

Figure 1. A–G. Anther culture in *Sambucus nigra* L. (A) T.s. portion of anther showing uninucleate pollen grains, $\times 466$. (B) 15-day-old culture on MS + 2,4-D (1 ppm) + KN (0.5 ppm), showing the profuse callus, $\times 2.6$ (C) T.s. portion of anther locule showing pollen grains. Note a large vegetative nucleus and a small generative nucleus in one of the pollen grains (arrow), $\times 933$. (D) Squash preparation, showing a pollen (arrow) with two equal nuclei, $\times 1,866$ (E) Same, from 24-day-old culture, showing a multicelled pollen grain, $\times 1,166$ (F, G) Same, showing different stages of development of the pollen embryoids. F $\times 933$, G $\times 1,166$.

whitish in colour and histological preparations revealed that it originated from the connective region of the anther.

Acetocarmine squashes and histological studies of anthers at various stages of growth period, raised on MS + 2,4-D (1 ppm) + KN (0.5 ppm), showed that the pollen grains had followed two different pathways of development. Eight days after culture, in 70% pollen grains, nucleus divided to form a large vegetative and a small generative nucleus (figure 1C). However, in 20% of the pollen grains, the nucleus divided and two equal nuclei were formed (figure 1D). The remaining 10% of the pollen grains enlarged and were filled with starch grains as revealed by IKI staining. A few pollen grains were elongated during the course of division. In 14-day-old cultures, some pollen grains with four nuclei (3 large and 1 small) could also be seen. Twenty four days after culture, multicelled pollen were observed

(figure 1E). These have been referred to as pollen embryoids. Some pollen embryoids ready to exude out of the pollen wall were also observed (figure 1F, G). A few of them later produced incipient plantlets.

It has been demonstrated by several workers that the addition of both an auxin and a cytokinin to the basal medium is necessary for inducing divisions in the pollen grains³. Similar observations have also been made in *Sambucus nigra* (present work). However, these growth adjuvants induce proliferation of connective tissue as well.

In *Sambucus nigra* (present work), the growth of the callus from the connective is fast, which account for the failure of the pollen embryoids to develop further. Further experiments are being conducted to suppress the growth of the sporophytic tissue and to obtain the pollen embryoids and consequently androgenic haploids by manipulation of growth regulators in the medium.

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EXCEPTIONAL FEATURES OF STERILITY IN *HIBISCUS ROSA-SINENSIS* CV. SCARLET

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LEWIS¹ suggested that self-incompatibility is a device to conserve eggs for compatible pollination and the oddity to this rule is *Theobroma cacao* where incompatible pollen germinates, the tube grows at compatible rates and the male gametes fertilize the egg and endosperm nucleus but still the embryo aborts. We report a similar loss of ovules on self-pollination in a cultivar of *Hibiscus rosa-sinensis* although the failure of seed set here appears to be due to inhibitory factors operating both at pre- and post-fertilization stages.

About 1000 cultivars of *Hibiscus rosa-sinensis* are known² but these are generally sterile. However, of the

TABLE I

Comparison of the number of ovules fertilized and seeds set in the cultivars

| Cultivar and type of pollination | Sample* size | Ovules fertilized | | | | Seeds set | |
|----------------------------------|--------------|--------------------------------|------|--------|-------|--------------|-----------------|
| | | Total No. of ovules fertilized | Mean | Median | Range | No. of seeds | % of seed set** |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
| California Gold | | | | | | | |
| Self | 36 | 710 | 20 | 21 | 13-32 | 682 | 94.8 |
| Cross | 20 | 337 | 17 | 18 | 13-34 | 313 | 92.9 |
| Scarlet | | | | | | | |
| Self | 42 | 163 | 4 | 4 | 1-5 | .. | .. |
| Cross | 26 | 236 | 9 | 10 | 2-27 | 209 | 96.8 |

* after pollination, one half of the flowers were randomly selected (column 2) and marked for estimation of the ovules fertilized (column 3) while flowers of the other half were allowed to develop to fruits for the estimation of seeds (column 7).

** based on the estimation of ovules fertilized (see column 3).

50 selected cultivars raised in the University Botanical Garden at Kariyavattom, two are fertile—the self compatible California Gold and the self-incompatible Scarlet. Exceptional features that contribute to the sterility of self-pollinated flowers of Scarlet are listed here.

