

# How a Flower Becomes a Chestnut: Morphological Development of Chinese Chestnuts (*Castanea mollissima*)

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Chestnuts, members of the genus *Castanea*, family Fagaceae, are popular worldwide and consist of three sections with at least seven distinct species, but may include up to 12 species according to their classification (Bounous and Marinoni 2005). All species have noteworthy ecological, economic, and cultural importance in southern Europe, Anatolia, the Caucasus Mountains, temperate eastern Asia, and eastern North America (Conedera et al. 2004; Davis 2006). Chestnut species regularly bear sweet, nutritious nuts that are high in carbohydrate, but low in fat (Bounous and Marinoni 2005; McCarthy and Meredith 1988; Senter et al. 1994), which have historically been an important food source for people in remote, mountainous areas, and are highly valued in the cuisine of several cultures around the world. The nuts are also an important food source for wildlife (Burke 2013; Paillet 2006). Historically, American chestnut (*Castanea dentata* (Marshall) Borkh.) was an abundant tree species in eastern North America until it was essentially decimated in the early 20<sup>th</sup> century by chestnut blight, caused by the fungus *Cryphonectria parasitica*

(Anagnostakis 1987). Efforts are ongoing to produce and introduce blight-resistant, well-adapted chestnut back to the North American forest to regain its ecological and economic benefits (Thompson 2012).

Of the seven distinct species, three chestnut species, Chinese chestnut (*C. mollissima* Blume), Chinese chinquapin (*C. henryi* (Skan.) Rehder. and E.H. Wilson), and Seguin chestnut (*C. seguinii* Dode.) are native to China, Japanese chestnut (*C. crenata* Siebold and Zucc.) is native to Japan and Korea, European or Sweet chestnut (*C. sativa* Mill.) is found in Europe, Anatolia, and the Caucasus, and American chestnut (*C. dentata*) and the chinquapin (*C. pumila* (L.) Mill.) are native to North America (Mellano et al. 2012). Despite separation by seas and continents, chestnut species are similar in terms of their site requirements and climatic limits (Hunt et al. 2012; Fitzsimmons 2006; Fei et al. 2012). General biological traits of these species are also similar, including reproductive strategies and morphological development (Bounous and Marinoni 2005), and they easily interbreed when cultivated together. In their native forests, most

chestnut species are canopy trees with upright growth forms, while the chinkapin is a large shrub restricted to forest edges.

There is great interest in chestnuts for several reasons: forest and timber restoration of the American chestnut, reforestation and reclamation of strip mine lands, and commercial cultivation and production of other *Castanea* spp. (namely, Chinese chestnut) and hybrids. It is important to understand reproductive development in order to design effective breeding programs (Shi and Stösser 2005; Shi and Xia 2010).

We have assembled a collection of photos that show the sequence of morphological development of Chinese chestnut from flowers to mature fruit, and these photos illustrate growth of the structures that are the components of yield. Understanding these components may lead to more effective selection criteria and faster genetic gains because of the strong impact of the sequence and mechanics of development on economically important traits, natural and anthropogenic selection, and the interpretation of genetic ratios. The developmental sequence of flower and fruit affects gene expression, which has implications for commercial crop yields, selecting components to target for crop improvement, the mechanics of artificial pollination, and inheritance studies.

Specimens were photographed from orchard-grown Chinese chestnuts in Carrollton, Ohio, USA (40.5728° N, 81.0858° W). The timing of flower development reflects climatic conditions for that area in eastern North America.

Chinese chestnut flowers mid-summer, with flowers typically reaching anthesis from late-June through mid-July in Ohio (USA). Flowers are borne on catkins arising from the leaf axils of current season's growth (Fig. 1). Two types of catkins occur: bisexual catkins that bear one or more female flowers at the base and male flowers toward the tips, and unisexual male catkins, also called staminate catkins. Bisexual flowers occur toward the apex of the twig and tend to reach maturity later than the unisexual flowers, which occur toward the base of the twig (Bounous et al. 1992; Bounous and Marinoni 2005). On a typical flowering shoot, the catkins occur contiguously on a central flowering region of the shoot, with vegetative buds below and above the flowering region.

