Acta Bot. Neerl. 30 (3), May 1981, p. 219–229.

ONTOGENY AND VASCULARISATION OF THE FLOWER OF OENOTHERA (ONAGRACEAE)

O. C. DE VOS

Biologisch Centrum, Vakgroep Plantensystematiek, Rijksuniversiteit Groningen, Postbus 14, 9750 AA Haren (Gn)

SUMMARY

The ontogeny and the vascular pattern of the flower of *Oenothera biennis* L. and some other species of the genus were studied. The "ring" in the vascular system of the flower has been analyzed and appears to be composed of a partly degenerated system of nodal branches. The results affirm the theory that ovary wall and hypanthium are at least partly of axial origin.

The petal and the associated epipetalous stamen do not arise from the same primordium. They are not supplied from a common, single vascular strand.

Difference in size, shape, and stainability of cells of the inner and of the outer layers of the ovary wall are not related to a carpellar or an axial origin of these layers.

1. INTRODUCTION

The interpretation of the inferior ovary has long been in dispute (DOUGLAS 1944, 1957, LEINFELLNER, 1941).

DE CANDOLLE (1827), VAN TIEGHEM (1868, 1871), EAMES (1931) and SAUNDERS (1925) are protagonists of the general theory that the inferior ovary had developed by means of complete coalescence of the bases of calyx, corolla, stamens and carpels (coalescence theory).

On the other hand, the theory that the inferior ovary is a concave receptacle lined with carpels, was postulated by NAUDIN (1855) and GOEBEL (1886).

Finally SCHLEIDEN (1849) and PAYER (1857) proposed the theory that the entire ovary, its wall and placentae included, is a modified axis.

Evidence based on the ovary structure in the Onagraceae was involved in these discussions, and the interpretation of the hypanthium in *Oenothera* and *Fuchsia* forms an expansion of the discussions.

The coalescence theory received support from investigations on *Oenothera* and other genera of Onagraceae by BONNER (1948) and BAEHNI & BONNER (1949).

PANKOW (1966), BUNNIGER & WEBERLING (1968) and MAYR (1969) supported the theory of the concave receptacle. Previously, however, DUCHARTRE (1842) had postulated that the ovary of *Oenothera suaveolens* developed from an invaginated axis, lined with carpels, but he considered the placenta too to be axial.

BARCIANU (1975) adopted SCHLEIDEN's modified axis theory for *Oenothera*.

In publications of the last thirty years there is no agreement regarding the interpretation of the inferior ovary and the hypanthium in the Onagraceae.

PANKOW (1966), BUNNIGER & WEBERLING (1968) and MAYR (1969) studied the activity of the meristem in the floral apex, and the initiation and development of the floral parts. They concluded that the wall of the ovary and the hypanthium are at least partially axial. BONNER (1948) and BAEHNI & BONNER (1949) compared the vascular pattern of the flower of *Oenothera* with that in some other Onagraceae. They concluded that the wall of the ovary and the hypanthium develop by the coalescence of the bases of sepals, petals and stamens, the ovary being lined by the carpels. It seems therefore as if in the Onagraceae investigations of the vascular pattern of the flowers lead to interpretations which are at variance with interpretations based on studies of floral development.

This controversy led BUNNIGER & WEBERLING (1968) to state that not too much importance should be attributed to the vascular pattern in interpretations of the morphological nature of the flower.

2. MATERIALS AND METHODS

The following species were investigated: *Oenothera biennis* L., *Oenothera fruticosa* L., *Oenothera missouriensis* Sims., *Oenothera perennis* L.

Oenothera missouriensis was collected in the Hortus Botanicus of the University of Amsterdam. The other species were obtained from the Hortus "De Wolf" at Haren. About 50 buds and flowers of *Oenothera biennis* of successive ages were sectioned. The sections of 6 µm thickness were stained with auramin, astrablue and safranin (MAÁCZ & VÁGÁS 1963). Five additional buds of *Oenothera biennis* were cleared and stained. The number of buds sectioned from the other species were: of *O. fruticosa* 5, of *O. missouriensis* 3 and of *O. perennis* 3.

3. ONTOGENETICAL OBSERVATIONS

In *Oenothera biennis* the distance between the two groups of initials of the petals and the stamens is very small. Furthermore the time interval between the visible initiation of the two groups is very short. However, it is obvious that the first periclinal divisions initiating the development of the petal take place in the second tunica layer, and those which initiate the development of the stamen occur in the third cell layer. The first dividing cell in the third layer is certainly not adjacent to the first dividing cell of the second layer.

