**Floral organogenesis of *Prunus laurocerasus* and *P. serotina*
and its significance for the systematics of the genus and
androecium diversity in Rosaceae**

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Floral organogenesis of *Prunus laurocerasus* and *P. serotina* and its significance for the systematics of the genus and androecium diversity in Rosaceae

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Abstract: Phylogenetic studies have shown that most clades in *Prunus* are well-supported by the flower structure, but most taxa in the racemose group have not yet been re-evaluated and could contribute to the understanding of the systematic relationships of the subgenera. We examined the inflorescence and flower development in *Prunus laurocerasus* (subg. *Laurocerasus*) and *P. serotina* (subg. *Padus* I) using scanning electron microscopy. Our results indicate that they share several floral development characters but differ in the following aspects: (1) all flowers are fully developed and each flower is enclosed by a bract and two bracteoles, which later stop development (vs. the terminal flower degenerates and only a single bract subtends each flower), (2) the style protrudes from the floral bud (vs. the style is crooked and below the anthers), (3) the outer integument initiates close to the inner one (vs. in the middle of the ovule), and (4) an obturator appears after initiation of the two integuments (vs. simultaneously with the inner integument). Although our results are preliminary, differences in floral developmental characters support the different origins of *Prunus* subgenera *Laurocerasus* and *Padus* as based on molecular phylogenetic studies.

Key words: floral development, floral morphology, floral structure, *Laurocerasus*, *Padus*

1 Introduction

2 *Prunus* L. (subfamily Amygdaloideae of Rosaceae) is an economically important
3 genus that includes many temperate nut and fruit crops such as almonds, cherries,
4 peaches, and plums (Lee and Wen 2001; Potter et al. 2007; Zhao et al. 2016; Xiang et al.
5 2017; Zhang et al. 2017). There are different approaches to the classification of this genus
6 that has been divided into several genera or subgenera (Lee and Wen 2001; Kalkman
7 2004). Based on flower and inflorescence structures, *Prunus* is composed by: (1) the
8 solitary-flower group (subg. *Prunus*, *Amygdalus* and *Emplectocladus*), (2) the corymbose
9 group (subg. *Cerasus*), and (3) the racemose paraphyletic group (subg. *Padus*,
10 *Laurocerasus*, the *Maddenia* group, and the *Pygeum* group), in which *Padus* is
11 paraphyletic (Chin et al. 2014; Zhao et al. 2016; Fig. 1). However, in the racemose
12 groups *Padus* and *Maddenia* are deciduous and have terminal inflorescences, whereas
13 *Laurocerasus* and *Pygeum* are evergreen and have axillary inflorescences (Lu et al. 2003;
14 Kalkman 2004). The circumscription and affinities of the racemose group are disputed
15 and it has sometimes been treated as a single genus (*Padus*) or as of now, divided into
16 several genera (and subgenera) (see Kalkman 2004; Shi et al. 2013; Zhao et al. 2016).
17 Phylogenetic analyses have suggested that multiple independent allopolyploidy events
18 contributed to the origins of the racemose group. A widespread and early-diverging
19 lineage of *Prunus* is hypothesized to have served as the maternal parent(s) for these
20 allopolyploidy events, with several independently derived paternal lineages (Zhao et al.
21 2016; Fig. 1). The relationships between these subgenera and their affinities based on

molecular studies provide new opportunities to perform a comparative study of their flower and inflorescence structure and development.