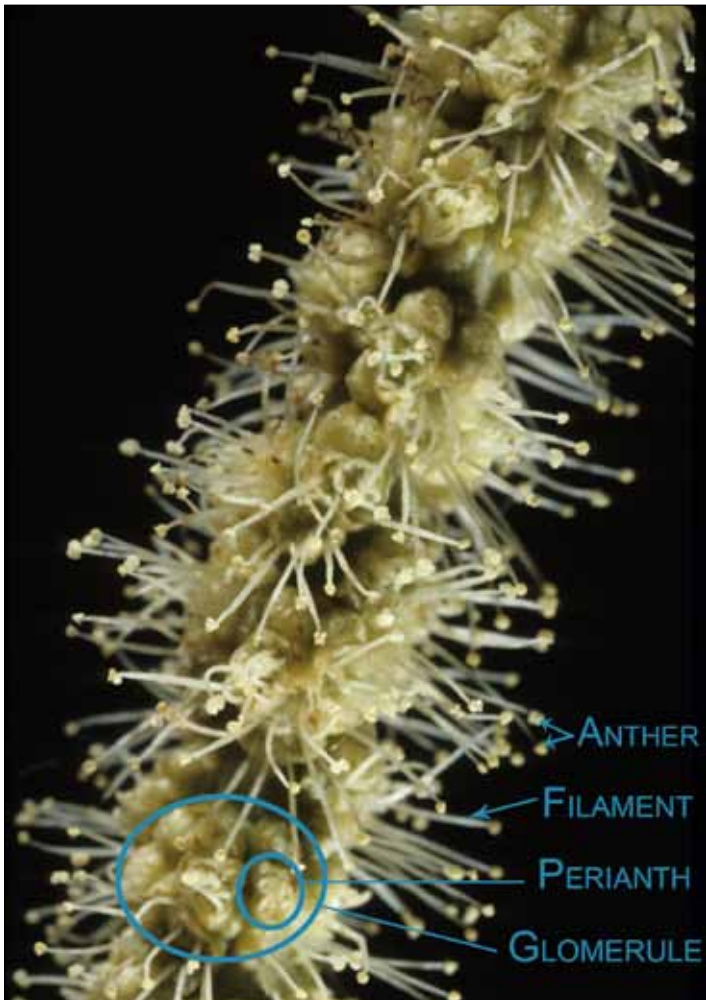
The male catkins occur in abundance and can produce a large quantity of pollen, which is typically wind-dispersed (Bounous and Marinoni 2005). Upon each catkin are glomerules; clusters of staminate flowers (Fig. 2). Each staminate flower is composed of a perianth and multiple stamens, with each stamen composed of a filament and anther. These filaments and anthers give the catkins their characteristic fuzzy appearance, as well as their characteristic pungent odor. Some interspecific hybrids are male-sterile, such as those



**Figure 1.** Twig with catkins at the onset of flowering.

produced from a Chinese chestnut father and American chestnut mother. Astaminate catkins exhibit the most common form of male-sterility manifested by a lack of anthers (Fig. 3). While glomerules and perianth occur as normal, development is halted before the production of stamens, and consequently pollen. Although trees exhibiting male sterility cannot be used as male parents, there seems to be an increased nut yield associated with chestnut trees that do not produce pollen.