The initials of the stamens are more centrally situated than those of the petals. At a later stage it becomes obvious that the stamen primordia show a two-layered tunica, while the petal primordia show only one distinct tunica layer. This renders it inaccurate to consider the stamen primordium as a branch of the petal primordium.

The carpels are initiated immediately after the epipetalous stamens. The first emergences are superposed to the petals and the epipetalous stamen: they grow out to form the roof and the stigma of the gynoecium. Alternating with these emergences are the four developing septae which later meet in the centre of the gynoecium and fuse with their margins post-genitally.

The view of DUCHARTRE (1842) that the placentae form part of an axial columna to which the septae are attached is based on an erroneous interpretation of the vascular supply to the ovules.

In older buds the tissues between the stamens and the top of the gynoecium show a strongly increased mitotic activity indicative of a rapid intercalary growth by means of which the floral tube is formed. In *Oenothera missouriensis* especially this tube attains a considerable length.

4. THE VASCULAR SYSTEM OF THE FLOWER

In the pedicel, just above the leaf-gap of the bract, a nearly closed sheath of vascular tissue can be discerned. At this level in *Oenothera biennis* the bundles are not bicollateral, as in the vegetative parts of the plant, but collateral. At the level of the leaf-gap the internal phloem moves through the xylem strands and comes to lie in the periphery of the stele. In *O. fruticosa*, *O. missouriensis* and *O. perennis* this displacement is much less obvious and in *O. fruticosa* in particular much internal phloem is discernible above the level of the leaf-gap. The bicollateral nature of these bundles hinders the interpretation of the orientation of the vascular system in this species.

In the pedicel 16 vascular bundles can be barely discerned. There are 4 broad bundles S, 4 smaller bundles C alternating with the bundles S, and 8 tiny bundles X alternating with the bundles S and C. Already below the base of the ovary the bundles X shift inward, move around the bundles S, and meet pairwise on the inner side of the S bundles (*fig. 1*). These pairs then coalesce to form the 4 bundles V. These bundles V remain clearly double over a considerable distance.

As a result of the coalescence of bundles in the pedicel 12 principal bundles (*fig. 2*) are present in the ovary wall. The large bundles S are clearly collateral with the phloem on the peripheral side. They give off numerous small side-

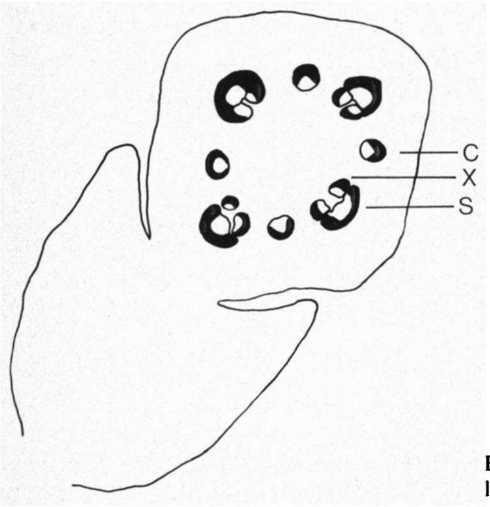

Fig. 1. *Oenothera biennis*, T.S. of petiole. Xylem drawn in black.

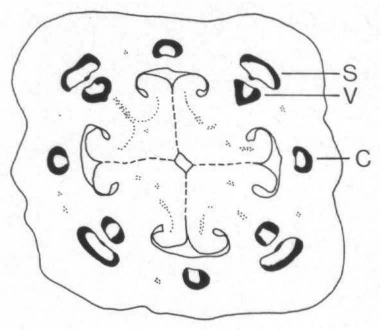

Fig. 2. *Oenothera biennis*, T.S. of ovary. Xylem drawn in black, lateral strands dotted.

branches, which run obliquely upward. The bundles V are double, the two collateral halves facing one another with their phloem, so that the whole bundle V sometimes seems to be amphicribral. These four bundles V give off the placental strands. These are given off in pairs at first running in a lateral direction, later in a radial direction when entering the septum. In the septum the two strands join each other and continue towards the placenta in an inward and slightly upward direction. In the placenta the two strands diverge again, each strand supplying a row of ovules.

After having given off the placental strands, bundle V continues through the hypanthium, at the apex of which it supplies the *episepalous* stamens.