Flowers and inflorescences have important taxonomic value, and their developmental process contains abundant information for systematics and evolutionary studies (Endress 2011). Previous studies on *Prunus* have focused on its anatomy (Sterling 1953*a, b*, 1964; Haskell and Dow 1955; Endress and Stumpf 1991; Zhang 1992; Chin et al. 2013), palynology (Hebda et al. 1991; Geraci et al. 2012; Shi et al. 2013), paleontology (DeVore and Pigg 2007; Benedict et al. 2011; Li et al. 2011), phylogenetics, and phylogeography (Bortiri et al. 2002; Wen et al. 2008; Chin et al. 2010, 2014; Liu et al. 2013; Zhao et al. 2016). Evans and Dickinson (1999*a*) compared the inflorescence and floral development of *P. virginiana* (subg. *Padus*) with that of other Amygdaloid genera. Stamen development in *P. padus* (subg. *Padus*), *P. avium* (subg. *Cerasus*), *P. mabaleb* (subg. *Cerasus*), and *P. domestica* (subg. *Prunus*) were reported by Lindenhof and Weber (1999*b*). Previous studies have also been conducted on the floral morphology and development of one or a few genera in Rosaceae (Sattler, 1973; Innes et al. 1989; Steeves and Steeves 1990, 1991; Endress and Stumpf 1991; Kemp et al., 1993; Evans and Dickinson 1996, 1999*b*, 2005; Lindenhof and Weber 1999*a, b*, 2000), providing significant contributions to a better understanding of the evolution of the family. Ontogenetic data may provide additional evidence for supporting hypotheses of the phylogenetic relationships between taxa. However, among most lineages of the racemose group of *Prunus*, floral organogenesis has not been studied in detail.

Here, we used scanning electron microscopy (SEM) to investigate inflorescence and flower morphology and development in *P. laurocerasus* L. (subgenus *Laurocerasus*) and *P. serotina* Ehrh. (subgenus *Padus* I) and assess their relationship, and also explore the systematic significance of the inflorescence and floral developmental characters in the family.

Materials and Methods

Flower buds of *P. laurocerasus* were collected in Washington, DC, USA (alt. 22 m, cultivated, voucher: *ZhaoLiang US20150202*, US) between February 2015 and May 2016. Flower buds of *P. serotina* were collected from natural populations in Rockville, MD, USA (alt. 43 m, voucher: *ZhaoLiang US20152001*, US) between April 2015 and March 2016. All plant materials were fixed in FAA (Formalin: acetic acid: ethanol: water = 10:5:50:35).

For SEM, 227 flower buds were first dissected and dehydrated in an ethanol and iso-amyl acetate series, followed by critical-point drying in CO₂, and finally were sputter-coated with gold and observed with a HITACHI S-3500 scanning electron microscope. The backgrounds of the SEM images were edited and details were colored using Adobe Photoshop. Photographs of mature flowers were taken with a Nikon D7100 digital camera (Fig. 2) against a black background. The description of the floral morphology was based on 30 mature flowers. The symbols used in the floral diagrams

and floral formulae followed Prenner et al. (2010), Ronse De Craene (2010) and Ronse De Craene et al. (2014).

Results

Inflorescence and floral development is described in detail for *Prunus laurocerasus*, and only significant variations are described for *P. serotina*.

Prunus laurocerasus

Inflorescence and flower structure

The racemose inflorescence is axillary with 30–35 flowers and the peduncle base is leafless. The mature flower is 0.8–1.2 cm in diameter. The five sepals are small, green, and triangular, the five petals are white, longer than sepals; the 17 to 20 stamens are in three whorls, with 10 antesealous and 5 antepetalous and 2 to 5 antesealous; the floral cup is 0.4–0.6 cm in diameter, the single carpel (rarely 2 or 3) has a glabrous superior ovary and long style, which protrudes from the flower bud before anthesis (Fig. 2A–H).

Organogenesis

On the inflorescence, bract primordia are initiated spirally and acropetally, followed by a single floral primordium in the axil. Each floral apical meristem is first flanked by a pair of bracteoles that later stop develop further (Fig. 3A–C). The sepals are initiated always clockwise in a Fibonacci spiral pattern with the first and third sepals on the abaxial side of the flower primordium, the second sepals in adaxial position, and the fourth and the fifth in lateral positions (Fig. 3D). The sepal primordia are crescent-shaped

and truncate (Fig. 3D), and followed by five alternate spherical petal primordia (Fig. 3E, F). With the development and deepening of the hypanthium, the first whorl of about ten narrow and hemispherical stamen primordia, is successively initiated as five antesealous pairs (sometimes single stamens appear, Fig. 3G–K, red). Then, a second alternate whorl of five antepetalous stamens appears between the five pairs of the first whorl (Fig. 3L, yellow), and is followed by a third alternate and often incomplete whorl of two to five antesealous stamens (Fig. 3L, blue). The gynoecium primordium is initiated at the center of the floral apex (Fig. 3H–L). In total, in 207 out of 227 flowers (91%), only one carpel develops (Fig. 3O), whereas in 14 and in 6 flowers, 2 or 3 are present, respectively (Fig. 3P).

