The Flowers of *Fragaria × ananassa*: Morphology, Response to Photoperiod, and Genetics of Induction

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ABSTRACT

The common cultivated strawberry (*Fragaria × ananassa*) is a healthy and popular fruit throughout the world, but its octoploid genetic structure poses difficulties to breeders, and the plant's flowering response to temperature and photoperiod has been challenging to predict, resulting in multiple flowering phenotypes throughout the commercial germplasm. This review assesses the morphology and physiology of these phenotypes, the cultural practices which are common to each flowering response, and focuses on recent efforts to map the genetic basis of day-neutrality within *F. × ananassa* and its progenitor *Fragaria vesca*. We summarize the recent consensus observing that the genetics of day-neutral flower induction in diploid *F. vesca* and octoploid *F. × ananassa* are not orthologous, and discuss the variance of findings regarding determination of day-neutrality in octoploid cultivars.

KEYWORDS: strawberry, day-neutral, diploid, octoploid

I. INTRODUCTION

The strawberry (*Fragaria* spp.) is one of the most widely distributed fruit crops in the world. Production of the fruit is present in almost every continent and has exceeded 4 million tonnes per year since 2007 (Wu *et al.* 2012). There is considerable genetic diversity within strawberry germplasm; wild diploid through decaploid plants have been discovered (Stewart and Folta 2010). This diversity leads to genotypic and phenotypic variance even within the same strawberry species. Perhaps the most commercially important variance is that of flowering habit within the commercially cultivated strawberry *Fragaria × ananassa*.

Because of its commercial value and popularity, the strawberry is a thoroughly documented fruit crop. The purpose of this review is to compile and contrast the morphologic and physiologic traits of *F. × ananassa* flowering types and review the most recent efforts to identify the underlying genetics behind flowering habit.

II. STRAWBERRY GROWTH, REPRODUCTION, AND COMMERCIAL MANAGEMENT

A. Vegetative Growth

The strawberry plant is an herbaceous perennial with short internodes forming a modified stem rosette (Savini *et al.* 2005). This modified stem is commonly known as a crown, where long-petiole trifoliate leaves and axillary meristems converge spirally around its axis, ending in a terminal inflorescence (White 1927). Strawberry leaves present a typical dicotyledonous structure with long petioles and foliaceous basal stipules (Savini *et al.* 2005). Leaf lifespan can exceed three months in favorable conditions (Poling 2012). Axillary meristems can differentiate into branch crowns, which stay near and are structurally identical to the original crown, or *stolons* (also called *runners*), which give rise to separate daughter plants (Fig. 1.1) (Demchak 2010). Crowns typically

Fig. 1.1. Leaves and axillary meristems of cultivar 'Portola.' Photograph taken August 9, 2016, in Minnesota.

produce one to two branch crowns in a season, but have been known to produce more than five; from a production standpoint, three to four total crowns per plant is desirable, as more can result in decreased fruit size (Poling 2012).

B. Flower Structure

Inflorescences have two internodes, and develop terminally on the crown or branch crown of the plant in a structure known as a *dichasial cyme* (Savini *et al.* 2005). Dichasial cymes have a terminal, primary flower branch with opposite secondary branches beneath the terminal bud, leading to secondary flowers. In strawberry, the inflorescence is commonly known as a *flower cluster*, and the primary flower, known as the "king flower," typically bears the largest fruit. Secondary branches begin at the juncture of the first and second internodes; some inflorescences also have tertiary and quaternary branches and flowers (Fig. 1.2).
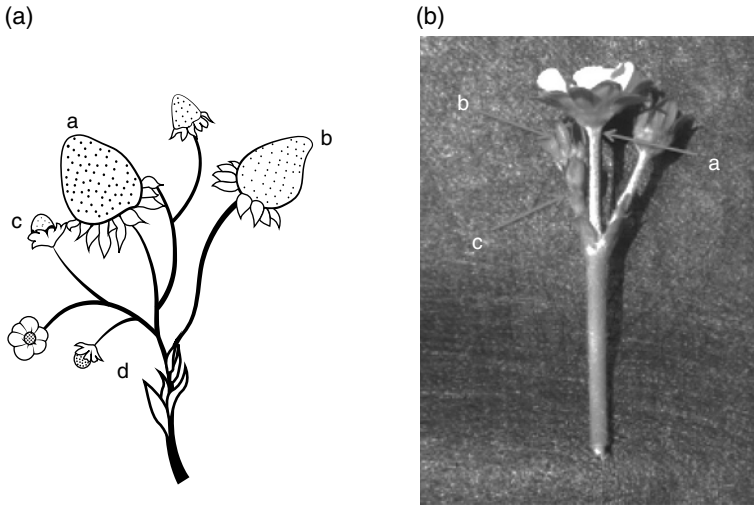
Fig. 1.2.   (a) Diagram of fully developed flower cluster with (a) primary flower, (b) secondary flower, (c) tertiary flower, and (d) quaternary flower http://www.hort.cornell. edu/grower/nybga/pdfs/2012berryproceedings.pdf (from Poling 2012). (b) Picture of a flower cluster of the cultivar 'Portola', with (a) primary flower, (b) secondary flower bud, and (c) tertiary flower bud. Photograph taken July 10, 2014, in Minnesota.
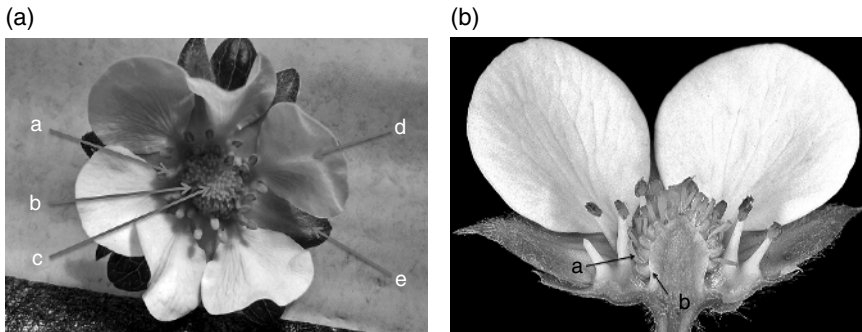

Fig. 1.3.   (a) Principal flower parts of cultivar 'Evie-II,' including (a) stamen, (b) pistil, (c) receptacle, (d) petal, and (e) sepal. Photograph taken July 10, 2014 in Minnesota. (b) Cross-section of *F.* × *ananassa* showing (a) pistil and (b) receptacle. Photograph obtained, with permission, from G.D. Carr, December 9, 2015; http://www.botany. hawaii.edu/faculty/carr/images/fra_sp.jpg.

The principal parts of the flower itself are shown in Fig. 1.3. Strawberry flowers have five sepals; fleshy green structures beneath the petals which enclose the flower at bud stage and eventually become the "calyx," or cap of the berry. Stamens discharge pollen and fertilize the pistils, which are secured on a conical stem known as the receptacle.
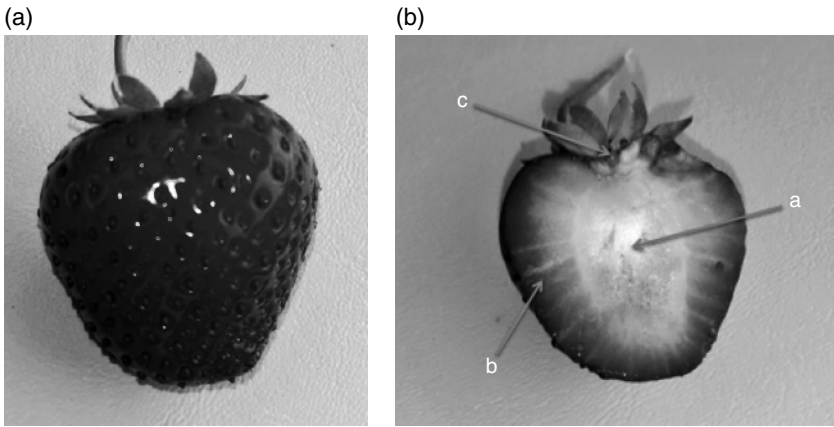
Fig. 1.4. (a) Profile of mature fruit of the cultivar 'Amandine,' with embedded achenes. (b) Cross-section of an 'Amandine' fruit, with (a) interior receptacle, (b) fibrovascular tube and (c) calyx. Photograph taken July 10, 2014, in Minnesota.

