members of the perianth are to some extent fused at the base, forming a floral tube in which sucrose-rich nectar is secreted from a gynopleural nectary located at the base of the ovary (Hartl and Severin, 1980; Smets and Cresens, 1988; Kirchoff, 1992).

Floral structure is fairly well-conserved within *Heliconia* (Schumann, 1900; Andersson, 1981; Kress, 1984; Andersson, 1985). While there is extensive variation in certain mature traits, particularly those associated with specific pollination relationships (e.g., size and curvature of the perianth), overall floral morphology is similar in even distantly related species, especially when compared to the floral diversity that characterizes other ginger families (Petersen, 1889; Loesener, 1930b). By extension, we hypothesize that early floral development is likely to be similar in species throughout the genus.

Recent phylogenetic analyses of the Zingiberales have placed the Heliconiaceae as sister to the ginger families (Kress, 1990, 1995; Kress et al., 2001) to the exclusion of the other banana families. However, this relationship is not well supported, and the analysis of different data sets (e.g., molecular vs. morphological, or different molecular data sets) result in different topologies often with low support (Kress et al., 2001). The uncertainty of the placement of Heliconiaceae is further demonstrated by a more recent phylogenetic analysis (Johansen, 2005), which places Heliconiaceae as sister to Musaceae, an association reminiscent of taxonomic studies that placed *Heliconia* as a genus within Musaceae (Schumann, 1900; Winkler, 1930; Andersson, 1981). This uncertainty highlights the importance of finding characters that can resolve the phylogenetic placement of the banana families. DNA sequence data and mature plant morphology have not yet been sufficient for this purpose. Developmental characters may provide additional phylogenetic signal.

The Heliconiaceae is a particularly important group in which to study floral development because its floral structure coupled with its potential placement as the sister group of the ginger families suggests that it represents an important evolutionary transition of floral form in the order (Kress et al., 2001; Rudall and Bateman, 2004) (Fig. 1). Studies of flower development have been used to reveal homology, homoplasy, and heterochrony of organs or organ parts within a comparative context (Erbar, 1988; Tucker, 1992; Leins and Erbar, 2003). Comparative studies of development have also been used to establish transitions between seemingly distinct mature states and to reveal unique transitional characters (Mabee, 1993). However, developmental data, like most character data, does not always provide unambiguous phylogenetic signal. Kirchoff (1983b) demonstrated that certain aspects of floral development may be more similar between genera than within a single genus. Because mature *Heliconia* flowers have characteristics that have helped place the genus as sister to the ginger families, detailed developmental studies may provide the data necessary

to clarify the placement of the family. Assessment of homology of developmental stages in morphologically and phylogenetically divergent taxa is not trivial (Jaramillo and Kramer, 2007). Rigorous and precise assessments of homology are achieved only in the light of comprehensive comparative developmental series (Buzgo et al., 2004). New techniques for establishing visual homologies make it possible to define homologous characters and character states using all developmental stages (Kirchoff et al., 2007). However, complete developmental sequences for representative taxa from all families in the Zingiberales are necessary to realize this potential. Developmental series exist for *Costus scaber* (Costaceae), *Scaphochlamys kunstleri*, *Hedychium gardnerianum*, *Globba unifolia* (Zingiberaceae), five species of Marantaceae, *Canna indica* (Cannaceae), and *Orchidantha maxillarioides* (Lowiaceae) (Kirchoff, 1983b, 1985, 1988, 1997, 1998; Kirchoff and Kunze, 1995; Box and Rudall, 2006). While some stages of development have been described in Musaceae (White, 1928; Fahn, 1953), Strelitziaceae (Kirchoff, 2003), and Heliconiaceae (Kirchoff, 2003), there has been no comprehensive study of flower development in any of these families. A comprehensive study of flower development in all three families is necessary to determine the pleisomorphic states in the order. The current study begins to fill the gap for floral developmental data in these families.

In this study, we characterize early floral development in *Heliconia latispatha* Benth., a member of an ecologically important tropical family (Stiles, 1979). The significance of *Heliconia*'s flowers with regard to the evolution of floral development and floral symmetry in the Zinigiberales is discussed.

MATERIALS AND METHODS

Young *Heliconia latispatha* flowers and inflorescences (Fig. 2) were collected at Waimea Arboretum, Oahu, Hawaii (accession 74p1142; voucher *Kirchoff 87-107* at BISH) and at Fairchild Tropical Garden (accession 59-1065). The material was preserved in FAA (50% ethanol, 10% formalin, 5% acetic acid) (Berlyn and Miksche, 1976) and stored in Kew Fluid (50% ethanol, 40% H_2O, 5% glycerol, 5% formalin).

Development of *H. latispatha* (ca. 20 inflorescences) was studied with the epi-illumination light microscopy technique of Sattler (Sattler, 1968; Posluszny et al., 1980; Charlton et al., 1989; Bartlett et al., 2008). Preserved material was removed from the fixative, dehydrated to 100% ethanol, and stained for several days in fast green (Johansen, 1940; Charlton et al., 1989). Destaining was carried out in 100% ethanol for a period of 2 d to several weeks. Photographs were taken with Kodak (Rochester, New York, USA) Technical Pan film on a Leitz (Wetzlar, Germany) Ortholux 2 photomicroscope equipped with an Ultropak illuminator. Kodak Dektol was used to develop the film for 3 min at 68°C. The negatives were scanned to disk with a Nikon (Tokyo, Japan) Super Cool Scan LS1000 slide scanner, or onto Kodak Photo CDs by one of several commercial photographic laboratories. Plates were laid out and adjustments made to the exposure of the photographs with Adobe (San Jose, California, USA) Photoshop CS and CS3. In some cases, the overexposure of some areas was adjusted by the digital equivalent of the photographic technique of burning. Drawings were made in Adobe Illustrator CS and CS3 and imported into Photoshop for inclusion in the plates. For ease of interpretation, all images of floral apices in the figures are oriented so that the flowers appear to lie on the right side of a cincinnus (Fig. 3A), regardless of their original orientation.

For paraffin sections, flowers of the correct age to show gynoecial formation were rinsed in two changes of 50% ethanol and dehydrated with 2,2-dimethoxypropane (Postek and Tucker, 1976). The specimens were transferred to 100% *t*-butyl alcohol and embedded in paraffin (Berlyn and Miksche, 1976). Sections were cut on an American Optical (Buffalo, New York, USA) rotary microtome or a Reichert-Jung 2040 Autocut microtome (Leica; Wetzlar, Germany) at 4 μm and mounted on slides using Bissing's modified Haupt's adhesive (Bissing, 1974). The sections were stained with tannic acid, ferric chloride, safranin, and fast green (Berlyn and Miksche, 1976), dewaxed using ClearRite III (Thermo Scientific, Waltham, Massachusetts, USA) in place of xylene, and mounted with Permount. Photomicrographs were taken with a Zeiss (Carl Zeiss; New York, New York, USA) Axiophot microscope equipped with a 5 megapixel QImaging (Surrey, British Columbia, Canada) Micropublisher low-light, cooled CCD color digital camera in the Biological Imaging Facility, College of Natural Resources, University of California-Berkeley.

