

# Common primordia and the double-headed inflorescence in 'Renova' red clover (*Trifolium pratense* L.), a papilionoid legume

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## SUMMARY

Double-headed inflorescences subtended by one pair of bracts were commonly observed on 'Renova' red clover grown under experimental conditions. The early ontogeny of these double-headed inflorescences was investigated using scanning electron microscopy. The inflorescence was initiated as an outgrowth of the vegetative apical meristem in the axil of the penultimate bract. This outgrowth (the inflorescence meristem) was elongated along an axis drawn between the stem centre and the bract. While still small and before floret initiation had begun, it became divided by a median furrow into two inflorescence meristems. These two inflorescence primordia then developed asynchronously and with mirror symmetry. The primordium proximal to the penultimate bract showed enhanced growth and initiated florets earlier than its counterpart. The other primordium enlarged and initiated florets only after the ultimate bract primordium had formed. The inflorescence apices were progressively reduced by the initiation of florets as hemispherical protuberances. These two remnant apices were each ellipsoidal in shape and their long axes were tilted relative to the flowering stem axis, such that they faced each other. Florets were initiated in elliptical whorls and their organogenesis was zygomorphic. At maturity, the mirror symmetrical pattern of development was not evident, and no vegetative tissue could be seen between the adjacent inflorescences.

*Key-words:* common primordium, inflorescence ontogeny, red clover, *Trifolium pratense* L., 'Renova' cultivar.

## INTRODUCTION

In developing flowers, organs sometimes arise from common primordia. For example, in *Saururus cernuus* and *Houttuynia cordata* (Saururaceae), floral apices and bracts arise from a single initial primordium, which later splits (Tucker 1975, 1981). Similarly, in the Primulaceae, there is a common petal–stamen primordium (Sattler 1967; Sundberg 1981), and in *Caulophyllum thalictroides* (Berberidaceae), a common primordium gives rise to both a stamen and a nectary (Brett and Poluszny 1982). Tucker (1984) has suggested that, in some cases at least, common primordia may be useful phylogenetic clues, indicating precocity of expression of a feature, and therefore relative advancement.

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In red clover (*Trifolium pratense* L.), inflorescences arise in basipetal sequence along flowering stems as they elongate. Such inflorescences are usually single (Payer 1857; Picklum 1954; Taylor 1985) but at times a double-headed inflorescence arises. Aitken (1960), in a study of both *T. pratense* and *T. alexandrinum*, suggested that these arise separately from the vegetative apex because a remnant of the shoot apex seemed to be present between the two inflorescences. While conducting a comparative study of inflorescence development among red clover cultivars (Retallack & Willison 1990), we noticed that the 'Renova' cultivar had an unusual propensity to produce double-headed inflorescences. Here we report that this double-headed inflorescence arises from a common primordium, rather than from two separate initiations.

## MATERIALS AND METHODS

### *Plant material*

Seeds of eleven *Trifolium pratense* L. cultivars were obtained from Dr T.-M. Choo (Agriculture Canada Research Station, Charlottetown, Prince Edward Island, Canada) and were germinated and grown in sterile potting compost in a Conviron PCV 36 walk-in growth chamber at Dalhousie University at 21°C, 16/8 h light/dark at 330 mol/m<sup>2</sup>/s photon flux density. Flowering occurred after 40 days. All the cultivars showed only single-headed inflorescences except for 'Renova' in which about 70% bore flowering stems with a terminal double-headed inflorescence.