This receptacle becomes the full, fleshy "berry" at fruit maturity. Despite this plant's common name, the fruit itself is not botanically classified as a berry. The seed-like organs embedded on the epidermis of the receptacle are actually modified dry fruits known as *achenes*. The achenes are each connected to the interior of the receptacle by fibrovascular strands, and hold the true seed within their pericarp (Fait *et al.* 2008) (Fig. 1.4). In *F. vesca*, auxin and gibberellin biosynthesis occurs in the endosperm and seed coat of the developing achenes, which in turn triggers maturity of the surrounding receptacle (Kang *et al.* 2013). Because the strawberry fruit contains multiple achenes, and is comprised of a receptacle in addition to its ovaries, it can be classified both as an aggregate and as an accessory fruit.

C. Flower Induction, Initiation, and Development

Flower induction, initiation and development are highly variable by cultivar, and dependent on genotypic responses to temperature and photoperiod (Savini *et al.* 2005; Stewart and Folta 2010). These responses are commonly grouped into three flowering categories: June-bearing; everbearing; and day-neutral. Strawberry cultivars are typically classified under one of these three categories based on their photoperiodic flowering habits, and it was originally assumed these habits remained constant over a wide range of temperatures (Darrow and Waldo 1933). However, further research led to the discovery that the photoperiod response of many cultivars would be altered if

temperatures were either sub- or supraoptimal (Guttridge 1985; Nishiyama and Kanahama 2000; Sonsteby and Heide 2007). This interaction of temperature with photoperiod, known as *thermo-photoperiod*, adds a quantitative factor to the original categorical classifications. Indeed, some believe it incorrect to assign broad flower habit categories to strawberry at all, as photoperiod responses appear to be cultivar-specific (Durner 2015). However, as the vast majority of strawberry-based publications use these classifications, this review will utilize them as well, with the implicit understanding of variance and interaction even within each flowering type. In this section, photoperiod response and common cultural practices of the three groups assuming optimal temperature conditions will first be discussed. The way in which the responses have been observed to change under different temperature ranges will then be explored.

1. June-bearing. Natural flowering patterns of cultivated octoploid strawberry, *F.* × *ananassa*, are of the June-bearing type (Darrow 1966). June-bearing cultivars are predominantly grown for commercial purposes in the Upper Midwestern United States, where other flowering types have historically performed poorly (Durner *et al*. 1984; Luby *et al*. 1987; Luby 1989). June-bearing cultivars induce flowers under shortening daylengths, optimally from 9.5 to 13-h days, depending on cultivar (Darrow and Waldo 1933). The change in daylength over time in the United States Upper Midwest (specifically using Minneapolis, MN 44.9833° N as a representative point) compared to a more southern latitude, where strawberries are also grown (specifically using Santa Maria, CA 34.5914° N as a representative point), is shown in Fig. 1.5. The figure implies that flower induction would typically occur in mid-September for June-bearing cultivars in the Minneapolis area, until temperatures induce plants into dormancy. Savini *et al*. (2005) noted that June-bearing cultivars will also have flower initials before they enter dormancy. For many June-bearing cultivars the dormancy-inducing temperature is a high of 10 °C (Kronenberg *et al*. 1976). On average, this threshold temperature will be reached in early November in the United States Upper Midwest (Fig. 1.6).

As daylength and temperatures increase the following spring, June-bearing plants stop flower induction and divert resources into flower development (Salisbury and Ross 1992; Nishizawa and Shishido 1998). This induction-to-development shift leads to June-bearing plants bearing high fruit yields until the induced flower buds are depleted, typically in late June or early July. Thus, June-bearing strawberry plants can be considered to have short-day induction requirements

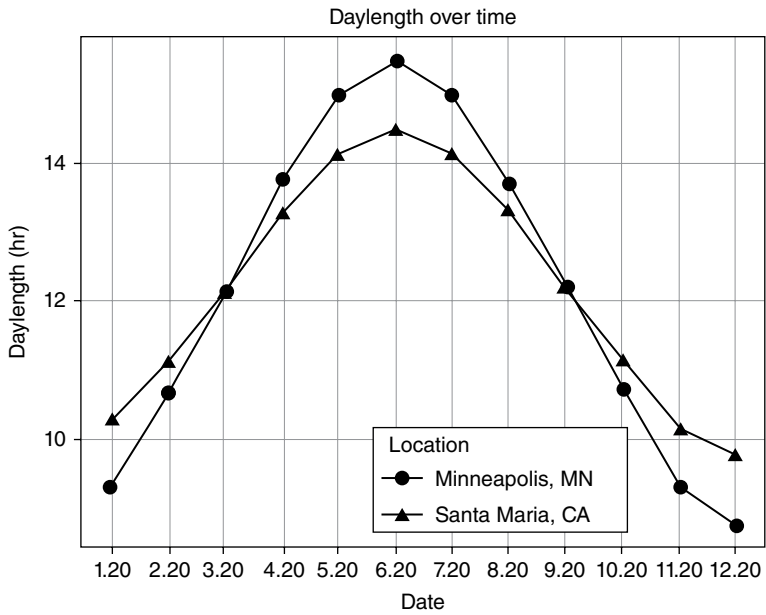**Fig. 1.5.** Average daylengths of Minneapolis, MN and Santa Maria, CA, taken on the 20th of each month. Raw data acquired from Time & Date AS: http://www.timeanddate.com/worldclock/astronomy.html?n=3857&month=12&year=2014&obj=sun&afl=-1&day=1.
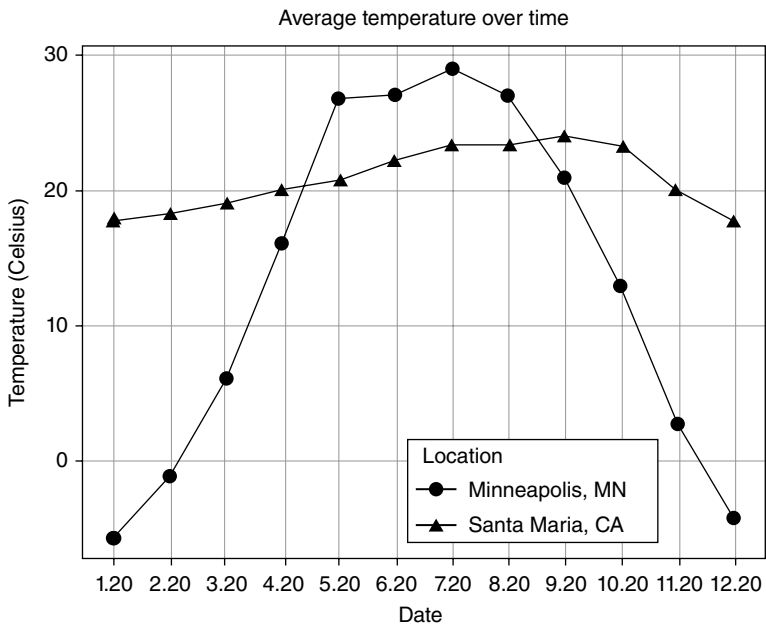

Fig. 1.6. Average high temperatures in Minneapolis, MN and Santa Maria, CA, taken on the 20th of each month. Raw data acquired from Intellicast: http://www.intellicast.com/.

and long-day development requirements. Under high temperatures (>30 °C), June-bearing plants will experience severely reduced flower development, even in optimal photoperiods (Serce and Hancock 2005). Savini *et al.* (2005) also noted that the morphology and differentiation time of inflorescences is based on the thermo-photoperiod that the plant is exposed to; June-bearing plants growing in warmer, short-day conditions tend to have faster and more prolific flower differentiation and shorter petiole lengths than plants exposed to long-day, cooler conditions.

Common cultural practices treat June-bearing strawberries as a perennial crop, typically using a "matted row" system. Rooted plugs of the June-bearing crop are planted in the spring of the first year (the "establishment" year). Flower clusters are typically removed during this entire first season, allowing the plant to divert more reserves into crown/branch crown development, root development, and runner production (Eames-Sheavly *et al.* 2003). June-bearing cultivars rarely establish runners during early season flower development. However, both flowering and runnering take place as daylength increases, and finally runners alone are developed during the hottest, longest photoperiods of the summer (Stewart and Folta 2010). Growers often arrange runners spatially from the crown to eventually root themselves, creating a thick, matted row of plants (Fig. 1.7) (Archbold and MacKown 1995). The plants then overwinter, and flower clusters induced during the short daylengths of fall are left on the plant the following spring for the first harvest. In this system, the number of leaves on each plant at the beginning of overwintering can be correlated with fruit production the following year (Poling 2012).