Terminology—The median plane of the flower bisects the flower and the axis that bears it (Weberling, 1989). The transverse plane is perpendicular to this axis. The adaxial side of the flower lies toward the lower-order axis that bears the flower. The abaxial side lies away from this axis. In most angiosperms, the adaxial–abaxial plane is the median plane of a flower and is also the plane of floral symmetry, but not for the Heliconiaceae, in which floral symmetry is determined by the placement of the antisepalous staminode (Figs. 1, 3A) (Kirchoff, 2003). For this reason, we use the terms posterior and anterior to identify the sides of the flower that are closest to or farthest from the main florescence axis, respectively (Fig. 3A). While the adaxial–abaxial plane is the median plane of the flower, it is the posterior–anterior axis that bisects the flower through its plane of symmetry (Figs. 1, 3A).

Organography—Plants of *H. latispatha* are 1.5–4 m tall with an erect, banana-like growth habit and are found natively in disturbed lowland forests from southern Mexico to Ecuador (Andersson, 1992). The inflorescences are comprised of 3–15 spathose, distichous primary bracts (Fig. 2A), typically orange, each subtending 10–15 flowers arranged in cincinni (monochasia) (Figs. 2B, 3A). One flower per primary bract opens each day. Within each cincinnus, the flowers are arranged in a zig-zag pattern from oldest to youngest, forming two rows (Fig. 3A). Within a row, the flowers have the same symmetry, while between rows the flowers are mirror images of one another (Kirchoff, 2003).

Flowers of *H. latispatha* are perfect with a trimerous perianth of one whorl each of poorly differentiated sepals and petals, five stamens in two alternating trimerous androecial whorls, a single posterior staminode, and an inferior, trilocular ovary. The perianth, which is straight, is approximately 34–51 mm long and yellow-green, while the petaloid posterior staminode is 3.9–8.4 mm long (Andersson, 1992). A floral tube formed by the fusion of the perianth members creates a nectar reservoir at the base of the perianth, into which sucrose-rich nectar is secreted from gynopleural nectaries. At anthesis, the distal regions of the posterior sepal become free from the floral tube.

RESULTS

Cincinnus development—Cincinnus apices are borne in the axils of the primary bracts. They give rise to higher-order bracts (prophylls) that subtend the individual flowers and to continuation apices that form the next higher-order prophyll, a new continuation apex, and a single flower (Fig. 3A, B). A new floral meristem arises in the posterior portion of each continuation apex (Fig. 3C, *f0*), and produces the next flower of the cincinnus. This pattern of prophyll, continuation apex, and flower initiation is repeated up to 15 times, resulting in a cincinnus with two rows of flowers (Fig. 3A).

The position of the first-formed prophyll determines the symmetry of the cincinnus. A cincinnus is left-handed if the first prophyll forms to the left when viewed abaxially, and right-handed if it forms to the right. However, the handedness of a cincinnus is not readily apparent until after four or five flowers have formed.

Floral development—*Early floral apex*—During its split from the continuation apex, the floral meristem is elliptical to deltoid (Fig. 3B, *f1*). Shortly after its separation, the apex becomes more distinctly triangular, with a flat or slightly convex top (Fig. 3C, *f1*). The vertices of the triangle are the sites of sepal initiation (Fig. 3A, D). Coincident with sepal initiation, a slight depression is visible at the center of the floral apex (Fig. 3D, *fc*). This depression will eventually be the site of gynoecial initiation.

Fig. 2. Mature reproductive morphology of *Heliconia latispatha*. (A) Inflorescence with spathose primary bracts (b), partial inflorescences of cincinni, and two flowers at antithesis (*). (B) Primary bract (b) enclosing partial inflorescence with one flower at anthesis. The single posterior sepal (1) initiates first during flower development and is free from the other perianth members (pe) above the short flora tube (not shown). s, stamens.

Sepal initiation—The triangular apex expands and forms the first sepal in the posterior position (Fig. 3A, D, E), followed soon by initiation of the second sepal on the lateral, anterior side of the flower, adjacent to the next younger flower of the cincinnus (Fig. 3A, E). The third sepal also forms anteriorally and laterally, opposite the second sepal (Fig. 3A, E, F). Sepals are thus formed in a spiral pattern with a handedness determined by the position of the flower in the cincinnus. The spiral is counterclockwise in flowers on the right, clockwise in those to the left (Fig. 3A). The first sepal forms more toward the periphery of the floral meristem than the other two sepals (Fig. 3D–G), allowing space for the subsequent formation of the staminode primordium (Fig. 3H, I, *stm*). The central depression, the floral cup, continues to deepen throughout sepal formation (Fig. 3D–G).

Petal and staminode primordia—During sepal initiation, conspicuous petal primordia appear around the periphery of the floral cup (Fig. 3E–G). The number of distinct primordia that form during this stage of development varies. In some flowers, there are four relatively separate primordia corresponding to the organs of the corolla (three) and the posterior staminode (Fig. 3H, *p1–3*, *stm*). Other flowers possess a single semicircular common primordium that gives rise to the two lateral petals and the staminode (Fig. 3G, I). Still others have states that seem to be intermediate between these two conditions (Fig. 3F). In all cases, the posterior region of the floral meristem, interior to the first sepal, enlarges and forms the staminode (Fig. 3E, G–I, *stm*). Petal formation is nearly simultaneous. The first petal forms in a posterior, lateral position between the first and second sepals (Fig. 3F–H, *p1*). Formation of the second and third petals occurs in the anterior and lateral regions of the apex, respectively, and continues the generative spiral established by the previous organs (Fig. 3G–I). In some apices, the petal primordia are relatively distinct (Fig. 3F), while in others they are confluent with the staminode, at least in their lower regions (Fig. 3E, G–I). During petal formation, the positions that will eventually be occupied by other members of the outer androecial whorl remain empty of distinct primordia (Fig. 3F–H).

Outer androecial initiation—As the petal primordia expand and become distinct, the sterile staminode also becomes distinct (Fig. 3I–K, *stm*). The two fertile, anterior, outer whorl stamen primordia arise from positions on the rim of the floral cup,

opposite the anterior sepals (Figs. 3I–K, *arrows*, *oa2*, *oa3*). Initiation occurs almost simultaneously, with the stamen opposite the third sepal appearing slightly ahead of the one opposite the second sepal (Fig. 3I–K), the reverse of the sequence predicted by the phyllotactic spiral established by the perianth.