The sepals enlarge and gradually become ovate triangular with a ciliate margin, and protect the inner organs during later development (Fig. 3M). There are stomata at the dorsal base of the sepals, and the stomata are surrounded by strip-type epidermal cells (Fig. 4A–C). The petal primordium gradually differentiates into a large lamina and a short stalk. The petals are glabrous, with stomata at the adaxial base, surrounded by quadrate epidermal cells (Figs. 3N, O, 4D–H). Each young stamen quickly differentiates into a long filament and a much shorter four-locular anther. The anthers are basifixed and have median longitudinal dehiscence lines between the thecae (Fig. 4I–J). The pollen is tricolporate (Fig. 4K–L).

After the initiation of a single carpel, the carpel primordium continues to enlarge in height and a depression appears at the base of its ventral side (Figs. 3K, 5A). With the

1 progression of carpel development, the concavity becomes deeper and deeper. The sides
2 of the carpel gradually fuse from the base to the apex, forming the suture line, except for
3 the uppermost part (Figs. 3N, 5B–C). The carpel is entirely plicate from the base of the
4 ovary up to the tip covered with minute unicellular receptive stigmatic papillae. The
5 developing style continues to elongate and finally becomes curved (Fig. 5D–G) before
6 protruding from the floral bud before anthesis (Fig. 3O). Unicellular papillate stigma
7 tissue differentiates on the upper truncate portion of the carpel (Fig. 5I).

8 Two ovule primordia are initiated along the margins of the carpel (Fig. 5J, L). The
9 semi-annular inner integument is initiated in the middle of the ovule and gradually forms
10 a regular ring (Fig. 5M, N). The ovule and the funiculus begin to bend toward the apex of
11 the ovary and the outer integument is initiated near the inner one (Fig. 5O). The outer
12 integument gradually forms a ring, and at maturity almost encloses the inner integument.
13 An obturator initiates from the cells on the wall of the locule, gradually enlarging, and
14 consists of unicellular papillae appearing near the funiculus on the placenta but not near
15 the micropyle (Fig. 5P–R). At maturity, the two ovules are anatropous and the obturator
16 is near the micropyle (Fig. 5S, K). Approximately 2% of flowers have only one ovule in
17 the carpel (Fig. 5T).

18

19 *Prunus serotina*

20 Inflorescence and flower structure

The racemose inflorescence is terminal with 32–40 flowers on the current year's branchlets, and leaves are present at the base of the peduncle. The mature flower is 1.0–1.4 cm in diameter. The 20 stamens are arranged in three whorls. The first to the third whorl are composed of 5 pairs of antesepalous, 5 antepetalous and 5 antesepalous stamens, respectively. The floral cup is 0.4–0.5 cm in diameter, the style has a distinct fold at the base and is lower than the stamens (Fig. 2I–P).

Organogenesis

In the inflorescence, there is one bract below each floral primordium (Fig. 6A–D). However, the terminal floral primordium degenerates late in development, leaving a residuum visible at the axil of the bract (Fig. 6B, arrow). The sepal primordia are initiated almost simultaneously (Fig. 6E). The five petals are alternate with the five sepals (Fig. 6F). After initiation, intercalary growth of each sepal primordium results in two rings of sepals and petals surrounding the flat floral apex (Fig. 6G). Ten stamen primordia (red) are successively initiated, appearing similar in size and form to five antesepalous stamen pairs (Fig. 6H). Subsequently, five antepetalous stamen (yellow) and five additional antesepalous stamen (blue) primordia are initiated as the second and the third whorl, respectively (Fig. 6I–K). The center of the floral primordium then bulges and a ventral furrow marks the beginning of carpel formation (Figs. 6L, 8A).

