The intriguing sex life of Bolwarra: an Australian relic of ancient Gondwanan flora

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The plant and its history:

Bolwarra is an aboriginal name for *Eupomatia laurina*. It's an evergreen understory shrub (up to 4 m high) in rainforests and wet sclerophyll forests of the east coast of Australia, covering tropical, sub-tropical and warm temperate climates (Plates A & 1). The plant has a primitive xylem for transporting water, and hence, its distribution is confined to moist soils within forested areas of the continent (Plate 2). The most southern natural stand of Bolwarra is just north-east of the small village of Metung in the state of Victoria, where FG's garden is located (Plate 2).

Bolwarra has dark green, glossy leaves and it flowers during summer (over 8 weeks in southern Victoria). The flowers are unique in their morphology and biology. They are white-cream (2.5-3.0 cm diam.), have a very strong musky-fruity fragrance, and last only one day. They are bisexual (both male and female parts on each flower), but don't have sepals, petals, styles or nectaries. Caps covering flower buds (Plate 5) are abscised at flowering (Plate 6). The flowers have stamens on the outside and sticky staminodes (petal-like sterile stamens) on the inside (Plate 7). The stamens and staminodes are united at their base, and together form a circular ring (synandrium) around the female parts of flowers in a receptacle. The positions of the stamens and staminodes of flowers are continually changing during most of the day, making it difficult for botanical artists to draw or paint the flowers.

Bolwarra is a member of a lineage of plants, which existed when the supercontinent, Gondwana (Plate 3), was still breaking up around 80 million years ago. Fossils indicate that the plant appeared between 72 to 66 million years ago. It is thought that a weevil pollination system of the plant was developed by the end of the Cretaceous period (66 million years ago).

Part of the intrigue of Bolwarra is its unique reproductive biology, involving asexual and sexual reproduction, and its highly specific symbiotic relationship with small weevils in the genus *Elleschodes*.

Plate A. Mature Bolwarra shrub in the Maranoa Botanic Gardens in Melbourne.

Asexual reproduction:

Bolwarra produces shoots from the base of the trunk and suckers from its roots, resulting in spreading shrubs with multi-trunks (Plate 4.). This enables the plant to regenerate after bushfires, and therefore assists with the survival of established populations.

Some plant species are capable of producing seed from unfertilised ovules through a process called apomixis. However, it is not known whether this form of asexual reproduction occurs in Bolwarra.

Sexual reproduction via cross-pollination:

Cross-pollination is achieved by a number of ingenious mechanisms. Firstly, the male (pollen) and female (stigmas) parts of the flower are separated physically (Plates 8 & 11), and mature at different times. The flowers are in a female phase (i.e. stigmas exposed and receptive to pollen) in the early morning, in a neutral phase for part of the afternoon, and in a male phase (i.e. pollen released) during the early evening. Early in the morning, the staminodes open exposing the stigmas (Plate 9), and the stamens are reflexed away from the stigmas. In the afternoon, the staminodes move inwards and cover the stigmas (Plates 11 & 12). In the early evening, the stamens move forward and the anthers release pollen through longitudinal slits (Plates 13, 14 & 15).

The development of flowers on individual plants is synchronised during the day (i.e., all flowers are at the same morphological stage). This is also a neat mechanism to facilitate cross-pollination. In addition, plants flower in flushes with a one or two day period without flower production following a day of flowering.

Flowers of natural stands of bolwarras are pollinated exclusively by species of small weevils (2 mm long) in the genus *Elleschodes*. In the early morning, the weevils are attracted to the flowers by their pervasive fragrance, and feed on the staminodes during the day (Plate 10). They lay their eggs at the bases of the staminodes and the inner gyne of the stamens, but not on the floor of the floral chamber. Staminodes provide food for the weevils and also protect them from predators during the day.

When the weevils emerge from the staminodes at dusk, they feed on the pollen and their sticky bodies (due to an exudate on the staminodes) become contaminated with pollen grains. The weevils then fly away from the plants. At dawn the next day, the weevils are attracted to flowers on other Bolwarra bushes (different populations), where they burrow into the staminodes and deposit the pollen grains (Plate 16) on the stigmas. Also, early the next morning after flowering, synandria are abscised from the receptacles (Plate 17) and fall to the ground (Plate 18). The eggs of the weevils in the fallen synandria hatch, and the larvae feed on the synandrial material before pupating in the soil. Adult weevils emerge from the soil and seek Bolwarra flowers. The weevils are not reported to be attracted to other flowers, and their entire life cycle is dependent upon the flowering of Bolwarra plants.

Pollinated flowers set fruit (2.0-2.5 cm in diam.; Plates 19 & 20) and produce seeds, which are dispersed by mammals and birds.

This fascinating system of outcrossing results in genetic diversity, which has enabled Bolwarra to adapt to environmental changes over a very long period (i.e., a major benefit of the plant 'sleeping around' with the aid of the weevil).