Inner androecial initiation—The inner whorl of the androecium begins its initiation very shortly after the appearance of the outer androecium. The inner stamen primordia are initiated along the inner margins of the floral cup, opposite the petal primordia (Fig. 4A, B). Their initiation occurs in a more rapid succession than seen in the other whorls, and the order of initiation is somewhat plastic. In some apices, the primordia opposite the second (anterior) and third petals appear to form simultaneously (Fig. 4A). In others, the primordium opposite the anterior petal forms first, followed by the one opposite the third petal. In all apices, the primordia opposite the first petal always form last (Fig. 4B, *ia3*). The posterior inner androecial members remain confluent with the staminode primordium throughout the initiation of the inner androecium (Fig. 4A–D).

By this point in development, the sepals have greatly enlarged. The largest is the posterior sepal, which will be distally free in the mature flower (Figs. 1, 2, 4C, D). Throughout early development, the floral cup has become narrower and deeper, though the gynoecium has not yet initiated (Fig. 4A–D).

Gynoecial initiation—The gynoecium initiates as three primordia along the inner margins of the floral cup, below the point of attachment of the outer androecial primordia (Figs. 4H, 5A–C). All other floral organs have initiated and begun enlarging by this time. The gynoecial primordia first become apparent as densely cytoplasmic packets of cells along the inner margin of the floral cup and become bilobed (conduplicate) very early in development (Figs. 4H, 5B, C). The primordia form opposite the sepals in very quick succession so that it is difficult to determine the order of initiation. They extend basipetally to form the septa and nectaries and acropetally to produce the style and stigma (Fig. 6F, G, I). In the earliest stages we observed, nectary development is not yet apparent (Fig. 5A).

Ovary development—As they grow downward, the marginal lobes of the conduplicate primordia enclose a central cavity between the lobes and fuse at the center of the floral cup (Figs. 5C, 6C–E). This fusion results in the formation of a locule at the center of each of the three primordia. Appression of the outer margins of the conduplications of neighboring primordia form the septae (Figs. 5C, 6B–E). However, the margins of the conduplications do not fuse and gynopleural nectaries (interlocular and infralocular septal nectaries; Fig. 6I) form in the septa, between the lateral margins of neighboring gynoecial primordia (Fig. 6A–E). These nectaries extend upward to the base of the style as nectary ducts and basipetally to a level below the closure of the locules, terminating in highly branched nectary tissue (Fig. 6A, B, I). The gynoecial primordia are solid, not conduplicate, below the locules (Fig. 6A, B). Each locule contains a single, axial ovule that projects upward into the locule to produce what is sometimes called the basal ovule of the family (Fig. 6D, I).

Style and stigma initiation—The style forms from the axial fusion of the conduplicate primordia at the apex of the ovary (Figs. 4I, 6F, G). The external and lateral margins of the gynoecial primordia are free in this region (Fig. 6F, G). Fusion occurs only where the primordia meet at the center of the flower. Initially, there is little fusion between the primordia as they elongate above the floral cup. Instead, they tightly abut against each other but do not fuse (Fig. 4I). Postgenital fusion occurs later, but is never complete at the very base of the style (Fig. 6F, arrow). This incomplete fusion allows the formation of an exterior passage connecting the interlocular nectar canals to the base of the floral tube. The openings of these canals are seen as three furrows near the base of the style in the mature flower. As the gynoecial primordia grow acropetally, the inner margins of the conduplicate primordia also remain unfused, forming the stylar canals (Fig. 6F, G, I). The primordia enclose the stylar canals in positions vertically continuous with the locules. Lower in the style, there are three canals (Fig. 6F) corresponding to the three locules. More distally, these canals fuse to form a single stylar canal (Fig. 6G).

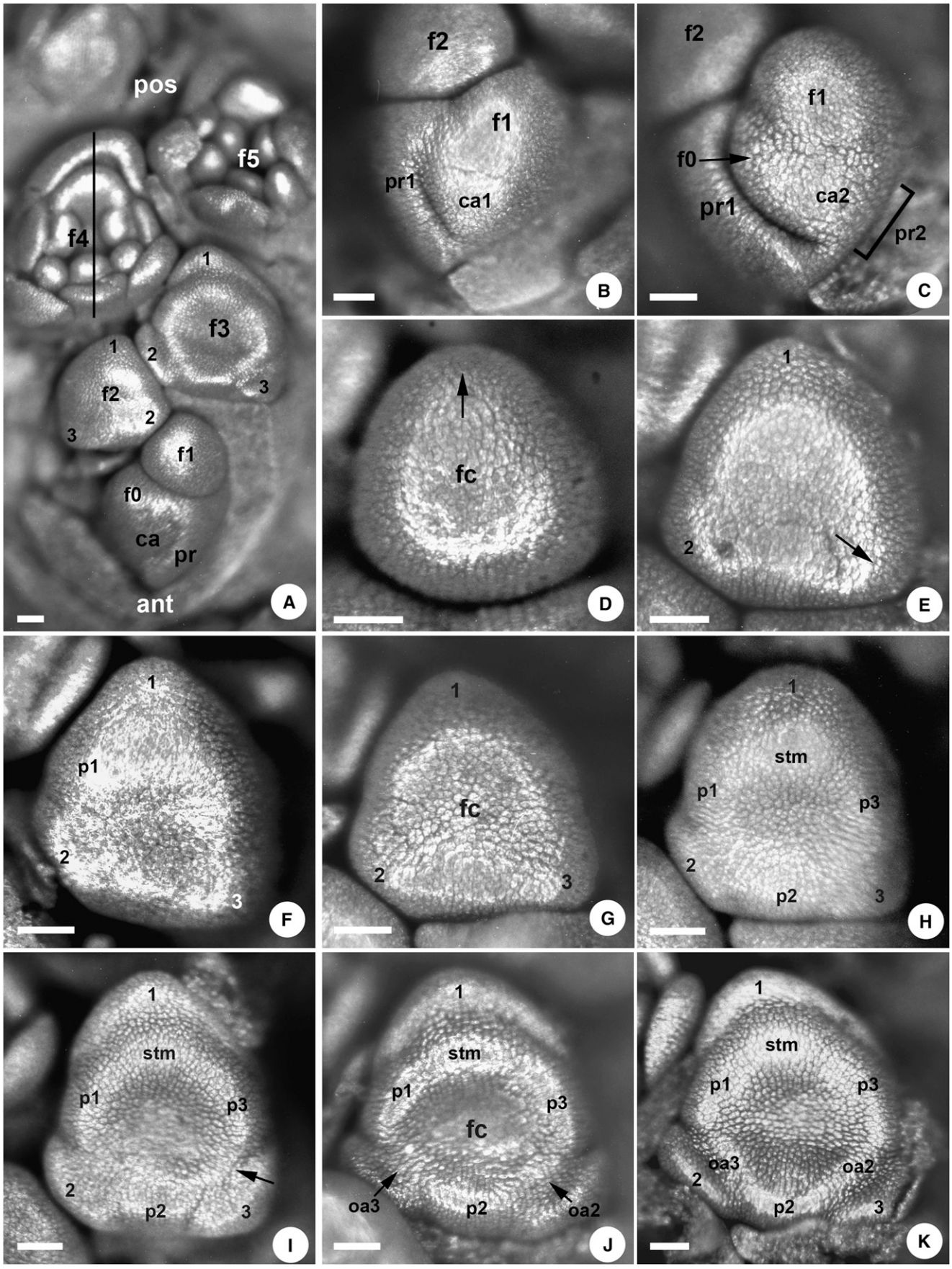
Enlargement/differentiation of the perianth and androecium—All floral members continue to expand and enlarge after their initiation. The petals and inner stamen become distinct (Fig. 4E, F), and the staminode, which was previously confluent with the lateral, posterior petal primordia, is now fused only to the inner, lateral stamens (Fig. 4E). As development proceeds, the staminode becomes free from the lateral stamens or at least is not as strongly connate (Fig. 4F, G).

