

A complete study of chestnut reproduction, from pollination to seed formation

C. Larue ^{1, 2*}

¹*Univ. Bordeaux, INRAE, BIOGECO, 33610 Cestas, France*

²*INVENIO, Maison Jeannette, 24140 Douville, France*

*Corresponding author: clement.larue@inrae.fr

Keywords: Fruit set, Insect pollination, Male-sterility, Orchard, Self-Interference

Sexual reproduction of plants is a key stage in their life cycle, on which the functioning of ecosystems and our food supply depend. For seed plants, sexual reproduction requires successful pollination, i.e. the transport of pollen from the stamens of a flower to the stigmas. Pollination can fail if the quantity of compatible pollen produced is insufficient (pollenizer limitation) or if pollen is not transported successfully (pollinator limitation), both mechanisms resulting in an insufficient quantity or quality of pollen received by the stigmas (pollen limitation). Once the pollen has been delivered, post-pollination mechanisms can result in ovule or seed abortion before maturity. The European chestnut (*Castanea sativa*, Fagaceae), an important forest and fruit tree, is used to explore and model pollination, from pollen production to fruit set. I used observations (phenology, floral architecture, insects), experiments (insect exclusion with insect-proof nets and emasculation), molecular analyses (characterization of genetic diversity and paternity analyses), and a spatially explicit Bayesian model of pollination and fruit set to study chestnut pollination. My objective was to identify the key reproductive mechanisms that allow fruit formation in orchards and forests. When I prevented insects to access female flowers with insect-proof nets, fruit production collapsed, thus proving that chestnut trees are insect-, not wind-pollinated, as was assumed before. Beetles and flies, not bees, are the main pollinators. Some European chestnuts are naturally male-sterile, i.e. they no longer produce pollen, indicating that this species is gynodioecious. I confirmed that male sterility is of cytoplasmic origin, with male-sterile trees producing more fruits than male-fertile ones. I used an emasculation experiment to investigate the impact of self-pollination on fruit set. The results show that the impact is massive, suggesting that one of the main mechanisms behind this sexual polymorphism is self-interference between maternal and paternal functions resulting in ovule discounting in bisexual trees due to late-acting self-incompatibility. The model also revealed variable and asymmetrical barriers between chestnut species, matching well with observed paternity and seed-set in production orchards. Finally, I discuss difficulties encountered in transferring these results to the public and to farmers and I outline possible solutions.

Reference: Larue C (2021) De la pollinisation à la formation des graines : le cas du châtaignier. PhD Thesis, Université de Bordeaux (all the results of this PhD thesis have now been published in international, peer-reviewed articles: <https://clementlarue1.github.io/>)