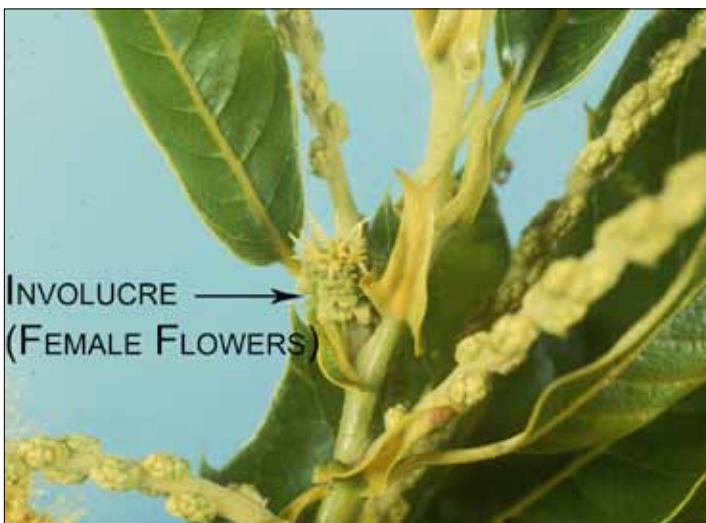
At the base of each bisexual catkin is one or more pistillate inflorescence, also referred to as the involucre (Fig. 4), which typically contains three female flowers (three pistils per involucre). On some trees, involucres with as many as seven pistils can be found. *Castanea* is generally not self-fertile; therefore, cross-pollination is needed for effective fertilization. The prickly involucre (Fig. 5) typically contains up to three pistils. Each pistil comprises an ovary and multiple rigid, pubescent styles, each with a glabrous stigma at the tip. Peak pollen receptivity of the three flowers is staggered in time, with the central flower becoming recep-



**Figure 2.** Fertile catkin.



**Figure 3.** Sterile catkin.



**Figure 4.** Pistillate inflorescence at the base of a bisexual catkin.



**Figure 5.** Involucre: outside (left) and inside (right).



**Figure 6.** Three ovaries inside the involucre.

tive several days before the adjacent ones. As each flower becomes receptive, the cluster of styles becomes visible. A cross-section of the involucre with the top half removed reveals the three distinct ovaries (Fig. 6). The center ovary becomes pollen-receptive first, followed shortly by the two adjacent ones. Each ovary contains 12–18 ovules (Shi and Stösser 2005), which remain equal in size until approximately one month after peak pollination.

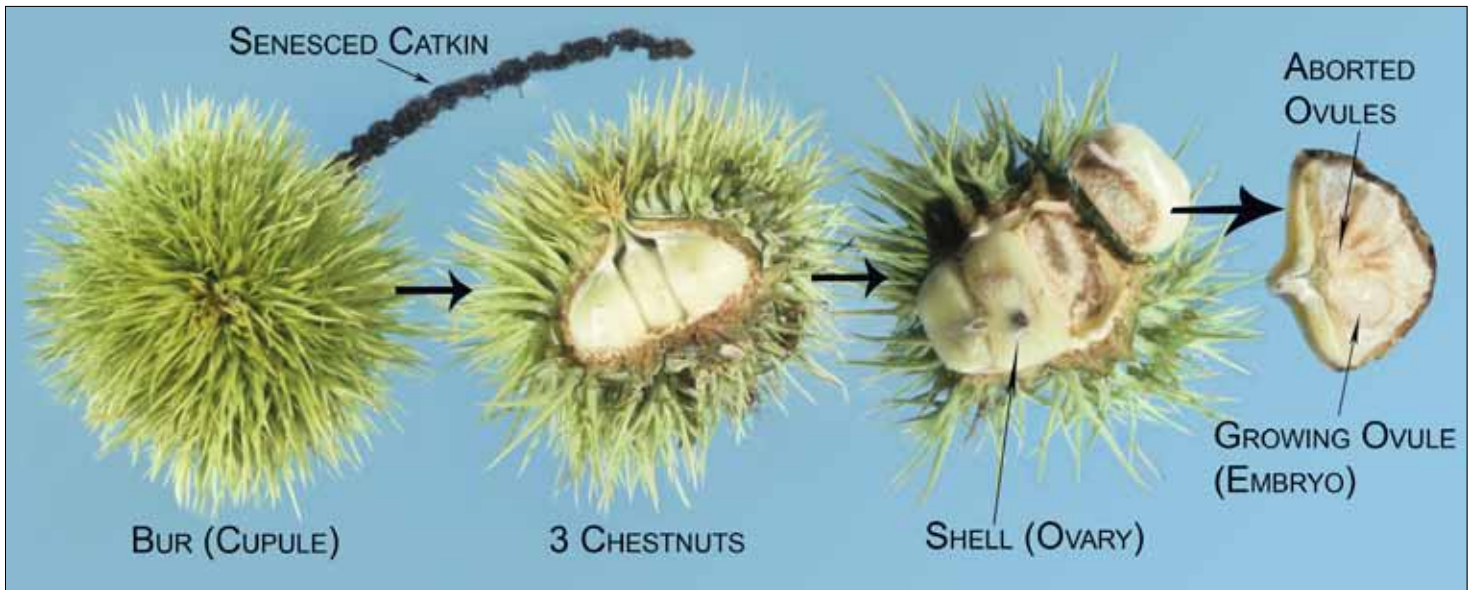
Roughly three weeks after the start of bloom, typically in mid-July in Ohio (USA), pollen production ceases, coinciding with senescence, browning, and drop of male catkins and the male portions of bisexual catkins. Simultaneously, female flower stigmas cease receptivity, the styles begin to darken, and the fertilized ovaries begin to grow. The soft, green involucre containing three female flowers now becomes the recognizable green, spiny cupule (the chestnut bur) (Fig. 7) containing the three growing ovaries. Since the ovaries develop into the nut shells, the nut shells are technically the fruits.

