Floral Biology and Embryo Development in Chestnut (*Castanea sativa* Mill.)

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**Abstract.** Floral biology of chestnut, from sporogenesis to mature embryo, is described. Microsporogenesis in flowers of unisexual catkins occurred in the first week of June 1991. Anthesis started in mid-June (≈70 days after budbreak) and lasted 2 weeks. In mid-June, in each pistillate flower, six to eight styles began to emerge, and 4 to 7 days later, they were extended fully (i.e., full bloom). In each flower, 10 to 16 anatropous ovules developed from the ovary axis. The megaspore mother cell had formed by the end of bloom. The mature ovule consisted of two integuments and a small embryo sac of the Polygonatum type. Zygotes were found 15 to 20 days after pistillate flower full bloom. Embryo development followed the Onagrad type, *Trifolium* variation. Seeds attained full size in mid-September, and fruit were mature in early November. The embryonal axis averaged 4.5 mm long × 2.1 mm wide. An apical meristem and the radicle were evident at opposite ends of the axis.

Chestnut species (*Castanea* spp., Fagaceae) are monoecious, but cross-pollination is necessary to ensure good yields due to gametophytic self-incompatibility (McKay, 1942; Pisani and Rinaldelli, 1990) and morphological sterility of male flowers, as reported in many cultivars (Bergamini, 1975).

There are two types of inflorescences: unisexual staminate catkins that develop at the base of the flower branch and bisexual catkins that are found near the apex. Catkins, or aments, are scaly, bracted, spike-like inflorescences of flowers grouped in clusters. As reported by Sander (1974) and Nienstaedt (1956), in chestnut, male flowers are spirally inserted along the catkin axis in clusters of four to nine. Pistillate inflorescences appear singly or in clusters of two to three at the base of the bisexual catkins. The aspects of chestnut floral biology that previously have been reported include flower differentiation (Bergamini and Ramina, 1971), male flower morphology (Bergamini, 1975; Bergougnoux et al., 1978; Solignat and Chapa, 1975), and the effect of pollen on fruit and seed development (McKay and Crane, 1938; Renxue and Mengting, 1990).

In our paper, chestnut floral biology in relation to phenological stages and embryo development is described to provide a complete description of gametogenesis, fertilization, and fruit set and development until maturity. Knowing the correlation between the phenological stages and flower and fruit biology can be helpful in choosing the right time for pollination in breeding and in experiments testing pollinizers for the chestnut cultivars.

**Materials and Methods**

From 1987 to 1991, we observed cultivated and wild chestnuts, including ‘Marron di Meana’ and ‘Madonna’, in various locations of Piemonte, Italy. The data presented are from samples collected in Italy; the period and frequency of sampling were chosen based on the observations in previous years. Unisexual male catkins and pistillate flowers at various phenological stages were collected in

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Fig. 1. Chestnut inflorescences: (a) unisexual staminate catkins at bloom, collected 25 June (×0.4) and (b) bisexual catkins and blooming pistillate flowers, collected 22 June (×0.7).
ally clusters of up to five inflorescences were found. As reported by Pisani and Rinaldelli (1990), pistillate inflorescences had stamen remnants (Fig. 2).

The time of male anthesis varied, depending on the arrangement of the flowers. In unisexual catkins (Fig. 3), meiosis occurred in pollen mother cells in the first week of June. Anthesis started 10 to 15 days later, in mid-June, and lasted 2 weeks. In contrast, in bisexual catkins, anthesis began 1 July and ended 11 July.

Based on fluorochromatic reaction, the mean pollen viability percentage was $81.3 \pm 6.1$. The germination percentage in hanging drops was $58.2 \pm 7.0$; on agar medium, it was $50.1 \pm 4.5$.

In mid-June, each pistillate flower showed six to eight styles emerging from the enclosing scales. Full bloom was reached in 4 to 7 days.

Fig. 2. Pistillate inflorescence at bloom showing stamen remnants, collected 25 June (×6).

Fig. 3. Section of flowers of unisexual catkins at meiosis, collected 7 June (×25).

Fig. 4. Style tips at bloom, collected 22 June (×45).