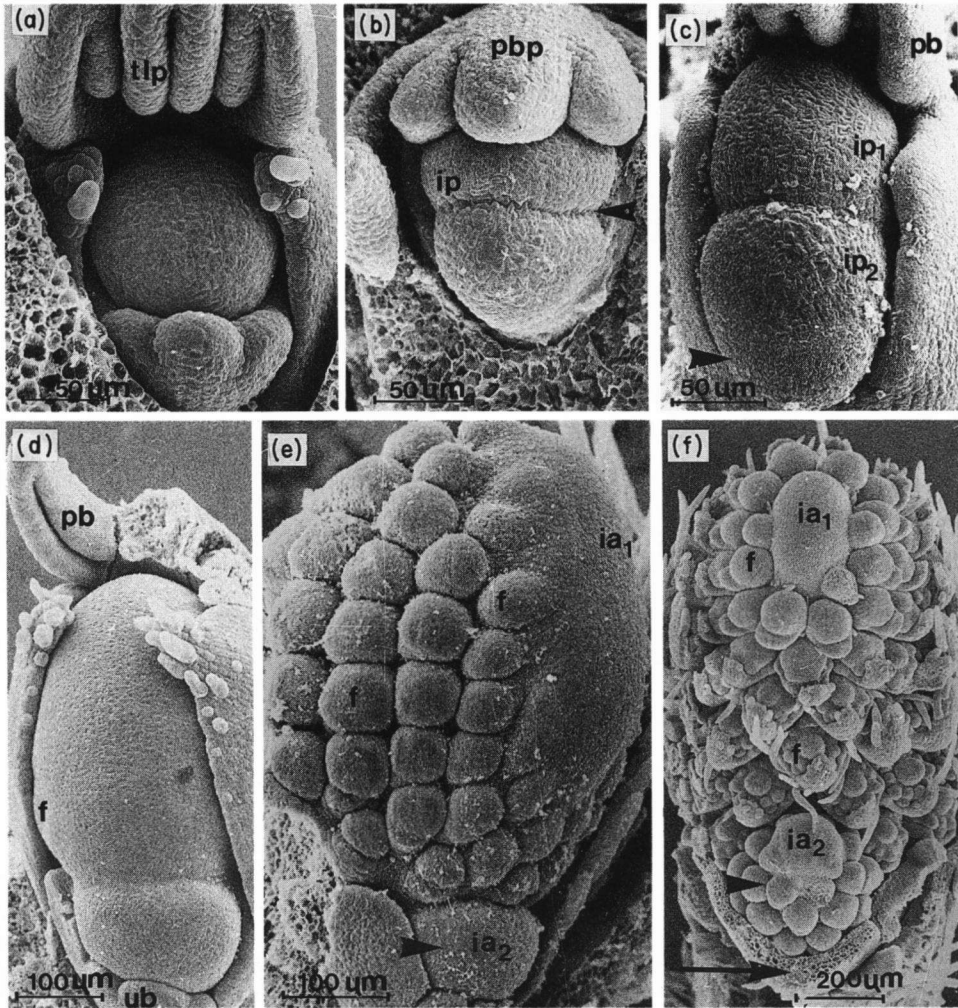
### *Scanning electron microscopy*

Apices of *T. pratense* cv. 'Renova' were dissected at room temperature in 3% (w/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.0), placed in fresh fixative for 3 h, prior to rinsing in buffer and post fixation for 1 h in 2% (w/v) osmium tetroxide. The specimens were acetone dehydrated and critical-point dried from liquid carbon dioxide in a Polaron Critical Point Drying apparatus. Dried samples were mounted on aluminium stubs covered with double-sided sticky tape and coated with a thin layer of gold palladium using a Samsputter II A coating unit. They were then examined with a Bausch and Lomb Nanolab 2000 SEM, operating at 15 kV.

## RESULTS

The single-headed inflorescence of the 'Renova' cultivar of red clover, like that of the 'Florex' and 'Ottawa' cultivars (Retallack & Willison 1990), is initiated in the axil of the penultimate bract, becoming evident as a dome which is distinctly bigger than its vegetative counterpart (Fig. 1a). The primordia of double-headed inflorescences, which arise commonly in this cultivar, can be recognized at a very early stage, being clearly divided into two sections by a furrow (Fig. 1b). The ultimate bract is cut off after bifurcation has occurred and arises opposite the penultimate bract, so as to cradle the entire double-headed inflorescence (Fig. 1d). As in the case of the single-headed inflorescences of the 'Florex' and 'Ottawa' cultivars (Retallack & Willison 1990), the ultimate bract arises as a ridge apparently from a remnant of the vegetative apex. This remnant lies to the side of the bifurcated inflorescence primordium (Fig. 1c), not between its two components.

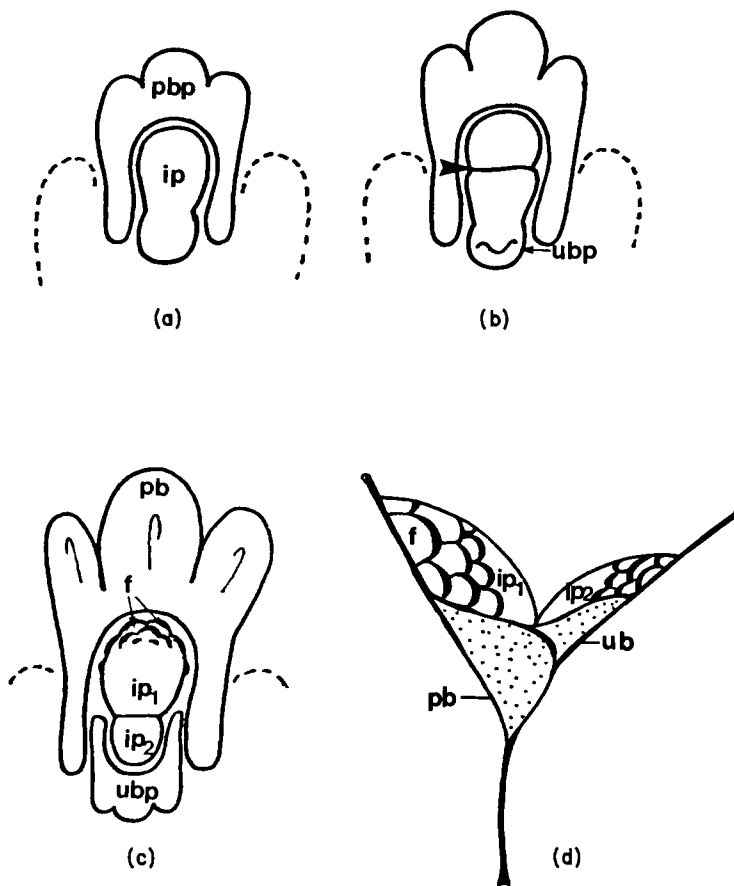
Both bracts develop the primordia of three blades and two stipules, the latter enlarging radially so as to enclose the base of the developing double-headed inflorescence (Fig. 1d). Usually, the component of the double-headed inflorescence that lies toward the penultimate bract is larger and somewhat more advanced in growth and development than its