Approximately two months after pollination, three separate nuts are visible nestled side-by-side (Fig. 8). Removing the shell from one of the nuts reveals the ovules. Typically, one of the ovules (now an embryo) grows and expands, while the others cease development. Occasionally, more than one embryo will develop resulting in two or more embryos per nut. A close-up view of the embryo and the internal structure of a nut, when the bur and shell are peeled away, reveals that one embryo develops into the kernel while the others abort (Fig. 9). The developing embryo, attached at the tip to a placenta, connects the tip of the nut to the base of the shell (the hilum). The hilum is the point of attachment of each nut to the inside of the bur and allows the transfer of carbohydrates and nutrients into the developing kernel from the bur and shoot. The embryo fills from the tip and expands downward, eventually filling the entire space inside the shell. As the kernel expands, the placenta is pushed to the side and eventually fits tightly between the kernel and the nut shell. The shell and bur size up before the kernel grows to capacity.

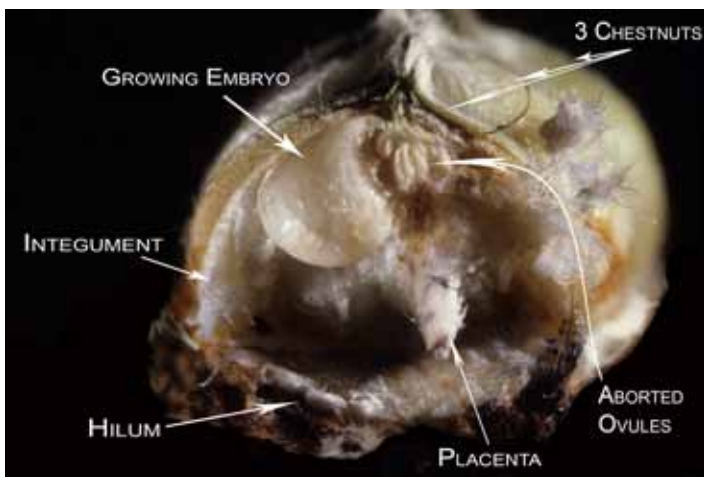
Pollination typically occurs in late June in Ohio (USA), but lack of significant kernel growth until August raises questions about carbohydrate storage and allocation during



**Figure 7.** Catkin senescence and the beginning of bur expansion.



**Figure 8.** Layers inside the expanding bur.



**Figure 9.** Expansion of the kernel inside the shell.



**Figure 10.** Expansion of the kernel inside the shell.

the season. Most of the carbohydrates that create the kernel are injected into the nut within the last two weeks before nut drop. Approximately eight weeks after pollination, the kernel is very small and most of the inside of the shell is filled

with the integument, appearing fuzzy and white (Fig. 10). By ten weeks, the yellow kernel has expanded significantly, but not yet filled the shell. The integument, still white and fuzzy, has been substantially compressed. Approximately twelve weeks after pollination, the typical time of ripening, the yellow kernel has fully expanded to fill the inside of the shell, and the integument has become a skin-like seed coat, brown and white in color (the pellicle). The ripe kernel is composed of two cotyledons and an embryonic axis with a radicle and plumule, which will form the first root, shoot, and leaves during germination.