The sepals enlarge and enclose other floral organs gradually in later development (Fig. 6M–P), with denticles at the upper margin and stomata at the abaxial base (Fig. 7A–C). The petal primordium differentiates into a large lamina and a short stalk. The

1 petals are glabrous, with stomata on the adaxial base (Figs. 7D–G). The ornamentation on
2 the petal epidermis is minute (Fig. 7G). Each young stamen has a long filament and a
3 much shorter four-locular anther. The anthers are basifixed and have longitudinal
4 dehiscence (Fig. 7H, I). The pollen is tricolporate (Fig. 7J, K).

5 The carpel has a distinct fold in the middle (Fig. 8D, E). A semi-annular inner
6 integument is initiated from the side of the ovule (Fig. 8J). An obturator appears on the
7 wall of the locule (Fig. 8K–N). The outer integument is initiated, and the obturator is
8 obvious, and at maturity may entirely enclose the micropyle (Fig. 8L–O).

9

10 **Discussion**

11 **Systematics of subgenera *Laurocerasus* and *Padus***

12 The lack of information regarding floral development in the racemose group, except
13 for *Prunus virginiana* (*Padus* I), complicates a comparison of the different subgenera
14 within this group (Evans and Dickinson 1999a). The questions addressed in this study
15 stemmed from the aim to gain a better understanding of the origin of the different
16 subgenera of the racemose group in *Prunus*, especially with reference to recent molecular
17 phylogenies (Zhao et al. 2016). Comparisons of floral ontogeny and mature morphology
18 may provide additional evidence for the support of recent phylogenetic hypotheses
19 (Evans and Dickinson 1999a).

20 Our study has shown that *Prunus laurocerasus* and *P. serotina* share several
21 morphological and developmental traits consistent with characteristics of the genus

1 *Prunus* (Evans and Dickinson 1999a) including centripetal initiation of floral organs,
2 hypanthium, stamens with longitudinal dehiscence, tricolporate pollen, bitegmic and
3 anatropous ovules, and the presence of obturators on the placenta. However, we do note
4 several floral developmental features that differ between the two species (Table 1).

5 Although the morphology of the mature raceme is similar in *P. laurocerasus* and *P.*
6 *serotina*, the early inflorescence development of *P. laurocerasus* differs from that of *P.*
7 *serotina*. In *P. laurocerasus*, the terminal flower develops well and two bracteoles have
8 their development arrested, leaving one remaining bract. In contrast, only one bract is
9 initiated and the terminal floral primordium degenerates in *P. serotina*. Thus, the similar
10 presence of a single bract is the results of two distinct developmental pathways, both
11 species have only one bract surrounding the flower at maturity.

12 *Prunus serotina* possesses a typical pattern of 10+5+5 stamens, similar to *P.*
13 *virginiana* (Evans and Dickinson 1999a). However, in *P. laurocerasus*, the third whorl is
14 often reduced and incomplete, with two to five stamens. The pollen grains of *P.*
15 *laurocerasus* and *P. serotina* are tricolporate with elongated striate sculpturing, which is
16 the similar to *Padus*, *Laurocerasus* and *Maddenia* species but different from the pollen of
17 *Pygeum*, which has rugulate exines with much shorter, rod-shaped muri (Hebda et al.
18 1991; Shi et al. 2013b).

19 The style of *P. laurocerasus* is long and protrudes from the floral bud before
20 flowering, perhaps facilitating outcrossing. On the contrary, the stigma of *P. serotina* is
21 lower than the anthers and there is a distinct fold at the base of the style.

1 Ovule characters are of great systematic significance (Wang and Ren 2008; Endress
2 and Matthews 2012). In *P. laurocerasus*, the initiation and development of the outer
3 integument is rapid and not obvious, and the obturator appears after the outer integument.
4 In contrast, the outer integument is obvious in *P. serotina*, and the obturator is initiated
5 before the outer integument, similar to *P. virginiana* (Evans and Dickinson 1999a).
6 Despite these developmental differences, the obturators are morphologically similar in *P.*
7 *laurocerasus* and *P. serotina* at maturity.