2. Everbearing and Day-neutral. The second and third flowering types, everbearing and day-neutral, are often considered synonymous, likely due to crossover in pedigrees. Everbearing cultivars include the diploid alpine strawberry *F. vesca*, along with various more common octoploids (Duchesne 1766; Fletcher 1917). Cultivars categorized as everbearing both induce and develop flowers under longer photoperiods, typically 12 h or more. Sironval and El Tannir-Lomba (1960) found that flower induction and development of *F. vesca* var. *semperflorens* was inhibited when plants were exposed to short-day treatments. Octoploid everbearing cultivars initiate most of their flowers on unrooted or recently rooted runners during the long days of summer, leading to fall harvests (Stewart and Folta 2010). The origin of the everbearing trait appears to have occurred separately in North America and Europe, as little crossbreeding occurred
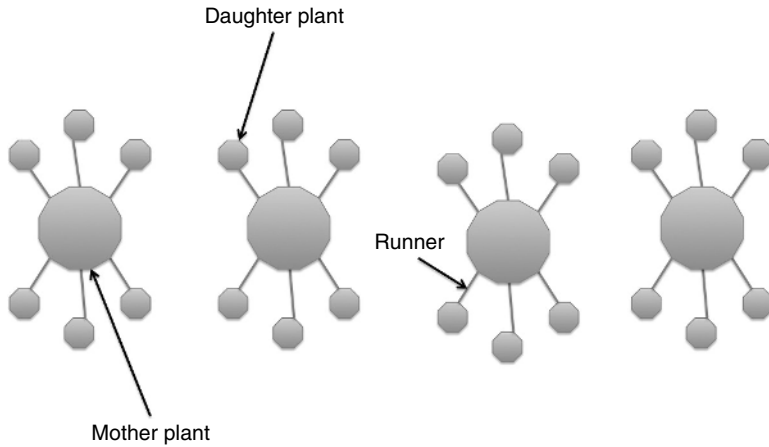
Fig. 1.7. Diagram of the matted-row system common to June-bearing cultivars.

between European everbearing *F. vesca* and North American everbearing *F. virginiana* cultivars (Stewart and Folta 2010). The North American everbearing phenotype is due to a single, unstable locus within the typical June-bearing genome (Stewart and Folta 2010), while the origin of the European everbearing trait is older and more difficult to identify (Darrow 1966).

The first recorded instance of a day-neutral phenotype was *F. virginiana* sub. *glauca*, and this was used as a parent in commercial everbearing breeding programs in the 1930s and 1940s (Darrow 1966). *F. vesca* may also display day-neutrality (Iwata *et al.* 2012). Many everbearing cultivars such as 'Arapahoe' and 'Ogallala' have day-neutral parents present in their pedigrees, which may contribute to why everbearing and day-neutral cultivars are sometimes thought to be the same (Hildreth and Powers 1941). However, true day-neutral cultivars often exhibit flowering habits that are phenotypically distinct from their everbearing relatives. The crowns of all day-neutral genotypes have a strong tendency to fruit proliferously in their first year, as opposed to most everbearing genotypes (Ahmadi and Bringhurst 1991). Day-neutral runners can also develop inflorescences before rooting occurs (Fig. 1.8). Just as important, day-neutral cultivars are historically documented as insensitive to changing photoperiods, fruiting at the same rate throughout a growing season of dynamic daylength (Durner *et al.* 1984). This distinguishes day-neutral cultivars from everbearing cultivars, which display long-day photoperiodism for flower induction and

Fig. 1.8. Day-neutral 'Monterey' runner, with developed inflorescence. Photograph taken July 10, 2014, in Minnesota.

development. These traits, in addition to increased heat tolerance (Stewart and Folta 2010), have contributed to abundant strawberry production in California, where day-neutral cultivars perform well. Other areas of the United States, such as the Upper Midwest, did not observe the same success, as day-neutral cultivars yielded poorly in Midwestern climates and were difficult to propagate (Durner *et al*. 1984; Luby *et al*. 1987; Luby 1989). This day-neutral market advantage allows California to account for 44% of the total national strawberry acreage and almost 90% of total yields, leading to a total revenue of $US 2.12 billion in 2012 (California Agric. Statistics Review 2014; National Agric. Statistics Service 2014).

In environments where they are commercially viable, day-neutral phenotypes are typically managed as annual plants in raised-bed systems with drip-tape irrigation and plastic mulch. An abundance of research has been conducted on cultivar/plastic combinations, with the consensus being that year-to-year and environmental variances across sites complicate the development of a single, optimal cultural practice for day-neutral production (Himelrick *et al*. 1992; Hughes *et al*. 2013). Recently, high tunnel structures that increase air and soil temperatures offer season extension potential, and have been shown to increase total

and marketable yields in day-neutral strawberry cultivars without pollination being inhibited by the closed structure (Kadir *et al.* 2006). However, there has been a documented increase in fungal disease incidence in high tunnel systems due to reduced air circulation (Kennedy *et al.* 2013).

   It is often considered good horticultural practice to remove flower clusters from June-bearing plants for the first four to six weeks after initial planting (Eames-Sheavly *et al.* 2003); this forces the plants to partition more metabolites into vegetative growth and runner production, making the perennial crop more productive in subsequent years. Flower cluster removal is also practiced in day-neutral production, even though day-neutral cultivars are often only grown as annuals. Interestingly, Lantz *et al.* (2009), when conducting a study in Garrett County, Maryland (39.2833° N), demonstrated no significant difference in total yield when day-neutral 'Seascape' plants did not have flower clusters removed compared to treatments where flower clusters were removed two and four weeks after planting.

3. Thermophotoperiod and Temperature Effects.   There is still some uncertainty regarding the photoperiodic nature of June-bearing, everbearing and day-neutral flowering habits. While the common consensus is that June-bearing cultivars display short-day flower induction, everbearing cultivars display long-day flower induction and day-neutral cultivars are truly photoperiod insensitive, additional research has led many to believe that the photoperiodic tendencies of strawberry cultivars can be altered with temperature (Durner *et al.* 1984; Sonsteby and Heide 2007). In many cases, cultivars classified under photoperiodic categories only display their classified flowering response in moderate temperature conditions; once a certain threshold temperature is exceeded, their photoperiodic nature changes. For example, Guttridge (1985) found that flower induction of certain June-bearing cultivars can occur under any photoperiod if temperatures are <15 °C. Nishiyama and Kanahama (2000) demonstrated that the day-neutral cultivar 'Hecker' had inhibited flowering at high temperatures (30 °C/26 °C) when long day lengths (>14 h) were not present. This implies that some day-neutral cultivars may display long-day flowering habits under high-temperature conditions. Indeed, Sonsteby and Heide (2007) found similar results when testing the cultivar 'Elan', leading them to conclude that "…everbearing strawberry cultivars, in general, whether of the older European-type or the modern Californian-type originating from crosses with selections of *F. virginiana* ssp. *glauca*, are qualitative (obligatory) LD plants at high temperature (27 °C), and quantitative LD

plants at intermediate temperatures. Only at temperatures below 10 °C are these cultivars day-neutral."

Such general statements should be avoided, however, since there is considerable variability in strawberry flowering and fruiting response to temperature, even within the June-bearing, everbearing, and day-neutral categories (Wagstaffe 2009). For example, Bradford *et al.* (2010) discovered that plants of the day-neutral cultivar 'Tribute' required long photoperiods for flowering after a threshold temperature of 26 °C was exceeded, while plants of the day-neutral cultivar 'RH-30' required short photoperiods for flowering once the temperature exceeded 23 °C. This variance of thermo-photoperiod within a flowering category suggests that study is merited on all cultivars of commercial significance, even if research has already been conducted on similar cultivars within their traditional photoperiod classification.

Temperatures can also affect fruit production in ways that are not related to photoperiod. Kumakura and Shishido (1995) observed that strawberry flower buds of everbearing cultivars aborted during periods of high temperature (30 °C), while Karapatzak *et al.* (2012) found that everbearing cultivars exposed to supraoptimal temperatures (30 °C/20 °C) experienced severely reduced pollen viability leading to significantly reduced yields. Similar supraoptimal temperature effects were observed with June-bearing cultivars (Ito and Saito 1962; Durner *et al.* 1984). Yield reductions likely manifest as a result of unviable pollen contributing to poor fertilization and misshapen fruit (Ariza *et al.* 2011). These reductions in pollen viability appear to be dependent on high night temperatures, as supraoptimal day temperatures with cool night temperatures did not result in reduced viability (Wagstaffe 2009). The effect of supraoptimal temperatures on flowering and yield in day-neutral cultivars is less thoroughly researched, though day-neutral cultivars have previously been regarded as being more heat-tolerant (Stewart and Folta 2010).