**Fig. 1.** Inflorescence ontogeny of the double-headed 'Renova' red clover inflorescence. (a) Vegetative apex in the axil of a trifoliate leaf primordium (tlp). (b) Median furrow (▲) develops in the young inflorescence primordium (ip) subtended by the penultimate bract primordium (pbp). (c) Enhanced developmental growth of that young inflorescence primordium (ip<sub>1</sub>) proximal to the penultimate bract (pb) accompanies the distal elongation (▲) of the other young inflorescence primordium (ip<sub>2</sub>). (d) Preferential development of that young inflorescence in the axil of the penultimate bract. Florets (f) are initiated as protuberances proximal to the penultimate bract. Growth of the distal ridge (Fig. 1c) has produced the ultimate bract (ub). (e) Florets are initiated from the periphery of the larger inflorescence apex (ia<sub>1</sub>) in elliptical whorls. Floret development of that inflorescence distal (▲) to the penultimate bract has not been initiated. (f) Both members of the double-headed inflorescence are developing such that the inflorescence apices are tilted so as to face each other. Florets are first initiated from that inflorescence distal to the penultimate bract in a zone (▲) proximal to the ultimate bract (only scar present, †). Florets on the inflorescence proximal to the penultimate bract are developmentally more advanced.

counterpart (Fig. 1e and f), and in the mature flower it becomes the upper portion of the double-headed inflorescence. This developmental pattern is summarized in Fig. 2.

Florets are initiated first on the larger portion of the inflorescence, beginning at the periphery of the dome-like meristematic apex and progressing to become elliptical whorls (Fig. 1e). The oldest florets rest proximal to the penultimate bract. The residual inflorescence apex is not placed symmetrically on the top of the young inflorescence but is tilted



**Fig. 2.** Stages in the origin and development of the double-headed inflorescence of *Trifolium pratense* L. cv. 'Renova'. (a–c) Show the polar view. (a) Origin of the common inflorescence primordium (ip) in the axil of the penultimate bract primordium (pbp). (b) Cleavage (▲) of the common primordium and initiation (↑) of the ultimate bract primordium (ubp). (c) Floret (f) initiation on the more mature primordium (ip<sub>1</sub>) associated with the penultimate bract (pb). (d) Lateral view showing floret initiation on both inflorescence primordia (ip<sub>1</sub> and ip<sub>2</sub>) subtended by their respective bracts (pb and ub) and the main axis.

toward the ultimate bract primordium (Fig. 1e). Floret development is initiated on the smaller inflorescence primordium proximal to the ultimate bract and progresses as a mirror image of the larger one, which is more advanced. Thus, the remnant portions of the inflorescence domes face each other, being tilted away from their respective bracts (Fig. 1f). At maturity, these remnant domes are no longer visible and we found no evidence of a residue of the vegetative apex between the adjacent inflorescences. Subsequent development of all florets on both inflorescences is characterized by the features already established for florets on single-headed inflorescences (Retallack & Willison 1990).

## DISCUSSION

The symmetry of the double-headed condition in the 'Renova' cultivar of red clover is interesting but its full significance has yet to be resolved. Inflorescence ontogeny in single-

headed red clover is characterized by zygomorphic symmetry (Retallack & Willison 1990), unlike that of white clover (*Trifolium repens* L.) which is actinomorphic (Retallack *et al.* 1990). Each inflorescence in the double-headed condition has the characteristic zygomorphic symmetry of red clover with its dorsiventral preference. There is also a zygomorphic symmetry between the two inflorescences of the double-head, but the plane of this symmetry is at right angles to the plane of zygomorphic symmetry in the single-headed flower (i.e. each inflorescence is symmetrical about a plane drawn between the centre of the stem and the subtending bract, but the symmetry between the paired inflorescences is about the line of the furrow which forms between them).