8 Although subgenus *Padus* is paraphyletic, the differences observed in floral
9 developmental features of *P. laurocerasus* and *P. serotina* support the hypothesis that
10 *Prunus* subgenera *Laurocerasus* and *Padus* (at least *Padus* I) have different origins. This
11 conclusion is in accordance with molecular phylogenetic inferences regarding the
12 relationship between these subgenera (Zhao et al. 2016).

13

14 **Comparison with other taxa in Rosaceae**

15 Early floral organogenesis of the first two whorls of *P. laurocerasus* and *P. serotina*
16 are mostly similar to other taxa of Rosaceae. Five sepals and petals are initiated
17 centripetally in multiples of five, followed by the development of the hypanthium (Kemp
18 et al. 1993; Evans and Dickinson 1996, 1999a). In Rosaceae, the initiation of stamens
19 mostly appears in a cyclic polyandry pattern with stamens in all whorls arising
20 independently of one another (Ronse De Craene and Smets 1987). In most cases the
21 stamens of Rosaceae species are arranged in three whorls, with 10 antesealous primordia

forming the first whorl (as parapelalous stamens), and five antepetalous and antesealous primordia forming the second and the third whorls, respectively (Lindenhof and Weber 1999a, b, 2000). The number of stamens in the first whorl is generally stable, and the variation observed for the stamen number always occurs in the innermost two whorls (Evans and Dickinson 2005). In our study, we showed that while the three whorls are complete in *P. serotina*, the third whorls is often not in *P. laurocerasus*, and like in other Rosaceae, variation in numbers of stamens whorls or per whorls is likely linked to the expansion of the hypanthium (Ronse De Craene and Smets 1993; Lindenhof and Weber 1999b).

In Rosaceae, it is thought that the petal and the two nearest stamens are initiated from a common primordium, as previously shown in *Fragaria*, *Filipendula* and *Crataegus* (Sattler 1973; Ronse De Craene and Smets 1993; Evans and Dickinson 1996).

In both *P. laurocerasus* and *P. serotina*, ten antesealous stamens appear after the petals, and these primordia are initiated in pairs but without a common primordium and free from each other and also separately from the stamens inner whorls. At the same time, there is sometimes a stamen pair being replaced by a single stamen in *P. laurocerasus*, which may be the result of secondary degeneration (Ronse De Craene and Smets 1993).

In *Oemleria cerasiformis*, antepetalous stamens primordia first initiated (Evans and Dickinson 1999a).

Amygdaloideae taxa always have one carpel. In male *Oemleria cerasiformis* flowers, there is no gynoecium initiation; however, in the female flowers, the five carpels are

1 carried up the inner wall of the hypanthium as it elongates. It has been argued that the
2 presence of a single carpel is a derived trait in Rosaceae (Ronse De Craene 2010). In
3 Amygdaloideae, ancestral character reconstruction supports that a larger number of
4 carpels is the ancestral character and the number of carpels decreased to one or two in
5 *Prunus* of the tribe Amygdaleae (Xiang et al. 2017). Both *P. laurocerasus* (also see
6 Sterling 1953b) and *P. serotina* have multiple carpels, indicating that they might have
7 been undergoing an evolutionary transition from multiple carpels to only one carpel.
8 Further sampling may provide insight into the extent to which the number of carpel
9 varies.

10 Ovule development shows great diversity in Rosaceae. For example, in most taxa a
11 pair of ovule primordia are initiated near the base of the ventral ovary margins. In
12 Rosoideae, only a single ovule appeared near the base of one ventral ovary margin.
13 Multiple ovule primordia are initiated near the ovary base (e.g. *Physocarpus*) or along its
14 length (e.g. in *Spiraea*) (Evans and Dickinson 1999b).

15 It has been reported that Amygdaloideae taxa have one ovule (Mabberley 1997).
16 However, in our study, two ovules were always observed in *P. laurocerasus* and *P.*
17 *serotina*, which is also the case in *P. virginiana*, *Exochorda*, *Oemleria*, and *Prinsepia*
18 (Evans and Dickinson 1999a). In *P. laurocerasus* and *P. duclouxii*, there is sometimes
19 only one ovule at maturity (this study, Sterling 1964).