Suboptimal temperatures can also affect fruit development. Ariza *et al.* (2015) conducted a thorough analysis of cold temperature on differentiating inflorescences, and observed that chilling events (24 h at 2 °C) can reduce pollen grain production and viability as early as 20 days before anthesis, and increase ovule abortion three to six days before anthesis. These events would be especially deleterious for June-bearing plants, as all June-bearing flower buds develop in the spring when chilling events are more likely to occur. A chilling event on day-neutral plants may also inhibit fruit production on developing inflorescences, but since day-neutral plants tend to produce inflorescences throughout the growing season it likely would not have as large as an

effect on cumulative yields. In the diploid *F. vesca*, Davik *et al*. (2013) observed the accumulation of alcohol dehydrogenase, dehydrins, and galactinol as biomarkers associated with cold tolerance.

III. INFLORESCENCE ARCHITECTURE

Strawberry flower cluster anatomy has been thoroughly researched, as possible differences in inflorescence architecture have been hypothesized to correlate with differences in yield and berry weight among cultivars (Webb *et al*. 1978). Savini *et al*. (2005) documented the most common flower cluster and inflorescence anatomy in an architectural model, with primary, secondary, and tertiary flowers (Fig. 1.9a). Inflorescences that follow this architectural pattern appear to display two primary internodes leading to the primary flower, secondary branch internodes that form opposite the primary node and lead to secondary flowers, and tertiary internodes that form at the node of secondary branches, leading to tertiary flowers (Fig. 1.10). Unlike the Savini diagram (Fig. 1.9a), tertiary internodes can grow much longer than secondary internodes, making tertiary flowers appear "ahead" of secondary flowers (Fig. 1.10). Thus, the best way to distinguish secondary flowers from tertiary flowers is to compare differences in flower development; secondary flowers should be further advanced along the development path to mature fruit than tertiary flowers (Fig. 1.10). Interestingly, the formation of inflorescences from new branch crowns after planting follows the same architecture as flowers forming on individual inflorescences, with secondary branch crowns branching from the primary crown, and tertiary branch crowns branching from secondary branch crowns (Fig. 1.9b).

There is, however, observable variability from this typical inflorescence pattern, and of the day-neutral cultivars only 'Seascape' inflorescences have been formally documented (Hancock 1999; Savini *et al*. 2005). Figure 1.11a shows the June-bearing cultivar 'Annapolis' displaying the most typical inflorescence architecture, with day-neutral cultivars 'Albion' and 'Seascape' displaying similar habits (Figs 1.11b, g and h). 'Monterey' and 'San Andreas' inflorescences will sometimes only form a single secondary branch (Figs 1.11d and f). Occasionally, more developed inflorescences displaying this habit will create additional secondary branches, but these branches display an alternate growth habit, as opposed to the opposite secondary branching pattern of the more documented habit typical in 'Seascape.' The inflorescences of 'Evie-2' and 'Portola' sometimes appear to form two separate primary
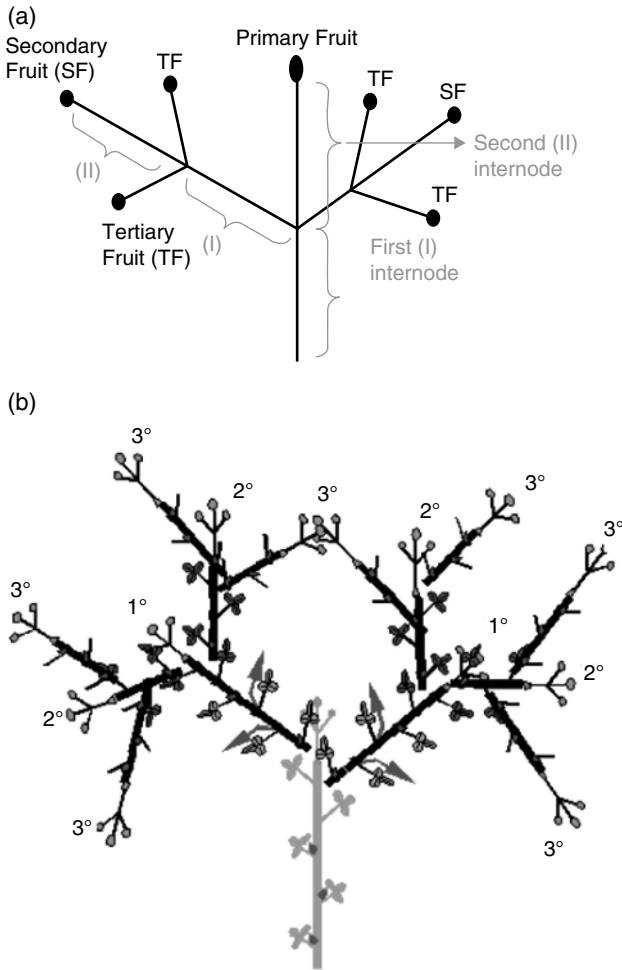
Fig. 1.9. (a) Diagram of a single, typical strawberry inflorescence, and (b) model of 'Seascape,' where 1°, 2°, and 3° represent primary, secondary, and tertiary inflorescences, which developed from buds after initial planting. From Savini *et al.* 2005; reproduced with permission from Taylor & Francis Ltd.

branches, forking off the first node (Figs 11c and e). Interestingly, 'Monterey' and 'San Andreas,' whose inflorescences typically only form a single secondary branch, were also the two lowest-yielding cultivars in 2013 University of Minnesota trials of day-neutral cultivars, while 'Evie-2' and 'Portola,' which seem to produce two primary branches, were the highest-yielding (Petran *et al.* 2016). While causation cannot be applied, these findings do raise the question of inflorescence architecture/yield relationships for further research.

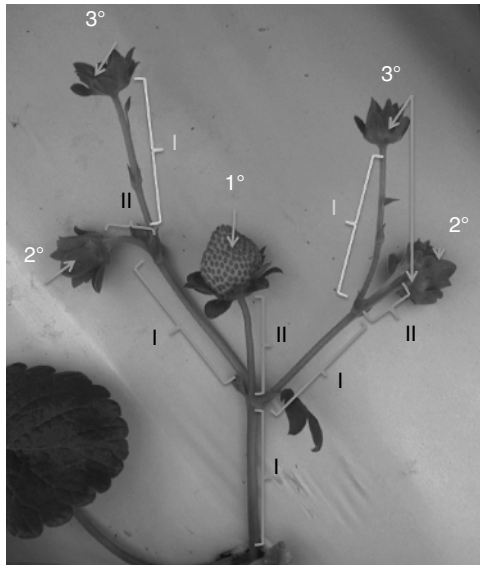**Fig. 1.10.** Photograph of 'Portola' inflorescence. 1°, 2°, and 3° represent primary, secondary, and tertiary flowers. Labeled brackets indicate primary (I) and secondary (II) internodes. Photograph taken July 15, 2014.
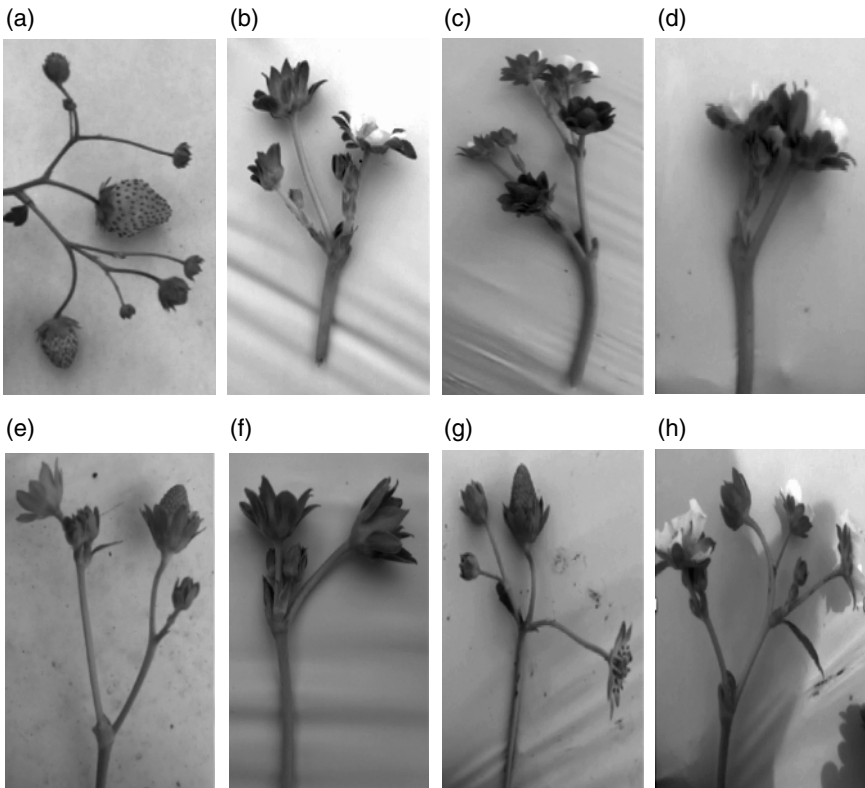

Fig. 1.11. Selected flower clusters of (a) 'Annapolis,' (b) 'Albion,' (c) 'Evie-2,' (d) 'Monterey,' (e) 'Portola,' (f) 'San Andreas,' and (g, h) 'Seascape.' Photographs taken July 15, 2014.