Fig. 5. Ovule development from megaspore mother cell stage to fertilization: (a) developed ovules are located on the upper part of the ovary axis; collected 5 July (×19); (b) longitudinal section of ovule showing the megaspore mother cell, collected 30 June (×228); (c) longitudinal section of mature ovule showing two integuments and elongated nucellus with a small embryo sac, collected 8 July (×76); (d) longitudinal section of ovule showing the zygote and degenerated synergids after fertilization, collected 8 July (×152); (e) longitudinal section of ovule showing a deeply stained endosperm nucleus of the same embryo sac of Fig. 5d, collected 8 July (×152); and (f) ovule development after fertilization: embryo sac enlarging outside nucellus, collected 11 July (×98.8).
At this stage, the tip of the style appeared hollow (Fig. 4). In the ovary, which consisted of seven (rarely six or eight) carpels, the ovules were differentiating. In each flower, 10 to 16 anatropous ovules developed from the ovary axis. They were located on the upper part of the axis due to ovarian growth below the ovules that occurred after anthesis (Fig. 5a). Under open-pollination conditions, female blooming ended =1 week after it commenced. At this time, megasporocytes were visible in the ovules (Fig. 5b). In 8 to 12 days, the megaspore developed into an eight-nucleate Polygonum-type embryo sac. Three nuclei were ephemeral antipodals, three formed the egg apparatus (two synergids and egg cell), and two polar nuclei fused just before the double fertilization. The mature ovule of chestnut consisted of two integuments and a long narrow nucellus (Fig. 5c) with a small embryo sac located at the micropylar end. The ovule structure resembled that reported for Quercus (Bonné-Masimbert, 1984).

In the same ovary, ovules were at several stages of development. Although we did not see fertilization, it must have occurred 12 to 15 days after full bloom because the synergids started degenerating (Fig. 5d) and the endosperm nucleus was evident in the center of the enlarging embryo sac around 8 July. In some ovules at this stage, a deeply staining endosperm nucleus was found surrounded by dense cytoplasm. Presumably, these ovules were going to abort (Fig. 5e).

Although a few ovules showed traces of pollen tubes through the micropyle, usually an embryo developed in only one ovule. The presence of the nucellus gave a bifurcate shape to the embryo sac, whose lateral branch was called “caecum” by Benson (1894) (Fig. 5f). The nucellus started degenerating in fertilized and nonfertilized ovules, but in the former, it was replaced by the enlarging embryo sac as these ovules grew.

At the end of the first week of July, 15 to 20 days after pistillate flower full bloom, the zygote and sometimes a four-celled pro-embryo were observed. The endosperm nucleus started dividing 2 to 3 days after synergid degeneration; two-celled pro-embryos were found when the endosperm was four-nucleate. The suspensor of the developing pro-embryo consisted of three to four cells; in ≈20 days from fertilization, the embryo became globular (Fig. 6), then heart-shaped, and finally torpedo-shaped. The endosperm became cellular at the globular stage.

By mid-August, cotyledons filled most of the seed, replacing the endosperm. Until this stage, empty fruit also were found, similar in length and width but with the transverse diameter shorter than in fruit with normal seeds. The empty fruit contained fibers and aborted ovules.

Chestnut growth, measured as width on 50 seeds, was almost linear from the end of June (3.63 ± 0.25 mm) to mid-September, when the seed had attained full size. At fruit maturity (7 Nov.), the chestnut average height, width, and thickness were 2.56 ± 0.23, 2.71 ± 0.27, and 1.83 ± 0.36 cm, respectively. The embryonal axis averaged 4.5 mm long × 2.1 mm wide and showed an apical meristem and the radicle (Fig. 7a and b).

Embryo development in Fagaceae follows the Onagrad type, probably the Trifolium variation, as reported in Quercus (Johansen, 1950). In C. sativa, this hypothesis is supported by the observation that the suspensor of the pro-embryo consisted of three to four cells. Besides the normal ovule development that we have described, anomalies were found, such as delayed or irregular embryo sac formation and endosperm or pro-embryo abortion. Davis (1966) reported secondary multiplication of antipodal cells in C. vulgaris. Although we saw degeneration of antipodals before fertilization in many ovules, at the same stage, embryo sacs were found with supernumerary nuclei that could have resulted from division of antipodals. The observed anomalies also were reported by Jialin et al. (1990) as some of the factors affecting empty bur formation in C. mollissima Bl. In our study, only one ovule per ovary developed in most of the observed nuts, as reported for the “marron” type, although in “normal” chestnuts, up to five seeds share the same nut. Bearing single-seeded nuts seems related in part to the high occurrence of anomalies, such as the delayed embryo sac differentiation in some ovules and the presence of supernumerary nuclei in the embryo sac. Yet, pro-embryo abortions, already reported by McKay and Crane (1938), can mean that...
growth-inhibiting or genetic regulatory factors are involved in preventing the development of more than one ovule per nut.

Literature Cited