The four bundles C split in the upper part of the ovary into inner and outer branches. The inner branches C′ join each other in the style. The outer branches C traverse the hypanthium to its apex, where they supply the *epipetalous* stamens. These branches are connected here to the vascular ring.

As in the ovary wall, in the hypanthium 12 main bundles are present (*fig. 3*). Four of them, the bundles S, are collateral, and eight of them, the stamen bundles V and C, are more or less amphicribral.

In the outer rim of the hypanthium a distinct ring of vascular tissue is present.

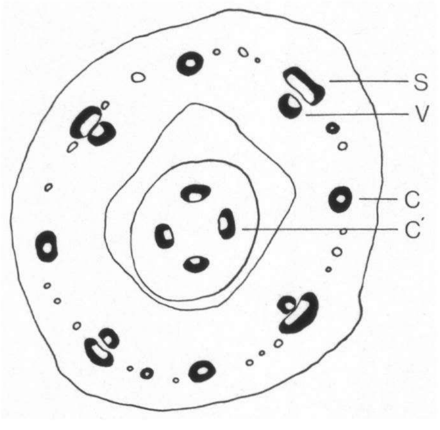

Fig. 3. *Oenothera biennis*, T.S. of hypanthium Xylem drawn in black.

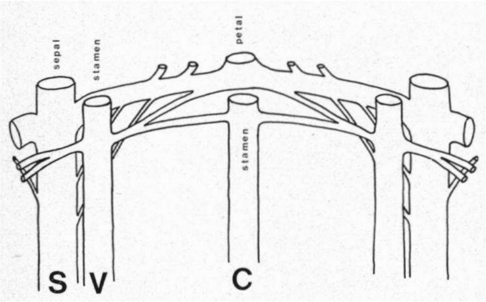

Fig. 4. Schematic view of the bundles in the rim of the hypanthium, seen from inside.

The ring is more or less continuous and forms numerous branches, both upwardly and downwardly. Observation of cleared flowers, in which the xylem has been stained, shows an irregular ring of bundles formed mainly by the bundles S and their subsidiary bundles. Sections of the hypanthium of a series of several buds and flowers at different stages of development show the structure of the vascular pattern in the ring more clearly. It becomes apparent that ancillary vascular bundles are present which lack a differentiated xylem. These are not visible in the cleared flowers.

A scheme of the vascular system of the ring in *Oenothera biennis* is shown in *fig. 4* and *fig. 5*. Each bundle S gives off two well-developend side-branches A 1, which form the bulk of the ring, while the main bundle continues into the midrib of a sepal. Small bundles, often without xylem, branching off from the main bundles S in the upper part of the hypanthium, join the side-branches A 1. The lateral veins of the petals split off from these side-branches. The petal bundles P, alternating with the bundles S, arise from the fusion of two adjacent branches A 1.

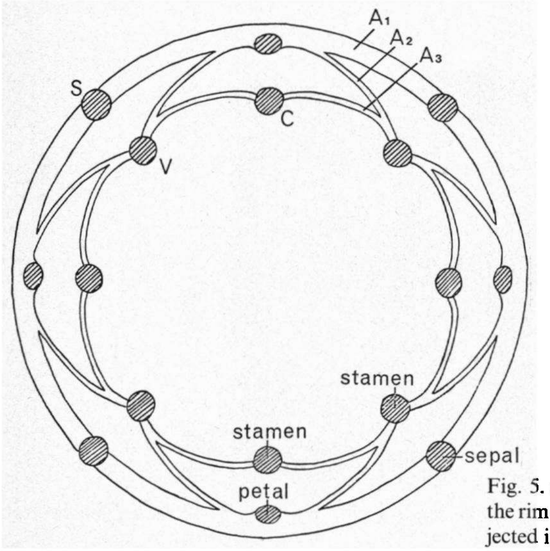

Fig. 5. *Oenothera biennis*, vascular pattern in the rim of the hypanthium, simplified and projected in a transverse plane.