Approximately six weeks after pollination, the shells appear green, heavily pubescent or fuzzy (Fig. 11). After ten weeks, the shells have grown to mature size and have become much shinier and more glabrous; however, the shells are white in color and very soft. At time of ripening, approximately twelve weeks after pollination, the shells have changed from shiny white to a familiar shiny brown. The brown coloration coincides with the hardening of the hilum and dehiscence of the ripe nuts from the bur. When ripe,



**Figure 11.** Maturation of the shells inside the bur.

Chinese chestnut burs usually open while still attached to the tree and nuts fall individually to the ground. Bur abscission from the tree typically happens later, although abscission can occur before nut dehiscence.

A mature Chinese chestnut comprises three layers: the shell, the pellicle, and the kernel. The shell is typically brown, light to moderately pubescent, with a white tuft of stylar remnants at the tip, and the lighter-brown, rougher-textured hilum at the base. Shells are somewhat soft and pliable, unlike the hard shells of other tree nuts. Peeling back the shell reveals the skin-like pellicle, the light brown seed coat covering the kernel (Fig. 12). The distinct groove seen in the kernel is residual evidence of the placenta's location between the kernel and the shell. Removing the pellicle reveals the bright-yellow kernel composed of two cotyledons and the radicle plumule.

Chestnuts ripen (Fig. 13) and harvest occurs sometime during mid-September and late-October in Ohio (USA), approximately 12 to 14 weeks after pollination. In contrast to many other tree nuts, chestnut kernels are mostly composed of carbohydrates (mainly starch) with smaller amounts of fat and protein (McCarthy and Meredith 1988; Senter et al. 1994). Chestnut kernels continue to undergo chemical and physiological changes after they ripen and fall from the tree, including the conversion of much of the starch to sucrose, making the nuts sweeter and more delectable post-harvest. The extent and nature of post-harvest changes in chestnut kernels vary greatly depending on environmental (storage) conditions (Miller 2006; Tzortzakis and Metzidakis 2012).

In conclusion, noteworthy attributes of chestnut development illustrated by this series of photos are as follows:

1. Chestnuts produce separate male and female flowers (monoecy) with an overwhelming preponderance of male flowers.
2. Each ovary (which becomes a chestnut) houses a large number of ovules at pollination time, but only one (sometimes two or three) develops into a kernel.



**Figure 12.** Layers of a ripe chestnut.



**Figure 13.** Ripe chestnuts and a dehisced bur.

3. Growth of the bur and ovary (chestnut shell) precedes growth of the enclosed embryo (kernel).
4. Half of the kernel's mass is acquired during the last two weeks before nut drop.

The same sequence of development and maturation occurs wherever chestnuts occur, although the timing of bloom varies with geographic location (earlier in the south, later in the north). However, these geographic differences get ameliorated during the season such that the time of harvest is not as variable as time of bloom.

The genus *Castanea* has long been useful in the ecology and economy of the human environment, and with a strong knowledge base and proper cultivation these species can be improved and utilized to help meet resource demands of the future.

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# A Chestnut Orchard in Pictures



Chestnuts in early September before ripening. Photograph by Amy Miller.

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Just as the burs begin to open. Photograph by Amy Miller.

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The orchard as the nuts fall. Photograph by Amy Miller.

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Post-harvest as the trees around the orchard turn bright colors. Photograph by Amy Miller.

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The dormant orchard in winter before another season. Photograph by Amy Miller.

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