The morphology of the staminode is markedly different than that of the other members of the androecium. Staminode shape is more reminiscent of a sepal or petal than of a stamen, and the staminode is taller, radially thicker and does not curve over the floral cup as strongly during the later stages of organogenesis as do the stamens (Fig. 4F, G). Just before the stage at which the sepals close over the flower, the staminode is approximately three times as wide tangentially as the stamens and 1.5 times as tall (Fig. 4G). As it expands, the staminode retains its position in the petal whorl, appearing almost as a fourth member of this whorl (Fig. 4F, G, 5E, 6H). At anthesis, the staminode is the smallest and most inconspicuous of the floral organs and does not strongly resemble a member of the perianth. It also has a single vascular trace, characteristic of polliniferous stamens (Fig. 6G).

The anthers enlarge throughout this period, and distinct thecae begin to form at approximately the stage of gynoecial initiation (Fig. 4G). The sepals and petals remain distinct throughout early development. A floral tube will eventually form below them, most likely through intercalary growth, from which the posterior sepal will ultimately detach itself in the mature flower.

DISCUSSION

Sequence of organ initiation—The pattern of perianth initiation follows Hofmeister's rule, an empirical heuristic derived from the observation that new leaf primordia are formed in the largest space between existing primordia (Hofmeister, 1849; Kirchoff, 2003). In *Heliconia*, the sepals initiate in an order influenced by the presence of the prophyll and the most recently formed flower. Sepals thus initiate on the side of the apex that is farthest from abutting tissue, proving the most space for development (Kirchoff, 2003). Because of this space limitation, sepal initiation is such that flowers in opposing rows of the cincinnus are formed with mirror-image symmetry (Kirchoff, 2003). This symmetry gives the flowers a chiral orientation within the cincinnus and dictates that the pattern of organ initiation will be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either clockwise or counterclockwise depending on the position of the floral meristem.

The petals also form in positions predicted by Hofmeister's rule, in a sequence that continues that of the calyx. However, the posterior staminode forms at the same time as the petals, which disrupts the sequence of later organ initiation. In fact, it is difficult to discern distinct petal and staminode primordia in some apices (Fig. 3I). In these apices, the posterior side of the flower resembles a ring-primordium such as is found in *Costus scaber* (Costaceae) (Kirchoff, 1988).

Hofmeister's rule is also partially observed in the initiation of the inner androecium. On the basis of prior whorl organ initiation patterns, one would expect both posterior, inner-whorl stamens to initiate before the anterior stamen. This successional pattern was only partially observed in our study, with the anterior stamen primordium often initiating simultaneously with one of the posterior stamen primordia. The timing of initiation of the posterior stamens may be influenced by the presence of the large staminode primordium, prompting the anterior stamen to initiate earlier than expected.

Staminode development—The Heliconiaceae is the earliest-diverging family in the Zingiberales that possesses a staminode (Kress et al., 2001). The more-derived ginger families have multiple petaloid staminodes that are an integral aspect of the mature floral architecture and display (Petersen, 1889; Schumann, 1902, 1904; Loesener, 1930a, b; Kennedy, 1978; Classen-Bockhoff, 1991; Kirchoff, 1991). In *Heliconia*, the staminode is small in size relative to the petals and stamen and does not form a significant part of the floral display, although it is hypothesized to play a role in guiding a hummingbird's tongue toward the nectar chamber (W. J. Kress, Smithsonian Institution and E. Temeles, Amherst College, personal communication).

Throughout early floral development, the staminode of *Heliconia* is more morphologically similar to the petal primordia than it is to the fertile stamen primordia. This similarity begins at the time of initiation, when the staminode arises simultaneously with the posterior petals and, in the majority of flowers studied, is confluent with these petals (although there is some degree of developmental plasticity at this stage). As subsequent stamen and gynoecial whorls are established, the staminode maintains its position in the posterior region of the petal whorl and is not, as would be expected, situated toward the center of the floral cup in line with the two fertile stamens of the outer androecium. The similarity continues through the latest stages of development studied and is arguably most apparent in the later stages (e.g., Fig. 6H, *stm*). This similarity disappears in the mature flower, in which the staminode is significantly smaller and of a different shape than members of the corolla (Andersson, 1992).

The affinity of the staminode to members of the androecium is demonstrated by its single strong vascular trace, a trait it shares with the fertile stamens of both the inner and outer androecial whorls. It can be concluded that despite the introduction of the staminode, the flowers of *H. latispatha* follow the "typical" monocot flower ground plan of three sepals, three petals, three members in each of two androecial whorls, and three carpels. Even though the staminode can superficially be described as an extension of the corolla in shape and position during floral ontogeny, it is anatomically a member of the outer androecial whorl. In this case, a simple comparative study of organ ontogeny could be misleading in establishing organ homology. It may, in fact, be inappropriate to assume that the staminode is morphologically homologous with either a petal or a stamen. Staminodes combine characteristics inherent to each of these fundamental organ types (Rutishauser and Sattler, 1985; Kirchoff, 2001; Rutishauser and Isler, 2001; Rutishauser and Moline, 2005).

From these results, it appears as though the staminode in *Heliconia latispatha* arose from a homeotic shift during early floral development. Characteristics normally associated with petal primordia, including the radial position of the primordium, size at initiation and developmental fate, have become associated with a primordium that is situated between two petals and is anatomically a member of the androecium. To validate this hypothesis, future studies will be aimed at localizing gene expression patterns in this organ as compared with surrounding petals and stamens, particularly with regard to floral organ identity genes and their downstream targets. Understanding the genes involved in the origin of the staminode will help elucidate the processes involved in floral evolution, particularly the evolution of the stamen whorls in Zingiberales.