20 The anatropous and epitropic ovules of *Prunus*, *Exochorda*, *Oemleria* and
21 *Chaenomeles* are initiated near the middle of the ventral ovary margin and the obturators

from the margins of the ovary wall above the ovule primordia, while the ovules of *Prinsepia* are pleurotropic and the integuments are free in *Oemleria* and *Exochorda* (vs. continuous in *Prunus* and *Prinsepia*) (this study, Evans and Dickinson 1999a, 2005). However, other Rosaceae taxa (e.g., *Physocarpus*, *Spiraea*) develop obturators from the funiculus of the ovules (Evans and Dickinson 1999b, 2005). On the contrary, the ovules of *Kageneckia* lack an obturator (Evans and Dickinson 1999b). Additional studies are required on more Rosaceae species to better understand ovule diversity within the family.

Conclusions

This paper is one of the first in a series to document the development of floral and inflorescence characters in *Prunus* and its close allies. This study again shows the interdependence of phylogenetics and comparative morphology. We have shown that information about the inflorescence and floral structure and development of *P. laurocerasus* and *P. serotina* provide support for the different origin for *Prunus* subgenera *Laurocerasus* and *Padus* I as retrieved in Zhao et al. (2016). However, further investigation on other congeneric species, as well as additional lineages within the racemose group of *Prunus* should be conducted to validate these findings. In addition, more molecular phylogenetic studies and comparative morphological studies of other subgenera in *Prunus* and other taxa in Rosaceae are needed.

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Table 1. Comparison of floral development between *Prunus laurocerasus*, *P. serotina* and other Rosaceae species.

Taxon	Subfamily	Inflorescence	Bract	Bracteole	Stamens	Pistil	Style	Ovule	Integument	Obturator
<i>Prunus laurocerasus</i>	Amygdaloideae	raceme, all flowers develop well	1	2 but degenerate later	10+5+ (2–5)	1–6	protrudes the floral bud	1–2, anatropous	2, the inner integument formed the micropyle	appears after two integuments
<i>Prunus serotina</i>		raceme, terminal flowers degenerate	1	absent	10+5+5	1	crooked and lower than the anthers	2, anatropous	2, the outer integument formed the micropyle	appears at the same time as inner integument
<i>Prunus virginiana</i>		raceme, terminal flowers absent	1	absent	10+5+5	1	wrapped	2, anatropous	2, micropyle is formed by the outer integument	appears at the same time as inner integument
<i>Exochorda racemosa</i>		raceme, all flowers develop well	1	2	20	5	?	2, anatropous	2, micropyle is formed by the inner integument	?
<i>Prinsepia sinensis</i>		raceme	1	absent	20	5	crooked and lower than the anthers	2, basal pleurotropic	2, micropyle is formed by the inner integument	?
<i>Oemleria cerasiformis</i>	Spiraeoideae	raceme, all flowers develop well	1	2	5+10	1	crooked and lower than the anthers	2, anatropous	2, micropyle is formed by the inner integument	appears after two integuments
<i>Physocarpus opulifolius</i>		corymb, terminal flowers absent	1	absent	10+5+5	3–5	wrapped	2–4 anatropous	2, micropyle is formed by the outer integument	appears at the same time as outer integument
<i>Sorbaria sorbifolia</i>		panicle, all flowers develop well	1	2	25–30	5	wrapped	6–8 apical epitropic	2, micropyle is formed by the outer integument	appears after two integument
<i>Spiraea trilobata</i>		corymb	1	absent	20	5	?	6–8 apical epitropic	1	same time as integument