The double-headed inflorescence of 'Renova' red clover arises from a single inflorescence primordium by bifurcation. Since we found no evidence of separate initiation, nor of a vegetative residue between the two inflorescence components, the initial primordium appears to be a common primordium. This implication of the existence of a common inflorescence primordium is in contrast to the situation reported by Aitken (1960) who considered that the double-headed inflorescence in *Trifolium pratense* and *T. alexandrinum* was, in effect, two inflorescences, separated in age and development with the remnants of a shoot apex between them. In view of the morphogenetic plasticity of *Trifolium* and our own observations of wild red clover (not reported), it is probable that 'Renova' red clover differs from the cultivars used by Aitken. Common primordia have not been reported previously for inflorescence apices but are well established for floral organs such as floral apices and bracts (Tucker 1975, 1981, 1989; Retallack *et al.* 1990).

The phylogenetic significance of common primordia is unresolved. Stebbins (1974) has suggested that precocity of expression of specialized features characterizes the more advanced taxa. Tucker (1984) has suggested that common primordia may represent such specialized features and so may serve as phylogenetic markers in the Leguminosae. If we accept the suggestions of Stebbins (1974) and Tucker (1984), the presence of the common inflorescence primordium in 'Renova' red clover is specialized and infers phylogenetic advancement. It is unlikely, however, that this concept was intended to apply at the cultivar level, but it is interesting to note that artificial selection for agricultural purposes should have selected a character which derives from a common primordium.

Bernier (Kinet *et al.* 1981) suggested that common primordia may be physiologically regulated in that they form when an apex is most active, basing this on the example in *Houttuynia cordata* (Tucker 1975) where common primordia forming floral apices and associated bracts were most prevalent in the physiologically active mid-zone of the inflorescence. Sundberg (1982) suggested that the range of expression for floral organs formed from common primordia in the Primulaceae suggested some sort of physiological stimulus. It is possible that a physiological explanation for the common inflorescence apex-apex primordia in 'Renova' red clover could be suggested, the inflorescence apex yielding the double-headed condition then being considered physiologically more active than its counterpart yielding the more usual single-headed inflorescence.

## REFERENCES

- Aitken, Y. (1960): Axial origin of the terminal inflorescence in the genus *Trifolium*. *Nature* (Lond.) **187**: 622-623.
- Brett, J.F. & Posluszny, U. (1982): Floral development in *Caulophyllum thalictroides* (Berberidaceae). *Can. J. Bot.* **60**: 2133-2141.

- Kinet, J.-M., Sachs, R.M. & Bernier, G. (1981): *The Physiology of Flowering*. Vol. III. *The Development of Flowers*, p. 18. CRC Press, Inc., Boca Raton, Florida.
- Payer, J.-B. (1857): *Traite d'Organogenie Comparee de la Fleur*. Paris. (Reprint, J. Cramer, Lehre, 1966.)
- Picklum, W.E. (1954): Developmental morphology of the inflorescence and flower of *Trifolium pratense*. *Iowa State Coll. J. Sci.* **28**: 477-495.
- Retallack, B. & Willison, J.M.H. (1990): Floral development, in Ottawa and Florex red clover, *Trifolium pratense* L. (Papilionoideae: Leguminosae). *Am. J. Bot.* in press.
- , Walker, N. & Fraser, J. (1990): Floral development in Sonja white clover, (*Trifolium repens*). *Ann. Bot.* **65**: 241-249.
- Sattler, R. (1967): Petal inception and the problem of pattern detection. *J. Theor. Biol.* **17**: 31-39.
- Stebbins, G.L. (1974): *Flowering Plants*. Belknap Press, Cambridge, Massachusetts.
- Sundberg, M.D. (1981): Apical events prior to floral evocation in *Cyclamen persicum* "F-1 Rosemunde" (Primulaceae). *Bot. Gaz.* **142**: 27-35.
- (1982): Floral ontogeny in *Cyclamen persicum* "F-1 Rosemunde Rose" (Primulaceae). *Am. J. Bot.* **69**: 380-388.
- Taylor, N.L. (1985): *Clover Science and Technology*. Soil Science of America, Inc., Madison, Wisconsin.
- Tucker, S.C. (1975): Floral development in *Saururus cernuus* L. Floral initiation and stamen development. *Am. J. Bot.* **62**: 993-1007.
- (1981): Inflorescence and floral development in *Houttuynia cordata* (Saururaceae). *Am. J. Bot.* **68**: 1017-1032.
- (1984): Origin of symmetry in flowers. In: White, R.A. and Dickison, W.C. (eds): *Contemporary Problems in Plant Anatomy*. Academic Press, New York.
- (1989): Overlapping organ initiation and common primordia in flowers of *Pisum sativum* (Leguminosae: Papilionoideae). *Am. J. Bot.* **76**: 714-729.