IV. GENETICS OF FLOWER INDUCTION

The underlying genetics that promote or inhibit flowering is complex and debated, and in order to appreciate that complexity in strawberry, the history of genetic flowering research in general can provide some background. The idea of *florigen*, a plant hormone (or family of hormones) responsible for flower initiation and development in all flowering plant species, was proposed by Chailakhyan (1936) after a series of grafting experiments. A quest to isolate and identify the florigen hormone took place thereafter, spanning the rest of the 20th century (Zeevaart 2006). The existence of florigen as a universal floral initiator was doubted after genetic research discovered multiple distinct flowering pathways in different species, but this dissonance was resolved after it was seen that the each separate pathway converged to a shared set of flower-promoting genes, the most well-known being *FLOWERING LOCUS T* (*FT*) (Koornneef *et al.* 1991; Samach *et al.* 2000; Simpson and Dean 2002; Putterill *et al.* 2004). Thus, florigens are indeed understood to be universal flowering inducers, but the production of florigen hormones is regulated by single genes in certain species and is polygenic in others. The protein produced by *FT*, now considered to be a florigen hormone, travels through the phloem to the shoot apical meristem and interacts with other proteins already present in the meristem to induce flower differentiation (Abe *et al.* 2005; Notaguchi *et al.* 2008).

Lifschitz *et al.* (2014) emphasized that, in addition to florigen, there are agents that act antagonistically to this pathway, known as *anti-florigens*. The protein of *TERMINATION FLOWER 1* (*TFL1*) in the model plant *Arabidopsis thaliana* is an anti-florigen that suppresses termination of the inflorescence and maintains vegetative growth in shoot meristems (Bradley *et al.* 1997). Lifschitz *et al.* (2014) proposed that not only florigens but also the florigen: anti-florigen ratio determines flowering times in short-day, long-day, and day-neutral flowering plants. Gene pathways that regulate florigen and anti-florigen production in strawberry must be discovered and understood before breeding efforts can be refined to select specifically for day-neutral habits.

While previous reviews have stated that no genes involved in strawberry flowering have been identified (Darnell *et al.* 2003), subsequent research has proposed specific floral promoters and suppressors within the strawberry genome. These discoveries are first reviewed within the diploid *F. vesca* genome, followed by the octoploid genome of commercial *F.* × *ananassa*.

A. *Fragaria vesca*

Fragaria vesca, commonly known as the woodland strawberry, shares morphologic and genotypic similarities with the more commercially significant, *F. × ananassa*. Despite its smaller size, the petal and sepal number, inflorescence architecture and early fruit development of *F. vesca* are notably similar to those of *F. × ananassa*, and the two species share a high degree of colinearity between their genomes (Rousseau-Gueutin *et al.* 2008; Hollender *et al.* 2012). Hollender *et al.* (2012) performed a thorough analysis of *F. vesca* floral development (Fig. 1.12), linking the genetic similarities to *F. × ananassa* with documentation of morphologic similarities as well.

To date, most strawberry genetic research has focused on *F. vesca* because of its relatively simple diploid genome compared to the octoploid *F. × ananassa* (Slovin and Michael 2011). *F. vesca* has an appealingly small genome size (~240 MB; $x = 7$), with a recently published reference genome (Shulaev *et al.* 2011) and several cultivars that have had success with *Agrobacterium*-mediated transformation (Folta and Dhingra 2006). Research within *F. vesca* has been undertaken with the belief that any genetic discoveries within *F. vesca* could be used as models for subsequent research within *F. × ananassa* (Weebadde *et al.* 2007; Hollender *et al.* 2012). Among the most commercially desired discoveries is that of flowering habit. Since *F. vesca* also contains multiple lines and cultivars with day-neutral flowering habits, it has been long hoped that understanding the genetic precursor to day-neutrality in *F. vesca* would serve as a springboard for similar discoveries in *F. × ananassa*; such a discovery would be highly beneficial to breeders looking to ensure a stable day-neutral habit in potential cultivar releases.

A genetic model for flowering habit in *F. vesca* was proposed by Brown and Wareing (1965) when F_1 and backcrossed progeny of seasonal × perpetual flowering parents segregated into 9:3:3:1 and 1:1:1:1 ratios, respectively. Such results indicated that a single gene controlled *F. vesca* flowering habit, with seasonal flowering as the dominant phenotype. Cekic *et al.* (2001) went further, using a type of microsatellite analysis (ISSR-PCR primer pair combinations) to identify two markers specific to *F. vesca* located near a single seasonal flowering locus. Finally, Iwata *et al.* (2012) discovered a specific anti-florigen *TFL1* homolog, *KOUSHIN* (*KSN*) within the genome of *F. vesca*, naming it *FvKSN*. A 2-bp deletion in the first exon of the *FvKSN* allele causes a frame shift, leading to a non-functional mutant *ksn*. All *F. vesca* strawberries displaying a continuous flowering habit were homozygous
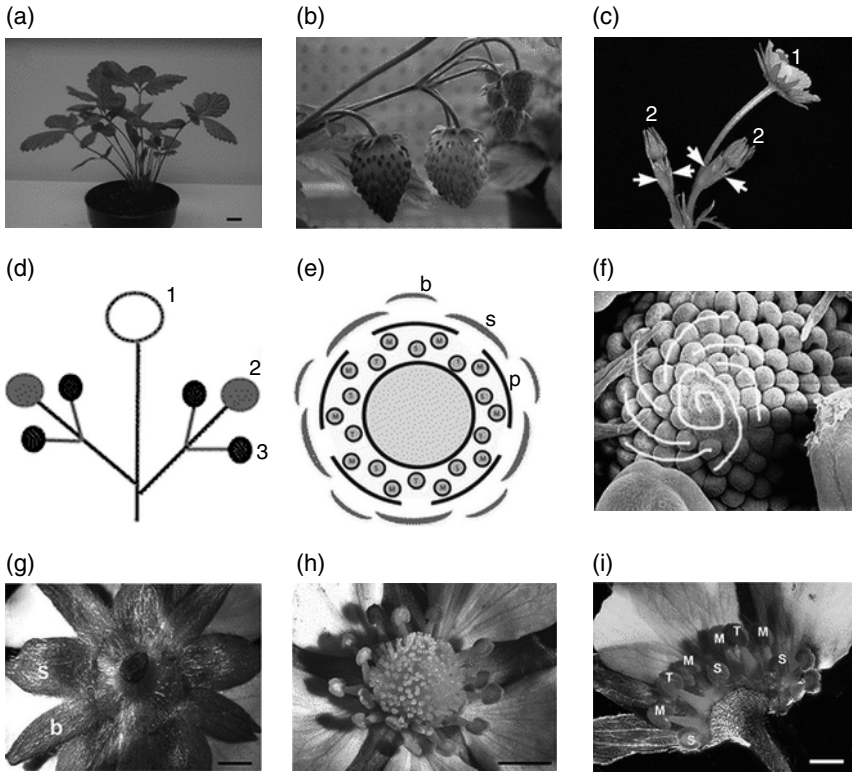
Fig. 1.12. *Fragaria vesca* shoot and flower development. (a) *F. vesca* YW5AF7 grown in a 10.2-cm pot; (b) YW5AF7 dichasial cyme bearing yellow berries; (c) Inflorescence with primary flower (*1*) and two developing secondary flowers (*2*). Young tertiary buds (*arrows*) are present beneath the secondary flower buds; (d) Diagram of shoot architecture. *Numbers* indicate primary, secondary, and tertiary flower buds; (e) Diagram illustrating floral organ arrangement. The two outer whorls are concentric rings of five bracts (*b*) alternating with five sepals (*s*). The third whorl consists of five white petals (*p*). Interior to the petals are two whorls of stamens. Stamens are arranged in a repeating pattern of five tall (*T*) and five short (*S*) in the inner whorl and 10 medium length (*M*) in the outer whorl. The *center circle* indicates a receptacle topped with numerous, spirally arranged carpels; (f) Scanning electron micrograph (SEM) of a developing floral bud, illustrating spirally arranged carpel primordial; (g) Abaxial view of a typical *F. vesca* flower with five narrow bracts (*b*) alternating with five wider sepals (*s*); (h) Adaxial view of typical *F. vesca* flower illustrating a whorl of five white petals, two whorls of ten stamens each, and an apocarpous gynoecium with ~160 pistils. (i) Dissected flower illustrating the "S, M, T, M, S" stamen pattern. Scale bars: (a) 2 cm; (g–i) 1 mm. From Hollender *et al.* 2012.