<i>Vauquelinia californica</i>		compound corymb, all flowers develop well	1	2	20	5	wrapped	2, basal apitropic	2, micropyle is formed by the outer integument	appears at the same time as inner integuments
<i>Crataegus</i>		cymose, all flowers develop well	1	2	10+5+5	5	?	?	?	?
<i>Gillenia trifoliata</i>		panicle, all flowers develop well	1	2	10+5+5	5	?	2, basal apitropic	2	appears after two integuments
<i>Photinia villosa</i>	Maloideae	panicle, all flowers develop well	1	2	10+5+5	3—5	?	2, basal apitropic	2, micropyle is formed by the inner integument	appears after two integuments
<i>Amelanchier alnifolia</i>		raceme, all flowers develop well	1	2	20	4—5	?	?	?	?
<i>Potentilla fruticosa</i>		single flower	2		10—50	20—120	?	?	?	?
<i>Rosa setigera</i>	Rosoideae	cymose-dichasium, all flowers develop well	1	2	10+10+10+10+10	majority	protrudes the floral bud (female flower) wrapped (male flower)	1	?	?

The taxonomic system is from Kalkman (2004). Flower morphological and developmental characters of *P. laurocerasus* and *P. serotina* are taken from this study; *Prunus virginiana*, *Exochorda racemosa*, *Prinsepia sinensis* and *Oemleria cerasiformis* from Evans and Dickinson (1999a); *Physocarpus opulifolius*, *Sorbaria sorbifolia*, *Spiraea trilobata* and *Vauquelinia californica* from Evans and Dickinson (1999b); *Crataegus* from Evans and Dickinson (1996); *Gillenia trifoliata* and *Photinia villosa* from Evans and Dickinson (2005); *Amelanchier alnifolia* from Steeves and Steeves (1990) and Steeves et al. (1991); *Potentilla fruticosa* from Innes et al. (1989); *Rosa setigera* from Kemp et al. (1993).

Figure legend

Fig. 1. Phylogenetic relationships in *Prunus* (simplified from Zhao et al. 2016).

Fig. 2. Mature inflorescences, diagram, flowers, floral diagrams and floral formula. A–H.

Prunus laurocerasus. A. Inflorescence subtended by modified leaves. B. Top view of the flower. C. Infructescence. D. Floral diagram and floral formula, with the different shading of the stamens showing different whorls. E. View of the flower from below. F. Lateral view of the floral bud. G. Vertical section of the flower. H. Diagram of the inflorescence of *P. laurocerasus*. Flowers are represented by circles. Aborting bracteoles are indicated with a broken outline. I–P. *P. serotina*. I, Inflorescence. J. Top view of the flower. K. Infructescence. L. Floral diagram and floral formula, with the different shading of the stamens showing different whorls. M. View of the flower from below. N. Floral bud, showing the stigma lower than the anther. O. Vertical section of the flower. P. Diagram of the inflorescence of *P. serotina*. Flowers are represented by circles. Aborting flower is indicated with a broken outline. Black arcs indicate sepals; grey arcs indicate petals; four closed circles indicate stamens; and ellipses indicate ovaries. Scale bars: B, E, F, G, J, M, O, 5mm; N, 1mm; A, C, I, K, 2cm.

Fig. 3. Inflorescence and floral organogenesis of *Prunus laurocerasus*. A. Top view of inflorescence, showing bracts. B. Lateral view, showing a flower surrounded by a bract and a pair of bracteoles. C. Later developmental stage of (B), two small bracts were

1 already degraded. D. Initiation of two bracteoles and five sepal primordia. E–F.
 2 Nonsynchronous initiation of petal primordia alternate with sepal primordia. G–H.
 3 Initiation of ten antesealous stamen primordia as the first whorl, composed by paired
 4 stamens or sometimes single stamen. I–K. Initiation of a single carpel. L. Initiation of the
 5 five primordia as the second whorl (yellow) and two (to five) as the third whorl (blue). M.
 6 Floral bud from above. N. Median longitudinal section of a floral bud, showing the carpel.
 7 O. Stigma extended in the floral bud. P. Several carpels in one flower. B, bract; b,
 8 bracteole; C, carpel; P, petal; S, sepal. Scale bars: A, C, O, P, 200 μm ; B, D–N D–M, 100
 9 μm .