Floral symmetry—The position of the staminode in *Heliconia* represents a shift in floral symmetry within the Zingiberales (Kirchoff, 2003). Within the order, two fundamental patterns of zygomorphy result from differential stamen suppression. In Strelitziaceae, Lowiaceae, and Musaceae, the adaxial, anti-petalous stamen is suppressed causing zygomorphy of the stamen whorl. In the ginger families, the abaxial, anti-sepalous stamen is either suppressed (Marantaceae, Zingiberaceae, Cannaceae) or incorporated into the staminodial labellum (Costaceae) (Troll, 1928; Maas, 1972; Kirchoff, 1988), resulting in a second pattern of zygomorphy (Fig. 1) (Kirchoff, 2003; Rudall and Bateman, 2004). In Marantaceae and Cannaceae, the flowers

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Fig. 3. Earliest stages of floral development in *Heliconia latispatha*. (A) Cincinnus with five flower primordia (f0–f5), occurring in two mirror-image rows. All but the youngest prophylls (pr) have been removed. The plane of floral symmetry is indicated on f4 (line). 1–3, sequentially formed sepals; ant, anterior side of the cincinnus and flowers (distal from the main inflorescence axis); ca, continuation apex; pos, posterior side of the cincinnus and flowers (adjacent to the main inflorescence axis). (B, C) Continuation apices (ca1, ca2) and adjacent flower primordia at two stages, showing the sequential formation of flowers (f2, f1, f0) and prophylls (pr1, pr2). (B) Continuation apex (ca1) before the formation of a flower and prophyll. (C) Enlargement of continuation apex ca1, and formation of new continuation apex (ca2) during the formation of a new flower (f0) and prophyll (pr2). (D) Young flower primordium as first sepal (arrow) is initiating. A floral cup (fc) has started to form at the center of the apex. (E) First and second sepal formation (1, 2), and third sepal initiation (arrow). The floral cup is deepening. (F) Third sepal (3) formation. 1, 2, first and second formed sepals; p1, location of first petal formation. (G) Deepening of floral cup (fc) before the appearance of the petals. 1–3, sepals. (H) Further deepening of the floral cup and separation of the petals (p1–p3) and first outer androecial member, the staminode (stm), from the raised periphery of the cup. 1–3, sequentially formed sepals. (I) Formation of the second outer androecial member (stamen; arrow). 1–3, sequentially formed sepals; p1–p3, sequentially formed petals. (J) Sequential formation of anterior outer androecial members (stamens; oa2, oa3). 1–3, sepals; fc, floral cup; p1–p3, petals; stm, staminode. (K) Later stage of outer androecial (stamen) formation. The periphery of the floral cup is beginning to be partitioned into distinct primordia. 1–3, sepals; oa2–oa3, sequentially formed outer androecial members (stamens); p1–p3, petals; stm, staminode. All scale bars = 100 μm.