ksn/ksn, while once-seasonal flowering habits were either *KSN/ksn* or *KSN/KSN*. These results coincided with the findings of Koskela *et al.* (2012) that a *TFL1* homolog, which they named *FvTFL1*, served as an anti-florigen, expressing itself in long-day photoperiods. Transgenically silenced *FvTFL1* lines and lines overexpressing a mutated *FvTFL1* with a 2-bp deletion both displayed day-neutrality, supporting the findings of Iwata *et al.* (2012) (Fig. 1.13). It was later found that *FvSOC1* activates the *FvTFL1* anti-florigen gene in the shoot apex during long-day conditions; thus, *FvSOC1* is currently alleged as the photoperiod control center of floral differentiation in *F. vesca* (Mouhu *et al.* 2013).

B. *Fragaria × ananassa*

F. × ananassa contains a complex octoploid genome, yet progress has been made in identifying specific florigens and anti-florigens within the species. When examining the June-bearing cultivar 'Nyoho,' Nakano *et al.* (2015) observed mRNA accumulation of the gene *FaFT3* in the shoot tip under short-day (8 h), cool-temperature (13 °C) conditions just prior to the induction of a floral meristem identity gene, *FaAP1*. The protein of *FaFT2* was found in the flower bud shortly thereafter, and both of these appeared to act antagonistically to *FaFT1*, which accumulated in plant leaves under long-day (16 h) conditions. *FaFT1* was also associated with the upregulation of *FaTFL1* in shoot tips in warmer temperatures (27 °C). From these observations, Nakano *et al.* (2015) proposed that FaFT1, FaFT2 and FaFT3, all of which contain amino acid residues similar to the florigen FT in *A. thaliana*, work to regulate flowering in June-bearing *F. × ananassa*, with FaFT2 and FaFT3 functioning as florigens in short-day, cooler temperatures, and FaFT1 being a precursor to FaTFL1 anti-florigen production in long-day, warmer temperatures. Further work should be conducted to analyze how these genes and proteins interact in day-neutral *F. × ananassa*.

While Nakano *et al.* (2015) proposed homologs of the FaFT proteins in *F. vesca*, their studies did little to elucidate the cause of flower habit differentiation in *F. × ananassa* and its relation to flower habit control in *F. vesca*, because it only focused on the June-bearing cultivar 'Nyoho.' In fact, despite its morphologic and genetic similarities, a preponderance of research has indicated that the single-gene models of flowering habit in *F. vesca* cannot be directly applied to day-neutrality in the more complex octoploid *F. × ananassa* genome. Inconsistent inheritance ratios among different field environments imply that day-neutrality may involve auxiliary genes interacting with a single, dominant locus in octoploid strawberries (Serçe and Hancock 2005; Castro *et al.* 2015).
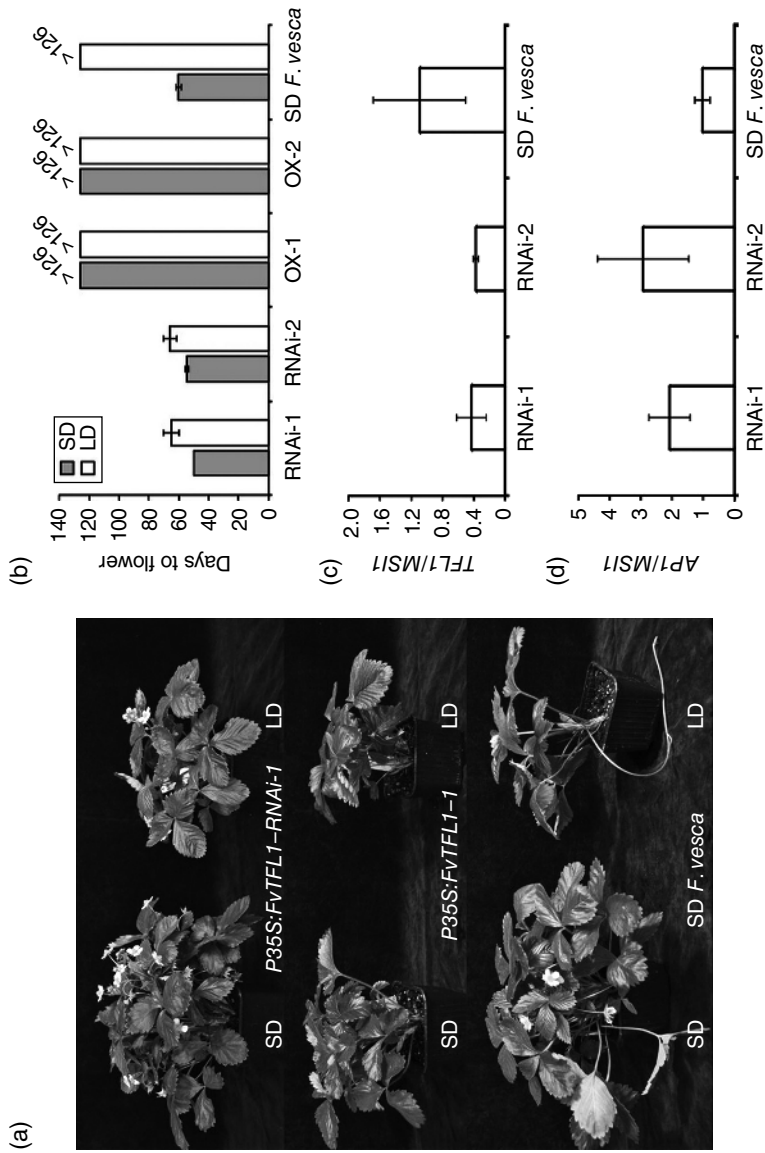
Fig. 1.13. Silencing of *FvTFL1* leads to daylength-independent flowering. (a) Phenotypes of *FvTFL1* RNAi silencing and overexpression lines in the short-day (SD) *F. vesca* background. Clonally propagated plants (runner cuttings) of SD *F. vesca* and *P35S:FvTFL1-RNAi-1* and *P35S:FvTFL1-1* lines were subjected to SD induction treatment for four weeks followed by long-days (LDs; left), or grown continuously under LDs (right); (b), Flowering time of SD *F. vesca* and *P35S:FvTFL1-RNAi* and *P35S:FvTFL1* plants (RNAi and OX, respectively) in SDs and LDs. Flowering time is indicated as days to anthesis from the beginning of the treatments. Treatments and plant materials were as described in (a). Values indicate mean±SD. $n=4$ (OX-1), $n=5$ (RNAi-2), $n=6$ (RNAi-1 and OX-2), and $n=7$ (SD *F. vesca*); (c,d) Expression of *FvTFL1* (c) and *FvAP1* (d) in the apices of two independent *P35S:FvTFL1-RNAi* (RNAi) lines. Values indicate mean±SD. $n=3$ (RNAi-1 and SD *F. vesca*) or $n=2$ (RNAi-2). From Koskela *et al.* 2012; © American Society of Plant Biologists, www.plantphysiol.org.

While this complexity certainly makes breeding for specific flowering habits more difficult, it is not a new phenomenon, as flowering is a polygenic trait in many other plant species (Hayama and Coupland 2004; Esumi *et al.* 2005).

In an effort to map the polygenetic sources of day-neutrality in *F. × ananassa*, Weebadde *et al.* (2007) conducted an amplified fragment length polymorphism (AFLP) marker analysis of the progeny of day-neutral 'Tribute' and June-bearing 'Honeoye' in five states throughout the United States. They found several (more than five) quantitative trait loci (QTLs) that were correlated with phenotypic variation in flower habit. Interestingly, no single QTL explained more than 36% of this variation, and the ability of a QTL to explain variation changed based on the location in which the cultivars were grown. For example, a QTL on LG 28 was a significant predictor of flower variation in plants grown in the hotter climates of every central and eastern state (Minnesota, Michigan, and Maryland), but the same QTL could not predict variation in California, where average temperatures are milder. Such a finding implied that not only may flower habit be a polygenic trait in *F. × ananassa*, but the genetic requirements for day-neutrality change based on other environmental factors. Weebadde *et al.* (2007) postulated that a minimum threshold of genes favoring day-neutrality must be present in order to achieve the habit, and the strength of a gene to contribute to reaching that threshold – that is, the necessity of a gene that confers heat tolerance in hotter climates – is dependent on its location. This genetic × environmental (GxE) theory helps understand previous research that found the photoperiodic nature of *F. × ananassa* to change based on temperature and light conditions (Guttridge 1985; Sonsteby and Heide 2007). Research investigating whether markers associated with flowering habit in *F. vesca* colocalize with flowering habit markers in *F. × ananassa* has yet to be conducted.