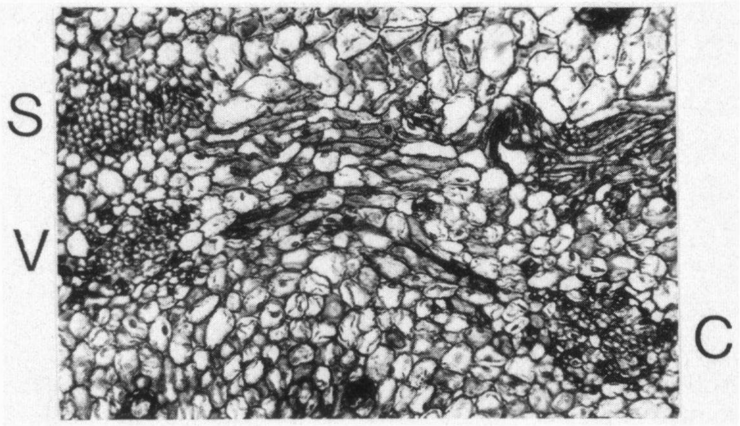

Fig. 6. *Oenothera fruticosa*, T.S. of rim of hypanthium. The part of the section shows an A 1 and an A 3 bundle.

Small bundles A 2, with little or no xylem, arise at the junction of A 1 and P and connect with the bundles V, which supply the episepalous stamens. These small bundles run almost concentrically with the rest of the ring along its inner side at the lower edge of the ring. For the most part these bundles are adnate to the main part of the ring and are almost indistinguishable from it. Only where they join the stamen traces do they diverge a little from the rest of the ring.

From the junction of A 2 and V further tiny bundles connect with the bundles C of the epipetalous stamens, which bundles also run almost concentrically with the rest of the ring and reach the bundles C at a point 10 μm below the origin of the bundle P in the ring formed by the A 1 bundles. These thin bundles are called A 3. They have little or no xylem.

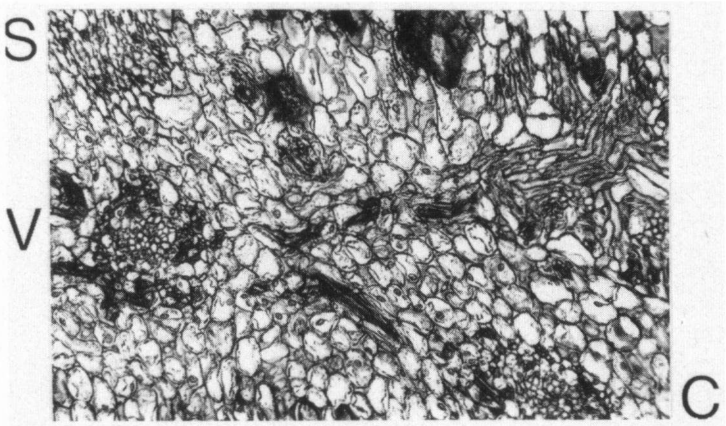

Fig. 7. *Oenothera fruticosa*, T.S. of rim of hypanthium. At this level an A 2 and an A 3 bundle are visible.

In the other species of *Oenothera* studied the vascular ring of the hypanthium does not seem to have attained a similar stage of complexity. Of the three species *O. perennis* has a vascular ring comparable to that of *O. biennis*. In *O. fruticosa* the connections between the stamen and the petal traces appear to be poorly developed, but are still conspicuous, while in *O. missouriensis* it was not possible to establish the presence of these connections with any degree of certainty.

4.1. Interpretation of the vascular system

Some authors treat the vascular system as an independent structure and use the evidence it provides for their comparative studies without any reference to other data. This may yield incomplete evidence since the vascular pattern develops in relation to the initiation and subsequent growth of the organs which it supplies. Vascular patterns may provide useful indications concerning the morphology and ontogeny of the flower, but they have to be interpreted exclusively as a registration of events taking place during the early ontogeny of the organs. Thus the innervation of an organ gives retrospective information concerning the time of its initiation and the location of its primordium with regard to previously initiated and innervated organs.

The large bundles S mainly provide the traces of the sepals. The petal traces appear to be offshoots of these bundles S. This is the consequence of the fact that in the floral apex the petal primordia are initiated after the sepal primordia. The point at which the bundles P and S unite is situated in the outer rim of the hypanthium, which indicates that between the points of initiation of the two whorls hardly any intercalary growth has taken place. On the other hand an extensive intercalary growth has occurred beneath the level of the sepal primordia resulting in a considerable elongation of ovary wall and hypanthium.

The vascular supply of the episepalous stamens is connected to the vascular system described above, thus showing that the stamen whorl is initiated later than the petal whorl. The same holds true for the epipetalous stamen whorl, which must therefore have been initiated at a still later point in time.