Sexual reproduction via self-pollination:

Self-pollination of Bolwarra has been reported in northern Australia by a number of pollination experts. A study using bagged flowers showed that 10% of flowers set fruit due to self-pollination, whereas 60% of open-pollinated flowers set fruit as a result of mainly weevil pollination. Self-compatibility enables isolated bolwarras in forests to produce fruit and seeds, which can be spread to

other areas, and increase the local population of the plants. In this way, self-fertilisation assists in the survival of Bolwarra in areas where populations are fragmented.

In contrast to natural stands of bolwarras in northern parts of Australia, fruit set is rare on cultivated plants outside their natural range. Less than 0.5% of flowers set fruit in gardens in Metung and Greater Melbourne. One reason for this is the absence of relevant weevils in the gardens, but the studies in northern Australia suggest that there should be a higher level of fruit set due to self-pollination. The temperatures in southern Australia are lower than those in northern parts of the continent, and differences in environmental conditions may cause the differences in the level of self-pollination. It has been shown that high-temperature treatments of stigmas of some self-incompatible plants, results in them becoming self-compatible. However, temperatures in Metung are unlikely to restrict the receptivity of stigmas to pollen of bolwarras because 48% of flowers set fruit when pollen of a clone of Bolwarra was hand brushed onto stigmas of flowers of the same clone. Therefore, the flowers have a high level of self-compatibility if the pollen reaches the stigmas under the environmental conditions at Metung.

The mechanism for and environmental conditions conducive to self-pollination are yet to be discovered.

Conclusions:

The reproductive biology of Bolwarra has enabled this primitive plant to survive for millions of years. It is remarkable that the mutually beneficial and highly specific relationship between *Eupomatia laurina* and weevil species of *Elleschodes* has been maintained for eons. Although there has been a long and successful association between Bolwarra and the weevils, the question is whether this relationship can be maintained when there are far more rapid changes in the climate than previously experienced. The danger is that Bolwarra and its pollinator weevils may respond differently to such dramatic changes. For example, climate change is causing bushfires to become more frequent and severe during summer in south-eastern Australia, and although Bolwarra will survive the fires via vegetative propagation, the intense heat may kill the weevils.

It's nice having plants with interesting stories like bolwarras in gardens in Melbourne and other parts of southern Victoria. Much of the reproductive system of Bolwarra in these environments is unknown at the moment, and this adds to the mystique of the plant. We are now having fun attempting to unravel this gap in knowledge of its sex life.

Plates 1 – 20. History and life cycle of Bolwarra. **Plate 1.** Foliage of Bolwarra; **Plate 2.** Forested areas in Australia – bolwarras grow naturally north of Metung in rainforests and wet forests within these areas; **Plate 3.** The supercontinent, Gondwanna, before it started to break up 180 million years ago; **Plate 4.** Multi-trunks of Bolwarra; **Plate 5.** Flower buds in leaf axils; **Plate 6.** Abscissing flower cap and emerging stamens (6.0am); **Plate 7.** Staminodes opening and stamens retracting (7.30am); **Plate 8.** Stamens retracting further (7.40am). **Plate 9.** Staminodes fully open and stigmas exposed during the female phase (8.30am); **Plate 10.** Small weevils visiting the flower and burrowing into the staminodes (Credit: R. Whyte); **Plate 11.** Staminodes folding inwards (early afternoon); **Plate 12.** Staminodes closing further (mid-afternoon): **Plate 13.** Stamens moving forward and anthers releasing pollen (7.00pm); **Plate 14.** Stamens clasping a tight 'ball' of staminodes (8.00pm); **Plate 15.** Longitudinal slits in the anthers (x40 magnification); **Plate 16.** Pollen grains (x400 magnification); **Plate 17.** An abscised synandrium (early the next morning); **Plate 18.** Fallen synandria on the ground; **Plate 19.** Developing fruit three weeks after artificial pollination; **Plate 20.** Mature fruit (Credit: R. Whyte).

Plate 1

Plate 2

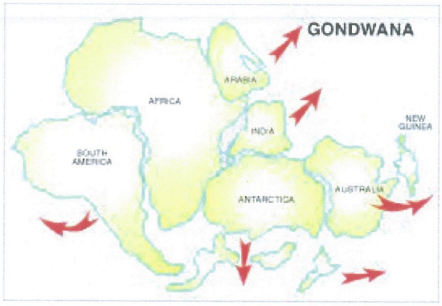

Plate 3

Plate 4

Plate 5

Plate 6

Plate 7

Plate 8

Plate 9

Plate 10

Plate 11

Plate 12

Plate 13

Plate 14

Plate 15

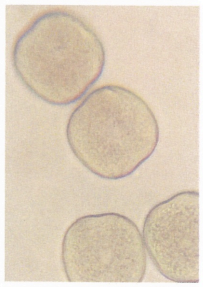

Plate 16

Plate 17

Plate 18

Plate 19

Plate 20