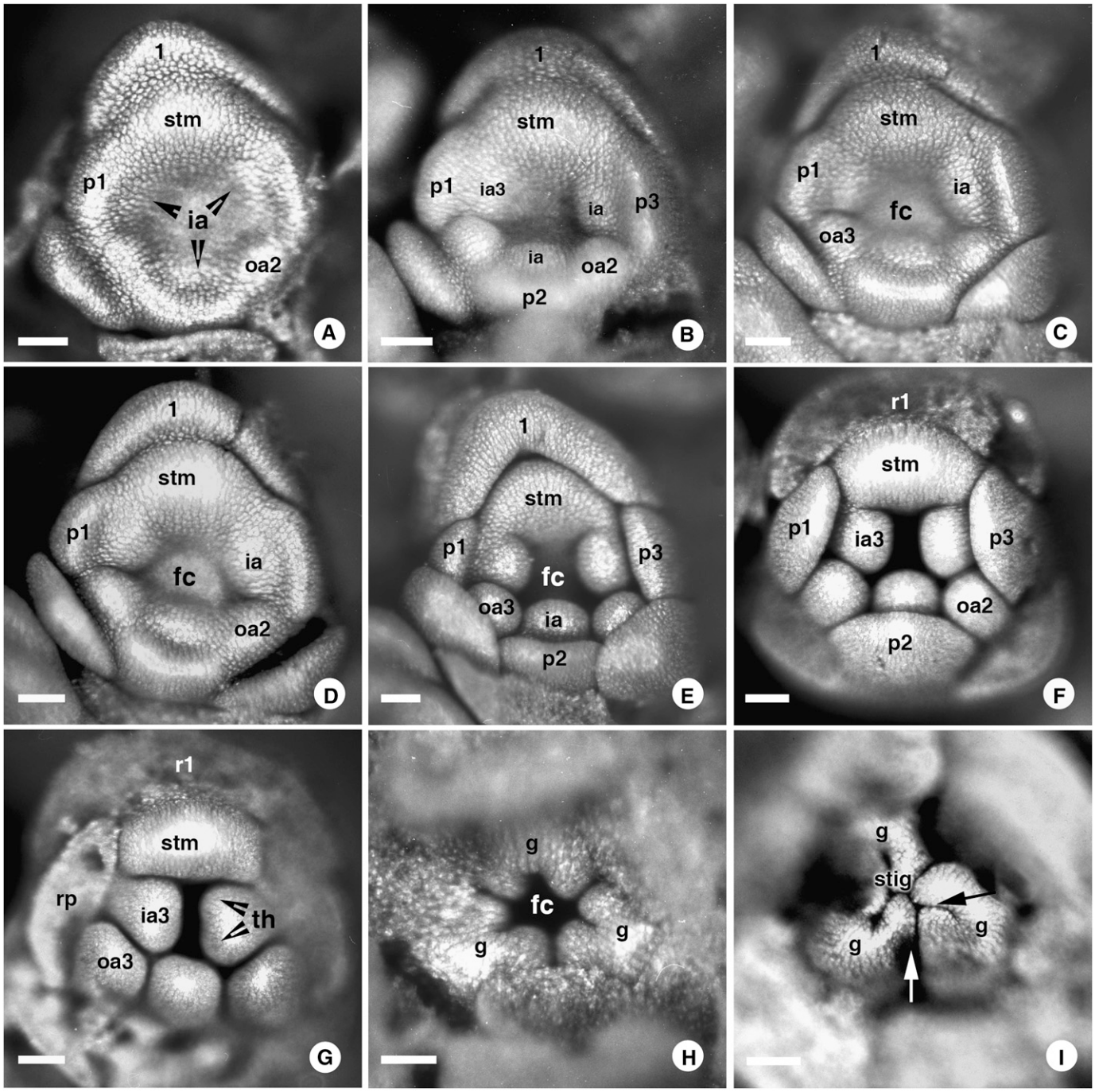

Fig. 4. Later stages of floral development in *Heliconia latispatha*. (A–C) Inner androecial formation. (A) Inner androecium initiation (ia). 1, first-formed sepal; oa2, second-formed stamen; p1, first-formed petal; stm, staminode. (B) Formation of all three inner androecial members (stamen). The initiation sequence of the first two stamens (ia) is variable. The third inner stamen (ia3) always forms last, in a posterior, lateral position adjacent to the midline of the cincinnus. 1, first-formed sepal; oa2, second-formed stamen; p1–p3, sequentially formed petals; stm, staminode. (C) Later inner androecial formation (ia). The petals (p1), staminode (stm) and outer androecial (oa3) primordia are still confluent, and the floral cup (fc) has further deepened. 1, first-formed sepal. (D–G) Enlargement of primordia. D. Acropetal growth of the petals (p1) is beginning to separate them from the staminode (stm) and outer androecium (oa2). fc, floral cup; ia, inner androecial member. (E). Organ enlargement has produced distinct sepals (1), petals (p1–p3), and outer androecial members (oa3). The posterior members of the inner androecium (unlabeled) remain laterally confluent with the staminode (stm). ia, inner androecial member. (F) The sepals have enlarged to cover flower and have been removed (r1). The staminode (stm) and posterior inner androecial members (ia3) remain confluent, though the apical portion of the staminode is becoming free. The staminode is the largest primordium at this stage and looks very similar to the petal primordia (p1–p3). oa2, second-formed outer androecial member. (G) Theca formation (th). The sepal (r1) and petal primordia (rp) have been removed. oa3, third-formed outer androecial primordium. (H) Gynoecial initiation. Three conduplicate gynoecial primordia (g) form on the inner margins of the floral cup (fc), opposite the sepals. They will extend basipetally to form the septa and acropetally to form the style and stigma. (I) Style and stigma formation. The three conduplicate gynoecial primordia (g) come into lateral contact (white arrow) to form the style and stigma (stig). Fusion between

are asymmetric, but the asymmetry is clearly derived from ancestral zygomorphy (Kunze, 1984, 1985). The situation in Heliconiaceae is different. In this family, the staminode is antisepalous but is not homologous to the abaxial, antisepalous stamen that is missing or transformed in the ginger families. A different antisepalous stamen has been modified to form the staminode (Kirchoff, 2003). This change, and the fact that there is only one staminode, causes the plane of zygomorphy to be dissociated from the medial plane of the flower. This condition is known as oblique zygomorphy (Eichler, 1878; Schumann, 1900; Winkler, 1930; Lane, 1955; Kunze, 1985; Kirchoff, 2003). Oblique zygomorphy also occurs in the Sapindaceae, Moringaceae, and Vochysiaceae, often in conjunction with flowers borne in partial cymes (Eichler, 1878; Ronse Decraene et al., 1998).

There is a strong correlation between zygomorphic flowers and racemose or similar inflorescences (Stebbins, 1951). Coen and Nugent (1994) hypothesized that this correlation could be due to either selective or developmental constraints. The developmental constraint hypothesis is supported by peloric mutants of *Antirrhinum* and other eudicots with zygomorphic flowers borne in indeterminate racemes. Terminal flowers of these racemes, when they occasionally occur in nature, are most often actinomorphic (Coen and Nugent, 1994; Rudall and Bateman, 2003). Additionally, the artificially induced *cen* mutant of *Antirrhinum* has a determinate inflorescence with an actinomorphic terminal flower, while all lateral flowers are zygomorphic (Coen and Nugent, 1994). These mutants, both natural and induced, suggest that the developmental environment of a laterally borne flower is necessary to generate zygomorphy. Oblique zygomorphy, however, suggests selective constraint. Oblique zygomorphy allows flowers to be borne at an angle appropriate for the approach of pollinators. For instance, in *Koelreuteria paniculata* (Sapindaceae), it has been hypothesized that oblique zygomorphy enables pollinator access to the flowers (Ronse Decraene et al., 2000). The situation in *Heliconia* is likely similar: the decoupling of the plane of symmetry from median plane of the flower allows for the correct orientation of the flowers with respect to pollination (Stiles, 1975; Kress, 1985; Pedersen and Kress, 1999).

Oblique zygomorphy is not merely a consequence of cymose branching within an inflorescence—examples of zygomorphic flowers in cymose inflorescences not displaying oblique zygomorphy exist throughout the angiosperms (e.g., Lamiaceae, Costaceae, Commelinaceae) (Eichler, 1878; Heywood et al., 2007). Taxa in the closely related Strelitziaceae do not have obliquely zygomorphic flowers although the inflorescence, including the enclosing bract, is of a similar form to that seen in Heliconiaceae (Kirchoff, 2003). In Strelitziaceae, final floral orientation appropriate for pollination is achieved by twisting of the pedicel (B. Kirchoff, unpublished observation; Eichler, 1878). Eichler (1878) also reported twisting of the pedicels of flowers in the Lamiaceae, which are borne in cymes. This twisting allows the flowers to be borne laterally, yet achieve the proper orientation for pollination. These examples suggest interplay between both developmental and selective forces in generating the correlation between zygomorphic flowers and inflorescence branching pattern.

Nectary structure and evolution—*Heliconia latispatha*'s nectary displays both interlocular and infralocular (sublocular) characteristics (Hartl and Severin, 1980; Kirchoff, 1992; Simpson, 1993). A major portion of the nectary occurs below the ovary (i.e., is infralocular or sublocular), while the rest extends up the length of the septae to the base of the style (i.e., is interlocular). Smets and Cresens (1988) coined the term "gynopleural nectary" to describe situations like this where elements of the secretory tissue form from incomplete fusion of the gynoecial primordia, but may not be solely interlocular. Gynopleural nectaries occur in the Cannaceae, Marantaceae, Strelitziaceae (Pai, 1963; Kronestedt and Walles, 1986; Kirchoff, 1992), and Musaceae (Kirchoff, 1992; Ren and Wang, 2007) and likely occur in the Costaceae (Newman and Kirchoff, 1992). Some doubt remains about nectary structure in the Costaceae due to their unusual anatomy. The nectaries of the Costaceae and of the female flowers of the Musaceae are supralocular, though they differ considerably in anatomy (Rao, 1963; Fahn and Kotler, 1972; Fahn and Benouaiche, 1979; Kirchoff, 1992; Newman and Kirchoff, 1992). The nectaries of the male flowers of the Musaceae (Ren and Wang, 2007) and of the flowers of the Cannaceae, Marantaceae, and Strelitziaceae are all interlocular. The Zingiberaceae are unique in the order in having epigynous nectaries that do not appear to arise from the fused margins of the gynoecial primordia (Rao, 1963; Newman and Kirchoff, 1992; Kirchoff, 1997, 1998). Despite reports to the contrary (Larsen, 1998; Bernardello, 2007), the Lowiaceae lack nectaries (Kirchoff and Kunze, 1995; Liao et al., 1998; Sakai and Inoue, 1999), though the presence of nectary canals has been reported (Wen and Liao, 1999).