All crosses were made in the morning following standard techniques. The pollinated flowers were guarded by nylon nets with 1 mm square meshes. Lactophenol aniline blue method³ was used to study the *in vivo* pollen germination and tube development. Embryo development was traced by squash and microtome techniques. Results of statistical analysis are summarized in table 1.

Both California Gold and Scarlet exhibit several common features in pollen development. Stigmas of both cultivars support self- and cross-pollen germination and about 40–60% of the pollen germinate *in vivo*. The rate of tube growth of self- and cross-pollen is 1.5 cm/hr. Yet, selfed flowers of Scarlet fail to develop to fruits.

The major events that lead to the sterility in Scarlet are as follows: Although 50–60 pollen tubes reach the base of the style in both selfed and crossed pistils only less than half of the self tubes pass through the central placenta while all cross tubes grow to the ovules without any inhibition. The mean, median and range of ovules fertilized in selfed pistils are only less than 50% of those in crossed pistils. Further, even the few selfed ovules do not develop to seeds. Day-to-day study of embryo development in crossed and selfed

pistils shows that in both cases the embryo grows normally during the first 6–7 days and reaches the globular stage after which the embryo in selfed ovule aborts and the flower dehisces. In contrast, embryo development is not inhibited in the crossed pistils which accounts for the high level of seed setting in them (table 1).

In the self-compatible California Gold, studied for comparison, the number of ovules fertilized in self- and cross-pollinated flowers showed no significant differences. About 92–94% of the ovules fertilized in both instances also developed to seeds (table 1).

Interestingly, a combination of pre-fertilization (placental inhibition) and post-fertilization (embryo abortion) events contribute to the sterility in selfed Scarlet. Normal tube growth in the stigma and style followed by inhibition at later stages have been reported in the literature. To take representative examples, self-tube reaches the ovule but does not enter the embryo sac in *Lilium*⁴. In *Theobroma*⁵, self-tube enters the embryo sac and fertilization takes place but the 'one celled embryo' aborts. Both these conditions seem to operate in Scarlet although in contrast to *Theobroma*, the embryo develops to the globular stage before degeneration sets in. Barnes *et al*⁶ have observed that no distinction is often made between failure of events prior to fertilization and post-fertilization failure. However, in this report, the inhibition operating at pre- and post-fertilization stages is recognised and histologically differentiated.

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A FIRST REPORT OF THE SYSTEMIC VALVE IN THE TRUNCUS ARTERIOSUS OF THE INDIAN FROG *RANA TIGERINA* (DAUD)

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THIS author¹ had earlier reported the presence of septum principale and the septum mediale in the truncus arteriosus of the frog *Rana tigerina*. Later the same author² reported the presence of a chondrioidal cardiac skeleton in the heart of the same species of frog. Since then several details of the truncus arteriosus have been worked out. This note is aimed to report the presence of a systemic valve in the same species of the frog and its possible role in the distribution of blood through the truncus arteriosus.

Systemic valve was first observed in a dissection of the truncus from the dorsal side, then confirmed through microtome sections of the truncus (figures 1, 2, & 3). The name was coined by this author because the valve was placed in the right systemo-carotid channel of the truncus. It is in the form of an obliquely placed muscular ring quite close to the septum principale and like the latter the systemic valve has its origin-base in the septum trunci (the horizontal septum dividing the pulmocutaneous channel from the systemo-carotid channel). The valve is about 350 μ away on the right side of the septum principale. Thus hanging freely from the septum trunci, the systemic valve stands as a 'collapsible door' between the right half of the systemo-carotid channel and the right systemic arch. For about 500 μ the systemic valve extends anteriorly into the right systemic arch. This is established from the fact that the tip of the valve begins to show up ahead of the base in serial microtome sections. The base of the valve is about 250

μ across and the valve itself is about 400 μ long. Barring the tip of the valve, the remaining distal part of the valve is merged in the ventral wall of the truncus. In the middle of the valve is a longitudinal lumen (figure 3) operated by strongly developed muscles around it. A thin endothelium covers the surface of the valve. Between the muscles and the covering endothelium is a thick coat of fibrous tissue.



Figure 1. Photograph of the dissection of the truncus of *Rana tigerina* showing the systemic valve (marked by an arrow) from dorsal $\times 150$.