5. THE LAYERING OF THE OVARY WALL

5.1. Introduction

PANKOW (1966) draws attention to the fact that the ovary wall consists of two layers with different cell sizes and cell shapes. He discribes the cells of the outer layer as larger and with a lesser affinity to stains than the cells of the inner layer, which according to him are more meristematic. The boundary between the two cell types lies in the region of the circle of vascular bundles. Although he observed these phenomena only in *O. glauca*, he considers that they may well be characteristic of the Onagraceae as a whole. He ascribes these differences in cell form and size to the axial origin of the outer layer and a carpellar origin of the inner, more meristematic layer.

Other authors have noted the same cell differentiation. BARCIANU (1875) shows in his drawings of the ovaries of *Epilobium dodonaei* and *Circaea lutetiana* two layers with different cell sizes and shapes. BUNNIGER & WEBERLING (1968) also mention the presence of two layers in *O. caespitosa*, *O. fruticosa* and *O. nocturna*.

5.2. Observations

In *O. biennis*, *O. fruticosa* and some other *Onagraceae*, the layering of the ovary wall is conspicuous, but in some species it is more pronounced than in others. The ovary wall of *O. fruticosa* shows the most marked difference between its inner and outer layers. In this species the boundary between the small-celled inner zone and the large-celled outer zone is so distinct that the distribution of the vascular bundles in the two zones can be defined. Nearly all bundles are situated in the outer zone, the bundles C excepted. The placental strands, which arise from the bundles V, penetrate the inner zone from the outer zone.

In *Clarkia elegans* and *Lopezia racemosa* the differences in cell characteristics between the inner and outer zones are small. Moreover, the inner zone seems to be much narrower than it is in *Oenothera*. In both *Clarkia* and *Lopezia*, on the other hand, the two zones can be discerned in the septae. The cells in the interior of the septae resemble those of the outer zone, whereas the cells of the lining at both sides of the septae exhibit the features of inner zone cells.

In young ovaries of *Gaura lindheimeri* the two layers are well defined, but at later developmental stages, when the roof of the ovary and the style are developing, there are no longer any differences between the cell layers; all cells resemble the cells of the outer layer of the young ovary wall rather closely.

Unlike PANKOW (1966), I do not consider that the so-called meristematic, inner layer of the ovary consists of tissue of carpellary origin. If one accepts PANKOW's hypothesis, the tissues I have studied in the Onagraceae must be interpreted as follows: The septae of *Clarkia* and *Lopezia* would be of axial derivation with the septal surfaces covered with tissue of carpellar origin; in *Gaura lindheimeri* the roof of the ovary and the style would be of axial origin; in *Oenothera fruticosa* the bundles V would be surrounded by axial tissue and this would be very remarkable since these bundles, which give off the placental traces, are supposed to be formed by the fusion of ventral veins of adjacent carpels.

It is clear, therefore, that the characteristic small-celled tissue lining the inner side of the ovary walls does not represent a distinct layer of carpellar origin. Conceivably the layering has some functional significance associated with the growth and development of the ovary. Whatever its origin and function may be, it is certain that the layering of the ovary wall cannot be regarded as evidence for the presence of tissues of both axial and carpellar origin in the same ovary wall.

6. GENERAL DISCUSSION

6.1. Floral organs, adnation or superposition of whorls

In *Oenothera biennis* the vascular system supplying the sepal, petal and two stamen whorls in the flower can be compared with the vascular system in a part of the stem with four nodes.

If the hypanthium is interpreted as an invaginated stem, the much abbreviated nodal system on top of it can be interpreted as having been turned over towards the inner side. Along the inner side of the invagination the characteristic nodal vascular system would be expected to continue into a fifth node bearing the carpel whorl. However, no such continuation can be discerned. If the vascular system were continuous here, this would mean that the course of the bundles in the extension would be different to that in the preceding internodes since the carpels are borne opposite the epipetalous stamens, whereas the four preceding whorls of floral organs alternate with one another.

BARCIANU (1975), BUNNIGER & WEBERLING (1968), DUCHARTRE (1942), GEERTS (1908) and PANKOW (1966) interpret all the whorls of flower-parts as alternate on the assumption that the petals and epipetalous stamens pertain to the same whorl. In this whorl each primordium would give rise to both a petal and a stamen. They therefore postulate four alternating whorls: one of sepals, one of petals plus epipetalous stamens, one of episepalous stamens and one of carpels.