The distribution of nectaries in the Zingiberales suggests a parsimonious evolution of this character. The ancestral state reconstructs as gynopleural with this state being retained in the Musaceae, Strelitziaceae, Heliconiaceae, Marantaceae, Cannaceae, and likely in the Costaceae. Nectaries have been lost in the Lowiaceae, and a transformation to epigynous glands has taken place in Zingiberaceae. Specializations in gynopleural nectary structure have occurred in the female flowers of the Musaceae (supralocular nectaries), Heliconiaceae (infralocular nectaries), and Costaceae (specialized supralocular nectaries). The unique structure of the nectaries in the Costaceae (Newman and Kirchoff, 1992) obviates any close relationship with the supralocular nectaries found in the female flowers of the Musaceae (Fahn and Kotler, 1972; Fahn and Benouaiche, 1979; Kirchoff, 1992).

Heterochrony in organ initiation—A major heterochronic shift is noted with respect to gynoecium development in *Heliconia latispatha*. As compared to other members of the Zingiberales, *H. latispatha* displays a later onset of gynoecial initiation (Kirchoff, 1983b, 1988, 1997, 1998; Kirchoff and Kunze, 1995; Box and Rudall, 2006). By the time the gynoecial primordia appear in *Heliconia*, the stamens have enlarged, and the floral cup is well developed, indicating a significant lengthening of the floral meristem. In the other families that have been studied, the gynoecium initiates immediately after androecial formation before any significant deepening of the floral cup (Kirchoff, 1983b, 1988, 1997, 1998; Kirchoff and Kunze, 1995; Box and Rudall, 2006). In *Scaphochlamys kunstleri* (Zingiberaceae), the

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primordia is incomplete at the base of the style, allowing flow of nectar from the gynopleural nectaries into nectar chamber. Fusion of the lateral conduplications in a single primordia is not complete (black arrow), forming the stylar canal that connects the stigma to the locules. All scale bars = 100 μm.

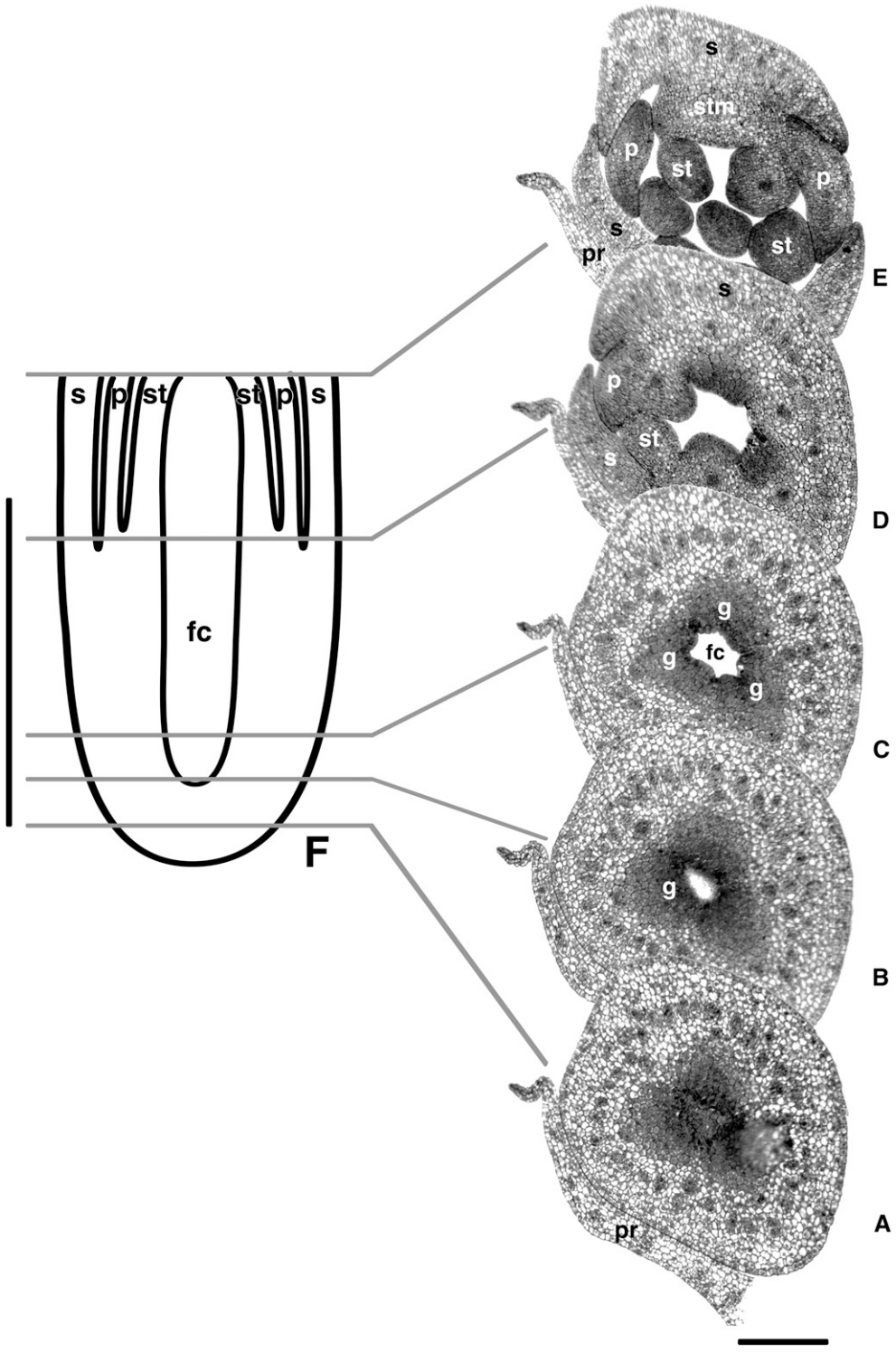

Fig. 5. Ovary development; cross sections at an early stage of gynoecial formation. (A–E) Sequential sections of young ovary showing conduplicate gynoecial primordia (g) surrounding the central floral cup (fc). (F) Schematic representation of the sectioned ovary showing the relative positions of the sections. p, petal; pr, prophyll; s, sepal; st, stamen; stm, staminode. Vertical and horizontal axes of (F) are not drawn to the same scale. Scale bars = 100 μm.

gynoecium initiates at approximately the same time as petal formation, while the floral cup is still quite shallow (Kirchoff, 1998). Further investigation of other species of *Heliconia* is needed to determine whether this heterochronic shift is specific to all members of the family and to identify a potential role in floral development.