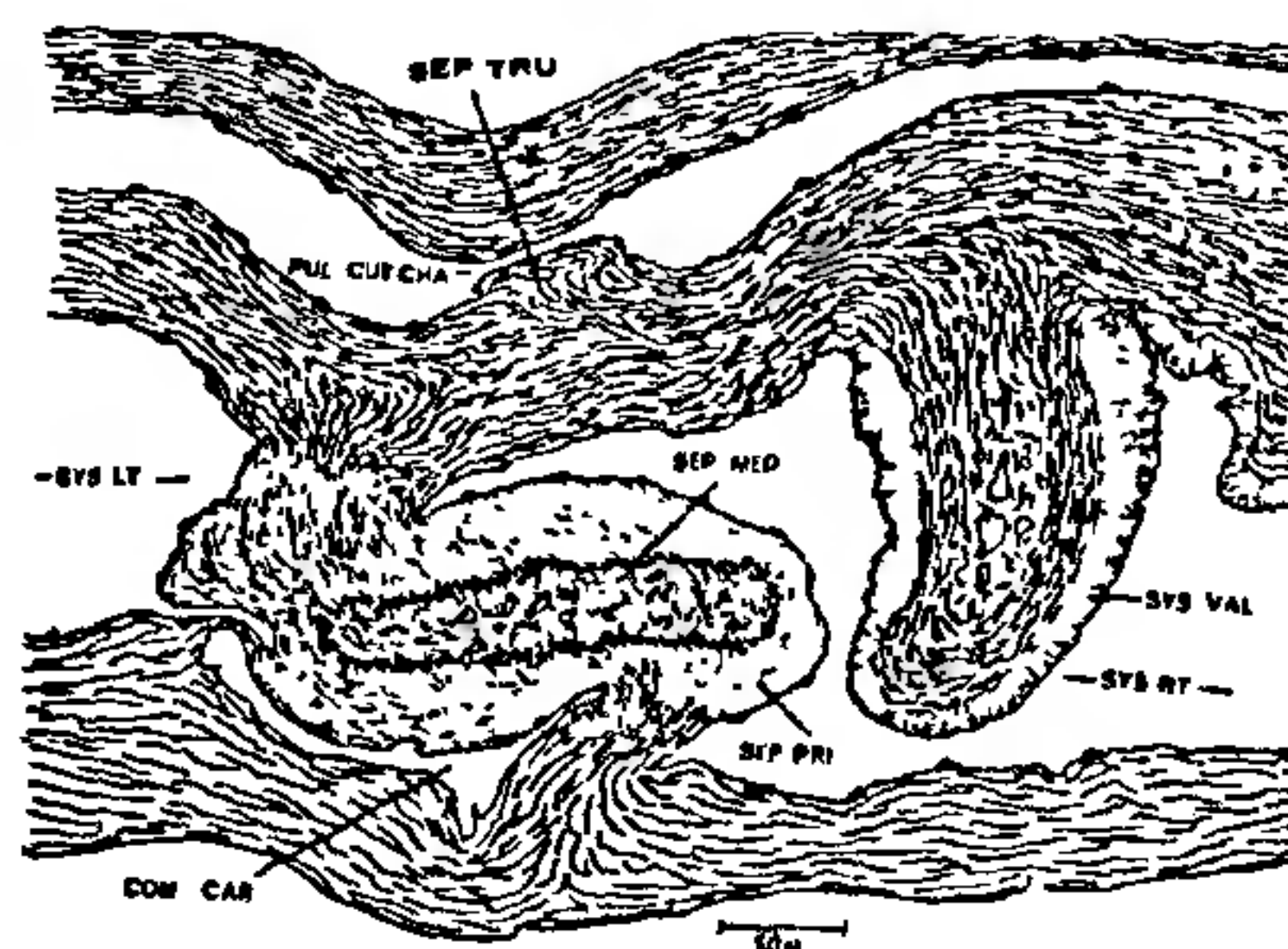


Figure 2. Transverse section of the truncus of *R. tigerina* through the septum principale and the systemic valve. *sys. lt.* left systemic arch; *com. car.* part of the ventral aorta leading into the common carotids; *sep. pri.* septum principale; *sep. med.* septum mediale; *sep. tru.* septum trunci; *sys. rt.* right systemic arch; *sys. val.* systemic valve; *pul.cut.cha.* pulmocutaneous channel.

The spiral valve in the conus of the frog's heart, to a certain extent, helps to keep the less-oxygenated blood from the right atrium and the well-oxygenated blood from the left atrium separated inside the conus. The position of the conus arising from the right dorsal side of the ventricle and its spiral twisting is such that upon ventricular systole, nearly all the less oxygenated blood finds its way into the cavum pulmocutaneum.