There is still some debate regarding the polygenic control of flowering habit in *F. × ananassa*. In a recent study, Gaston *et al.* (2013) used marker analysis to find a QTL named *FaPFRU* that they identified as the major controlling locus of day-neutrality and runner production in *F. × ananassa*. The study found day-neutrality to be dominant over seasonal flowering, suggesting that the genetic basis for flowering in *F. × ananassa* was not inherited from *F. vesca*, where seasonal flowering is dominant over day-neutrality. *FaPFRU* also appeared to control vegetative development, a discovery different from the locus controlling flower development in *F. vesca*, which does not control vegetative growth (Koskela *et al.* 2012). While acknowledging the polygenic evidence provided by previous research (Hancock *et al.* 2002;

Weebadde *et al.* 2007), Gaston *et al.* (2013) still proposed day-neutrality in *F.* × *ananassa* to be under single-locus control by *FaPFRU*. Indeed, *FaPFRU*, located on the IVb-f linkage group explained up to 59.3% of phenotypic variation in flower habit in that study (Table 1.1). Honjo *et al.* (2016) went on to affirm the findings of Gaston *et al.* (2013) while proposing their FxaACA02I08C marker, also located on LG IV, to be an accurate marker of day-neutrality.

The dissonance regarding genetic control of *F.* × *ananassa* flower habit in the current literature may be due to the GxE interaction effects described previously. The studies by Gaston *et al.* (2013) and Honjo *et al.* (2016) were each conducted in only one location (Villenave d'Ornon, France, 44.7806° N, 0.5658° W and Morioka City, Japan, 39.46° N, 141.8° E, respectively) and, while *FaPFRU* explained a majority of the phenotypic variation in the study of Gaston *et al.* (2013), the effect of this gene may be lessened in regions with different environmental conditions, as was observed with the LG 28 QTL by Weebadde *et al.* (2007). Before *FaPFRU* can be universally accepted as the single-control locus of flowering habit in *F.* × *ananassa*, its predictive strength would also have to be tested in areas such as Maryland and coastal California, where environmental pressures against day-neutrality appear to be high and low, respectively (Hancock *et al.* 2002; Weebadde *et al.* 2007). If *FaPFRU* remained a strong predictor of phenotypic variation in flower habit in each environment, the claim of Gaston *et al.* (2013) that *FaPFRU* is the major controlling locus of flower habit in *F.* × *ananassa* would be strengthened.

Indeed, such a project was conducted by Castro *et al.* (2015). Crosses of day-neutral 'Tribute' and June-bearing 'Honeoye' were qualitatively and quantitatively scored for day-neutrality in five different states in the United States with contrasting temperature conditions: Beltsville, Maryland (MD), East Lancing, Michigan (MI) and Saint Paul, Minnesota (MN), where average maximum summer temperatures are at least 28 °C; and Watsonville, California (CA) and Corvallis, Oregon (OR), where average maximum temperatures are at 22 °C and 26 °C, respectively (National Centers for Environmental Information, 2016). The authors also used 267 molecular markers in an attempt to construct a map of linkage groups associated with flower habit in each environment. As expected, even with the same parents, a higher proportion of progeny were scored as day-neutral in cooler CA than in hotter MD, reinforcing the role of GxE interaction in flower habit (Fig. 1.14). Similar to the findings of Gaston *et al.* (2013), the day-neutral:June-bearing inheritance ratio was 1:1 in the hotter MD, MI, and MN environments, suggesting single-locus control. However ratios were 3:1 in CA and 5:3 in OR,

Table 1.1. Map positions and genetic effect of QTL detected for the PF (perpetual flowering) and RU (runnering) traits for the female (f) parent 'Capitola' and male (m) parent 'CF1116.' QTL identification was based on composite-interval mapping analysis with LOD>LOD threshold (3.1) (α=0.05). From Gaston *et al.* 2013; reproduced with permission from Oxford University Press.

Trait	Linkage group	QTL name[a]	Year[b]	No. QTL[c]	Marker[d]	Position[e]	LOD[f]	r^2 [g]	Effect[h]
PF	Id-m	PF-LGId-m	2002	1	ccta185	6.01	3.2	10.3	+5.7
	Id-m	PF-LGId-m	2002	1	gata148	21.11	3.4	8.7	−7.6
	Id-m	PF-LGId-m	2007	1	ccaa267	43.71	3.7	8.5	+3.4
	IVb-f	PF-LGIVb-f	2002/2003/2003b/2004/2005/2007	6	gatt284	2.0/4.0	14.3–33.5	36.5–59.3	+6.0–10.7
	IVd-m	PF-LGIVd-m	2002/2003	2	tgac408/tgta115	60.06/55.57	3.2/3.7	10.5/13.4	+6.1/4.0
	Va-f	PF-LGVa-f	2007	1	u009180	20.01	3.3	5.9	−2.9
	vIc-f	PF-LGVIc-f	2007	1	v013200	84.47	3.1	4.0	−2.5
RU	IIc-m	RU-LGIIC-m	2005	1	gctg207	54.59	3.1	7.2	+1.2
	IIIb-m	RU-LGIIIb-m	2002	1	gtag280	54.86	3.7	7.4	+1.0
	IIId-m	RU-LGIIId-m	2002	1	tgac108	15.51	4.1	8.1	+1.0
	Ivb-f	RU-LGIVb-f	2002/2003/2005	3	gatt284	0.0/2.0	5.7–26.7	12.4–51.1	−1.5–4.8

[a]Name of the unique QTL.

[b]Year of observation of the QTL.

[c]Number of significant QTL.

[d]The left marker associated with the QTL is indicated.

[e]Position indicates the distance in cM of the QTL from the top of the chromosome.

[f]LOD is the log-likelihood at that position.

[g]r^2 is the percentage of phenotypic variation explained by the QTL.

[h]Mean effect on a trait mean value of the presence of one allele at a marker by comparison with the presence of the second allele. + and − indicate the direction of the additive effect. A positive effect means a higher value for the Capitola allele on the female map or a higher value for CF1116 on the male map.
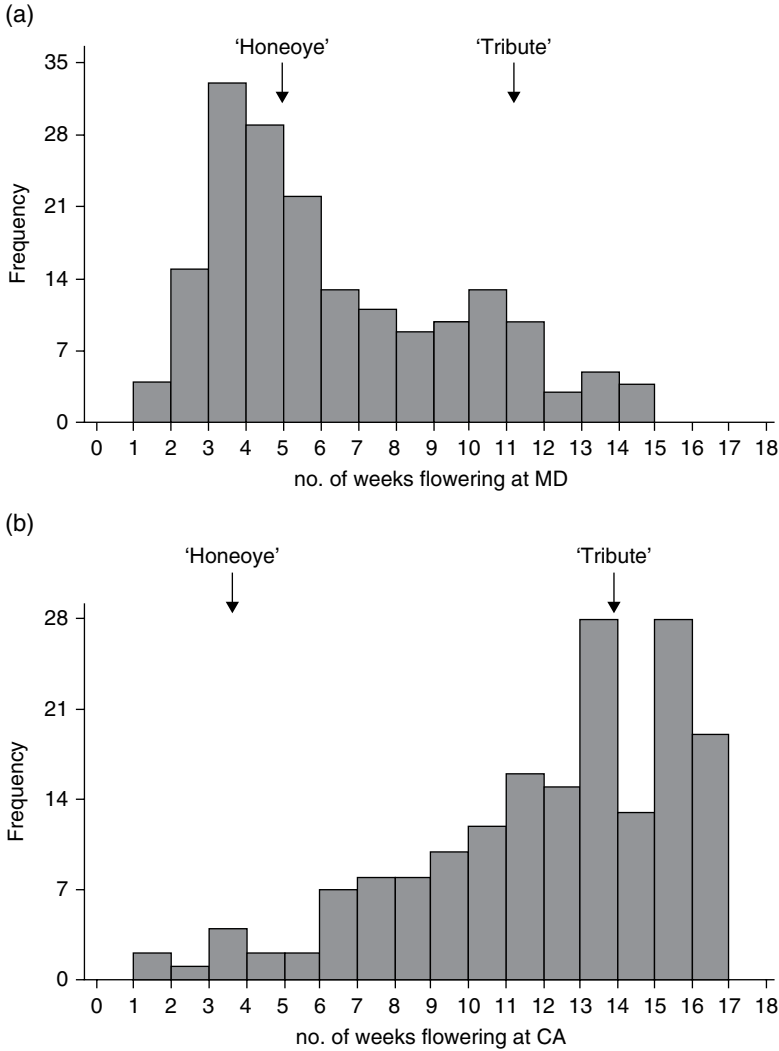
(a)

(b)

Fig. 1.14. Frequency distribution of number of weeks flowering at Maryland (MD) (a) and California (CA) (b). The phenotypic values of the parents, 'Tribute' and 'Honeoye' are indicated by arrows (Castro *et al.* 2015).

implying the presence of multiple alleles at one or more loci that only express themselves in cooler temperatures.