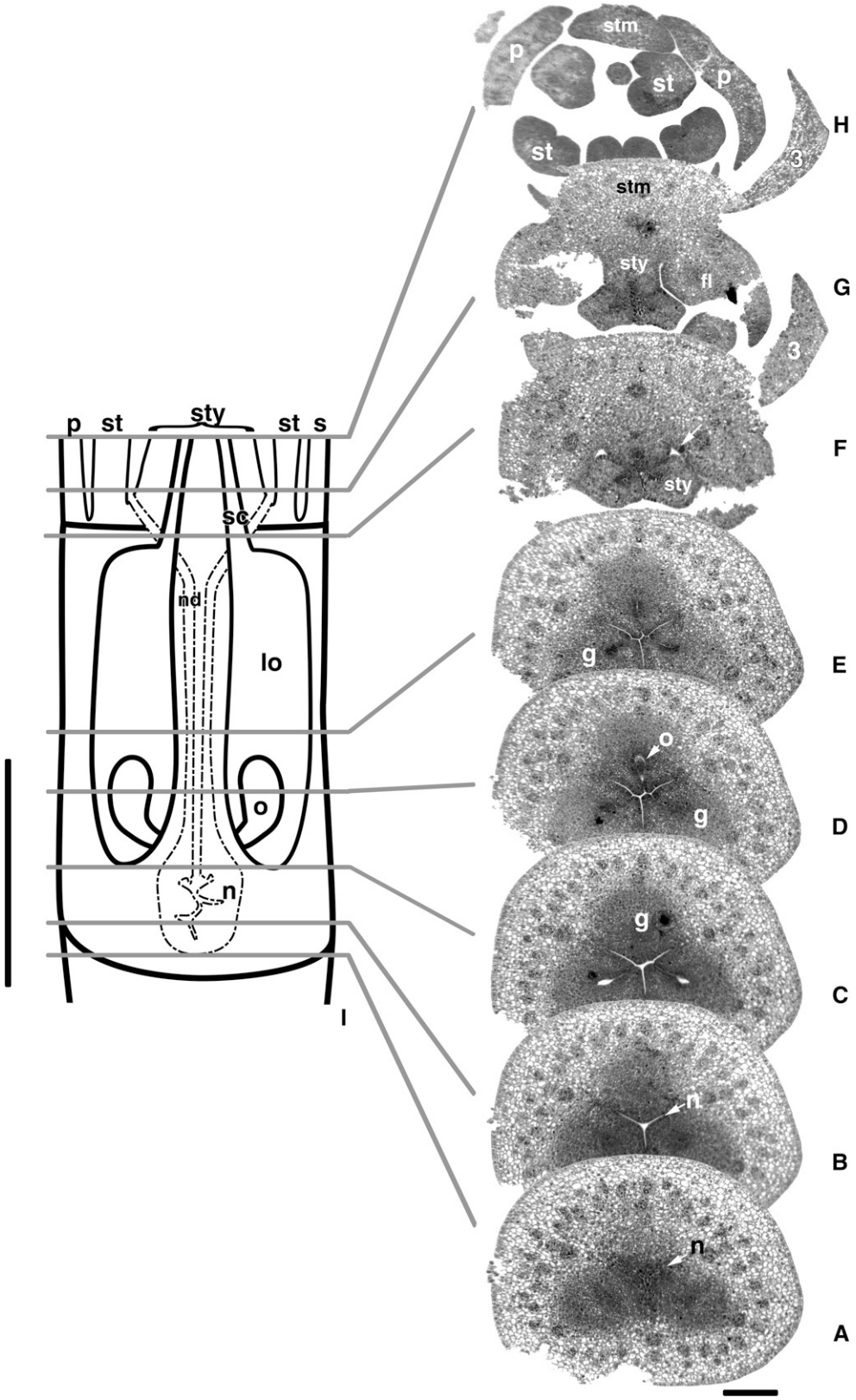

Fig. 6. Ovary development; cross sections during stigma and style (sty) formation. (A–H) Sequential sections showing nectary (n) formation, and conduplicate gynoecial primordia (g) each surrounding a single ovule (o). The young style (sty) extends above the top of the ovary. (I) Diagrammatic longitudinal section of a mature ovary showing relative positions of the sections. 3, third-formed sepal; arrow (on F), exit of the nectary duct at the base of the style; fl, filament; lo, locule; n, nectary duct; o, ovule; p, petal; s, sepal; sc, stylar canal ; st, stamen; stm, staminode. Vertical and horizontal axes of (I) are not drawn to the same scale. Scale bars = 100 μm.

Potential phylogenetic characters from developmental stages—Heliconiaceae is an important family in which to study floral structure and developmental evolution because of its combination of apomorphic and plesiomorphic characters at the level of the order. With respect to the androecium, *Heliconia* maintains the plesiomorphic characters found in the banana lineages, but it also possesses a mature staminode. The possession of staminodes is a well-characterized synapomorphy of the ginger families. The co-occurrence of plesiomorphic and derived characters within the stamen whorls of *Heliconia* provides us with the opportunity to tease apart the evolution of the stamen whorls across the Zingiberales (Kress et al., 2001; Rudall and Bateman, 2004).

Most phylogenetic analyses with broad sampling across the Zingiberales have placed Heliconiaceae as sister to the ginger families (Kress, 1990, 1995; Kress et al., 2001), a phylogenetic position supported by the possession of a staminode in the adult flower making this character a synapomorphy for the clade of Heliconiaceae plus the ginger families. However, molecular and morphological characters that help to unambiguously resolve the early divergence events within the Zingiberales have been difficult to find. While DNA sequences and mature plant morphology have not yet yielded sufficient data for this purpose, developmental characters may provide additional phylogenetic signal and may provide some insight into the earliest stages of floral developmental evolution across the Zingiberales.

Developmental studies have the potential to elucidate a great number of phylogenetically useful characters (Mabee, 2000; Wiens, 2000), but have been underrepresented in phylogenetic analyses due largely to the difficulty of gathering and analyzing comparative ontogenetic data (Scotland et al., 2003). Kirchoff (1988) described five useful characters for phylogenetic analysis gathered from floral development within each of the four ginger families and has more recently proposed new methods for extracting data from developmental sequences (Kirchoff et al., 2007). Many of these characters are not a direct manifestation of mature floral morphology and thus provide independent assessments of homology that could be useful in phylogenetic reconstruction (Poe and Wiens, 2000). This study of *Heliconia* adds to the body of knowledge of floral ontogeny within the Zingiberales. While our results do not disagree with the placement of the Heliconiaceae as sister to the ginger families, a full evaluation of our data in a phylogenetic context must wait until we have sufficient developmental data for all families to establish character homology, investigate character state evolution, and clearly identify apomorphic and plesiomorphic character states within the ingroup. Further research on Musaceae and Strelitziaceae will ultimately provide a comparative context for defining developmental characters and character states across the order. A future study utilizing developmental characters from all eight families may help to resolve some of the questions concerning phylogenetic evolution within the Zingiberales.

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