10
 11 **Fig. 4.** Floral development of *P. laurocerasus*. A–B. Sepal development. C. Stoma on a
 12 sepal (arrow in Fig. 4A). D–H. Petal development. H. Stoma on a petal (arrowhead in Fig.
 13 4G). I. Stamen. J. Longitudinal view of dehiscent anthers. K. Tricolpate pollen. L. Pollen
 14 aperture. P, petal; S, sepal. Scale bars: A, B, D–G, I, J, 200 μm ; 10 μm C, H, K; D, 100
 15 μm ; L, 5 μm .

16
 17 **Fig. 5.** Floral development of *P. laurocerasus*. A–I. Carpel development. A. Carpel
 18 primordium. B–F. Ventral slit formed. G. Mature carpel, with stoma (arrowhead). H.
 19 Stoma. I. Stigma. J–R. Ovule development. J, K. Abaxial view of partially dissected
 20 ovary with two ovules. L. Ovule primordia. M. Initiation of semi-annular inner
 21 integument. N. Inner integument becomes ring-like. O. Initiation of dehiscent outer

1 integument. P. Ovule bending inwards, with obturator appearing on placenta. Q–S.
 2 Mature ovule and obvious obturator. T. Only one ovule is present in the carpel. C, carpel;
 3 II, inner integument; N, nucellus; O, ovule; Ob, obturator; OI, outer integument. Scale
 4 bars: 100 μm .

5

6 **Fig. 6.** Inflorescence and early floral development in *P. serotina*. A. Top view of
 7 inflorescence to show terminal bract. B. Apical residual floral primordium (arrowhead)
 8 with a bract. C. Acropetal development of flowers along the inflorescence axis. D. Flower
 9 subtended by one bract. E. Sepal initiation. F. Petal initiation, alternate with sepal;
 10 initiation of first stamen primordia (arrowhead) G–H. Five pairs of antesepalous stamens
 11 (red) initiate as the first whorl. I. Five antesepalous stamens (yellow) as the second whorl.
 12 J. Five pairs of antesepalous stamens (red); antepetalous stamens hidden. K. Five
 13 antepetalous stamens (blue) as the third whorl. L. Single carpel initiation. M–O. Floral
 14 bud. P. Vertical section, stigma lower than the anthers. B, bract; C, carpel; P, petal; S,
 15 sepal. Scale bars: A, C, O, P, 200 μm ; B, D–N, 100 μm .

16

17 **Fig. 7.** Floral development in *P. serotina*. A, B. Sepal development, abaxial side. C.
 18 Stoma on the sepal (arrowhead in Fig. 7B, abaxial side). D–F. Petal development. G.
 19 Stoma on the petal (arrowhead in Fig. 7F, adaxial side). H. Stamen. I. Longitudinal
 20 dehiscent anther. J. Tricolpate pollen. K. Pollen groove. P, petal; S, sepal. Scale bars: A,
 21 B, D, 100 μm ; C, G, 10 μm ; J, L, 5 μm ; E, F, H, I, 200 μm .

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Fig. 8. Gynoecium and ovule development in *P. serotina*. A–G. Carpel development. A. Carpel primordium. B, C. Ventral slit formed. D. Mature carpel has a distinct fold. E. Two carpels in one flower. F. Stigma. G. Stigmatic tissue consisting of short papillae and pollen tubes. H–M. Development of ovule. H, I. Abaxial view of partially dissected ovary with two ovules. J. Initiation of semi-annular inner integument and obturator. K. Ovule begins to bend inwards and obturator enlarges. L. Initiation of semi-annular outer integument. M. Cup-shaped inner integument and hood-shaped outer integument. N. The obturator continues growing. O. Mature ovules. C, carpel; II, inner integument; N, nucellus; Ob, obturator; OI, outer integument. Scale bars: A–D, I, L–T, 100 µm; E–G, J, K, 400 µm; H, 10 µm.

Fig.1

136x76mm (300 x 300 DPI)

Fig.2

146x123mm (300 x 300 DPI)

Fig.3

147x158mm (300 x 300 DPI)

Fig.4

142x125mm (300 x 300 DPI)

Fig.5

146x149mm (300 x 300 DPI)

Fig.6

146x160mm (300 x 300 DPI)

Fig.7

146x145mm (300 x 300 DPI)

Fig.8

146x117mm (300 x 300 DPI)