Through their marker analysis, Castro *et al.* (2015) found that day-neutrality could be mapped to a specific genetic region on linkage group (LG) IV-T-1 of the 'Tribute' map – a region with multiple
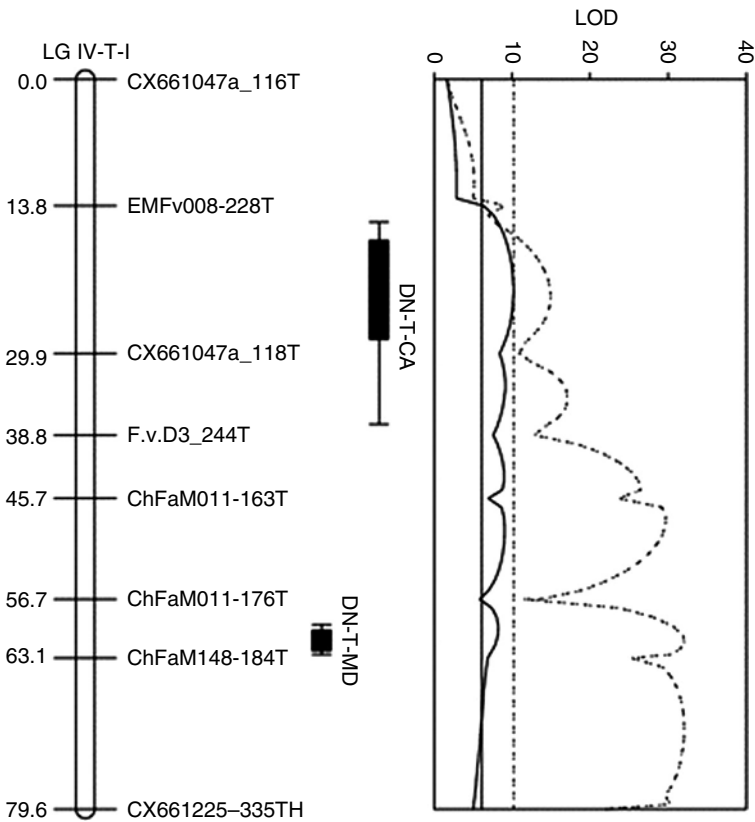
Fig. 1.15. Quantitative trait loci (QTL) for number of weeks of flowering detected in the octoploid strawberry (*F.* × *ananassa* Duchesne ex. Rozier) population 'Tribute' × 'Honeoye' evaluated in Maryland (*dotted line*) and California (*solid line*) using interval mapping (IM) (Castro *et al.* 2015).

QTLs – regardless of environment (Fig. 1.15). In MD, a QTL on LG IV-T-1 explained between 63.9% and 63.6% of the phenotypic variation, while in CA a separate QTL on the same LG explained between 51.2% and 50.1% of the phenotypic variation. These results imply that while day-neutrality may be under single-locus control in certain environments, multiple loci within the same genetic region may be expressed in cooler environments, and "…the distortion toward day-neutral progeny found in OR and CA presents a slight challenge to the single-locus theory and should be remembered in future research" (Castro *et al.* 2015). The single-locus control apparent in warmer regions seems to mirror the genetic findings of Gaston *et al.* (2013) and Honjo *et al.* (2016), but the QTL found on LG IV-T-1

could not be confirmed as *FaPFRU* because the studies did not use common markers to identify QTLs.

Taken together, research on the genetics of strawberry flower induction has shown *F. × ananassa* to contain more complexity than *F. vesca*. Control of day-neutrality in *F. vesca* is almost certainly achieved by a single locus (Cekic *et al.* 2001; Iwata *et al.* 2012; Koskela *et al.* 2012), and while the inheritance of flower habit in *F. × ananassa* is more uncertain, it appears that GxE interactions lead to single-locus control in hotter regions and potential multi-locus control (albeit on the same linkage group) in cooler regions (Weebadde *et al.* 2007; Gaston *et al.* 2013; Castro *et al.* 2015; Sooriyapathirana *et al.* 2015). While the understanding of induction in *F. vesca* was proposed as a springboard for induction research and consequent breeding efforts in *F. × ananassa* (Hollender *et al.* 2012), the support for this proposal remains unrealized; in fact, recent evidence suggests that the genetics of flower induction in *F. vesca* and *F. × ananassa* are not orthologous (Gaston *et al.* 2013). Regardless of this relation, continued research into the inheritance of day-neutrality in commercial *F. × ananassa* will assist breeders in releasing cultivars with extended yields in diverse environments throughout the world.

V. CONCLUSIONS

The strawberry is an extensively documented horticultural crop. There appears to be adequate information regarding the growth and reproduction habits of commercial *F. × ananassa* and its progenitors *F. vesca*, *F. virginiana*, and *F. chiloensis*. Within the literature there is a focus on *F. × ananassa* flowering habit, as this response represents the aspect of growth most commercially significant to strawberry growers. The three categories of flowering habit – June-bearing, everbearing and day-neutral – each respond differently to photoperiod and have separate cultural production practices endemic to the environments in which they are most easily grown. The day-neutral phenotype is the most desired because it offers the potential for extended seasons and increased yields, but until recently commercial day-neutral production has been limited to only a few regions such as Mexico, and California and Florida within the United States. This is apparently due to older cultivars having a narrow set of environmental tolerances, but newer cultivars are showing promise to increase the commercial range of day-neutral production (Petran *et al.* 2016).

Despite its value and popularity, little progress has been made in mapping the genetic basis of day-neutrality in *F. × ananassa* until recently. This is due to its complex octoploid genome and a history of inconsistent

inheritance ratios even within traditional breeding efforts. Because of these difficulties, most strawberry genetic research has focused on *F. vesca*, which features a simpler diploid genome, inheritance ratios that implied single-gene control of flower habit, and an assumption that any discoveries made within this species could be used as a springboard for genetic research in *F.* × *ananassa*. Indeed, it was discovered that a 2-bp deletion in a homolog of the anti-florigen gene *TFL1* resulted in day-neutrality in *F. vesca* (Iwata *et al.* 2012; Koskela *et al.* 2012).

Recently progress has been made in the mapping of day-neutrality in *F.* × *ananassa*, though it has little to do with any discoveries made within *F. vesca*; in fact, it has been proposed that genes controlling flower habit in the two species are not orthologous (Gaston *et al.* 2013). It appears that day-neutrality in *F.* × *ananassa* is influenced by GxE interactions, with single-locus control taking effect in regions where average maximum temperatures are above 28 °C, and potential multi-locus control in cooler regions (Castro *et al.* 2015). This may explain observed higher proportions of progeny displaying day-neutrality in milder versus hotter climates, even when parents are the same (Weebadde *et al.* 2007). While no research has discovered common markers between the flowering loci of *F. vesca* and *F.* × *ananassa*, the results of the induction research covered in this review imply that such work may not be as useful as previously believed. Instead, efforts may be better focused on investing a higher density of markers in linkage group IV-T-1 of the *F.* × *ananassa* genome, where QTLs determining day-neutrality appear to be focused. Designing markers for targeted genome regions will be made easier now that a reference genome for octoploid *F.* × *ananassa* has been released (Hirakawa *et al.* 2014), and will allow researchers to determine if markers highly correlated with day-neutrality in 'Tribute' will explain the habit in other parental lines as well. Despite its complexities, recent advances in understanding *F.* × *ananassa* flower habit have been promising, and increase the potential for the production of this valuable crop to be commercially viable throughout the world.

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