MAYR (1969) does not support the view that the petals and epipetalous stamens are initiated simultaneously. According to her, the primordia of the episepalous stamens are formed before those of the epipetalous stamens and develop at a faster rate than the latter. Because the episepalous stamens occupy more space than the epipetalous stamens, there would be more space for the carpels to develop opposite to rather than alternating with the epipetalous stamens. She refers to the work of HOFMEISTER (1868) in support of this theory.

My personal investigations are not conclusive on this point, but in general I do not endorse the view that petals and epipetalous stamens arise from the same primordia. I am inclined to support the view of Mayr that petals and stamens are inserted as separate whorls and that the primordia of petals and epipetalous stamens are merely closely approximate (see 3.1.).

My observations (chapter 4.1.) indicate that the petal and the epipetalous stamen are not supplied by the same single vascular strand, which does not support the viewpoints of BAEHNI & BONNER (1948, 1949), BARCIANU (1975), BONNER (1948), and DUCHARTRE (1942). I consider that each of them has its own vascular supply as was first observed by GEERTS (1908). The presence of an independent vascularisation of these organs supports the view that in Onagraceae the petal and the epipetalous stamen are distinct organs each arising from its own primordium.

6.2. The nature of the ovary: some different views

My study of the vascular pattern in the inferior ovary and the hypanthium supports the results of the ontogenetic studies of these organs by PANKOW (1966), BUNNIGER & WEBERLING (1968) and MAYR (1969), i.e., that in the species investigated the ovary wall and the hypanthium are at least partially of axial derivation, or in the formulation of LEINS et al. (1972), that a hypanthium is present which is partially gynoecial and partially perigynous in nature.

BONNER (1948) regards the ovary in Onagraceae as being only apparently inferior because it is only fused with the basal portions of the outer floral whorls.

To sustain this interpretation he gives a long list of arguments, some of which are based on evidence not confirmed by my investigations.

In addition to the vascular ring in the rim of the flower-tube, BONNER apparently detected a vascular strand running from each floral organ straight down towards the base of the flower. Some of these strands are said occasionally to join parallel strands. If these observations were correct, they would indeed confirm BONNER's view that the strands run within the basal parts of separate organs. In my material, however, the petals were never supplied by separate strands originating in the base of the flower. Consequently, there is no reason to suppose that the basal part of the petal forms a part of the flower-tube and the ovary wall. BONNER (1948) adduces as another argument the presence of the grooves on the outer surface of the *Epilobium* ovary. He considers that the position of these grooves corresponds to the continuation of the sepalar edges and accordingly interprets the ovary wall as being clothed with (parts of) sepals. In support of this interpretation he refers to the investigations and ideas of SAUNDERS (1922). In *Epilobium parviflorum* she demonstrated that petioles may show a continuation below their point of insertion on the stem. BONNER (1948) states: 'If such a feature is used as a proof of the leaf beyond its apparent end it can surely have an equal significance in the case of the floral leaves". With this quotation I fully agree, but it has in fact no relevance to BONNER's hypothesis regarding the nature of the inferior ovary. SAUNDERS states in the same paper: "The surface tissue of the spermophyte shoot axis is of foliar origin". She attempted to substantiate her thesis by the condition in *Epilobium parviflorum*, in which species the boundaries of the leaf-insertion clearly run downward along the stem as far as the next lower node. According to her these boundaries appear as two pubescent ridges. Many comparable structures on the periphery of stems were quoted by SAUNDERS in het attempt to demonstrate the foliar nature of the stem periphery in general. In relation to the foregoing, reference can be made to another part of the same paper by SAUNDERS (1922) where she states: "In the case of flowering stems the leaf-skin is formed by the bracts (when present) and the outermost sepals". By applying these ideas of SAUNDERS to the external structure of the onagraceous gynoecium my conclusion is directly contradictory to BONNER's. It is a common feature for a stem to bear leaf-like structures on its periphery. This applies to both vegetative and reproductive stems. Therefore the occurrence of apparent extensions of the sepals, appearing as ridges or grooves along the external surface of the inferior ovary, cannot be adduced as an indication of the foliar origin of the surface layer as a whole.

BUNNIGER & WEBERLING (1968) refer to the papers of VAN TIEGHEM (1868, 1871) and BAEHNI & BONNER (1949), to show the contradictory interpretations, based upon ontogenetical studies or upon studies of the vascular pattern. They criticized the value of the latter method of investigation. Also in my opinion one has to be very cautious when interpreting vascular patterns. Still, as shown in this paper, a more thorough study and careful interpretation of a vascular pattern may yield results at least in accordance with the results obtained by means of other methods.

ACKNOWLEDGEMENTS

The author is indebted to Dr. B. M. Moeliono for his suggestions and critical reading of the manuscript, to Mrs. A. Dijkstra-Huizenga and Mr. P. Dijkstra for their preparation of the slides and other assistance, and to Mrs. Y. Butler for the correction of the English text.

REFERENCES

Baehni, Ch. & C. E. B. Bonner (1948): La vascularisation des fleurs chez les Lopezieae. *Candollea* **11**: 305–321.

— & — (1949): La vascularisation du tube floral chez les Onagracées. *Candollea* **12**: 345–359.

Barcianu, D. P. (1875): Untersuchungen über die Blüthenentwicklung der Onagraceen. *Mitt. Gesammtgb. Bot.* **2**: 80–129.

Bonner, C. E. B. (1948): The vascular supply in Epilobium and related genera. *Candollea* **11**: 277–303.

Bunniger, L. & F. Weberling (1968): Untersuchungen über die morphologische Natur des Hypanthiums bei Myrtales-Familien I, Onagraceae. *Beitr. Biol. Pfl.* **44**: 447–466.

Candolle, A. P. de (1827): *Organographie végétale* Vol. I. Deterville, Paris.

Douglas, G. E. (1944): The inferior ovary. *Bot. Rev.* **10**: 125–186.

— (1957): The inferior ovary II. *Bot. Rev.* **23**: 1–46.

Duchartre, P. (1842): Observations sur la fleur et plus particulièrement sur l'ovaire de l'Oenothera suaveolens *H. P. Ann. Sci. Nat. II Bot.* **18**: 339–356.

Eames, A. J. (1931): The vascular anatomy of the flower with refutation of the theory of carpel polymorphism. *Amer. J. Bot.* **18**: 147–188.

Geerts, J. M. (1908): Beiträge zur Kenntnis der Cytologie und der partiellen Sterilität von Oenothera lamarckiana. *Rec. Trav. bot. néerl.* **5**: 91–206.

Goebel, K. (1886): Zur Entwicklungsgeschichte des unterständigen Fruchtknotens. *Bot. Ztg.* **44**: 729–738.

Hofmeister, W. (1868): Allgemeine Morphologie der Gewächse. *Handbuch der physiologischen Botanik* 1. Bd. 2. Teil. Leipzig.

Leinfellner, W. (1941): Über den unterständigen Fruchtknoten und einige Bemerkungen über den Bauplan des verwachsenblättrigen Gynoeciums an sich. *Bot. Archiv* **42**: 1–44.

Leins, P., H. Merxmüller & R. Sattler (1972): Zur Terminologie interkalaren Becherbildungen in Blüten. *Ber. deutsch. bot. Ges.* **85**: 294.

Maácz, G. J. & E. Vágás (1963): Untersuchung des Zugholzes mit dreifacher Färbung. *Acta Biol. Hung.* **13**: 341–346.

Mayr, B. (1969): Ontogenetische Studien an Myrtales-Blüten. *Bot. Jahrb.* **89**: 210–271.

Naudin, C. (1855): Observations relatives à la nature des vrilles et la structure de la fleur chez les Cucurbitacées. *Ann. Sci. Nat. IV Bot.* **4**: 5–19.

Pankow, H. (1966): Histogenetische Untersuchungen an den Blüten einiger Oenothera-Arten, *Flora* **156B**: 122–132.

Payer, J. B. (1857): *Éléments de botanique.* Masson, Paris.

Saunders, E. R. (1922): The leaf skin theory of the stem: A consideration of certain anatomico-physiological relations in the spermophyte shoot. *Ann. Bot.* **36**: 135–165.

— (1925): The inferior ovary. *New Phytol.* **24**: 179–185.

Schleiden, M. J. (1849): *Grundzüge der wissenschaftlichen Botanik*, 3. Aufl. Wilhelm Engelmann, Leipzig.

Tieghem, P. van (1868): Recherches sur la structure du pistil. *Ann. Sci. Nat. V Bot.* **9**: 153–226.

— (1871): Recherches sur la structure du pistil et sur l'anatomie comparée de la fleur. *Mém. des savants étranger à l'Institut II* **21**: 1–261.