

of characteristic cambium-like zones were prominent, as seen in the ovary-derived compact callus (arrows in figures 1 and 2 may be followed). In some cases, the filament along with the connective tissue proliferated into soft callus, in addition to the compact callus tissue of the anthers. After 60 days incubation on MS + 2,4-D medium, the anther lobes in some mature stamens showed tendency for soft callus formation. In such cases, the sporogenous tissue was observed to produce compact callus. Even 60 days after incubation, stamens with only the filament profusely proliferating into compact tissue and the anthers being normal was observed. When the filament proliferated, its vasculature was retained and found embedded in the neoformed compact callus (figure 3). The response of each of the three stamens varied in different florets. In some only one stamen responded producing either spongy or compact callus, whereas in others, two or all the three contributed towards callus production.

*Histological response on MS + NAA (2 to 6 ppm):* —

The response was almost similar to that on MS + 2,4-D. When the explant contained stamen primordia, they grew and fused together resulting in an irregular mass of compact tissue (figure 5). In some cases, the primordia independently developed into compact callus tissue.

When the explant contained partially developed stamens, the filament produced either a soft spongy or hard compact callus. The compact callus resulting from NAA treatment, instead of attaining globular shape as in 2,4-D-supplemented medium, proliferated irregularly. The anther did not always proliferate. When it did, usually compact callus resulted, and very rarely spongy callus was derived either from the anther wall or from the inner cells. Occasionally, one lobe of an anther attained its normal development whereas the other ended blindly or degenerated. On the other hand, when both the lobes developed normally, they were not equally responsive. Although there was soft callus formation from the anther wall and connective tissue, compact callus formation from the androgenous tissue was completely lacking. Hence most of the anther locules were empty. Partially-developed stamens, when responding, developed into roughly lobed compact tissue mass, recalling their destined structure, the stamens (figure 6).

Well-differentiated mature stamens and their parts generally produced soft callus. Compact callus formation was infrequently recorded in the inner core of the filament as on MS + 2,4-D treatment. The cambium-

like zones were completely lacking in callus derived after NAA supplementation.

As in the case of the ovary, the responses on NAA + 2,4-D supplemented medium, (depending upon the concentration of either), varied. If they were supplemented at equal levels the effects of 2,4-D dominated.

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## DOES POLLINATION EFFICIENCY SHAPE THE POLLEN GRAIN TO OVULE RATIO?

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VARIATIONS in pollen grain to ovule (P/O) ratios in plants have been intensively studied recently<sup>1-6</sup>. These variations have been mainly attributed to the efficiency of pollination system. Cruden<sup>2</sup> reporting increased P/O ratios from self to cross pollinated crops argued that the latter are less efficient in pollination and hence produce more pollen grains. Similarly, Uma Shanker and Ganeshaiah<sup>4,5</sup>, attributed the greater P/O ratios in the wild species over their domesticated species to greater risks of pollination encountered by the wild species. In another study, Uma Shanker and Ganeshaiah<sup>6</sup> found that the reduction in P/O ratio with advance in age of *Croton* was correlated to the increasing probability of success of pollen grains in reaching the stigma. These explanations assume that pollen grains are merely a means by which the females get fertilized and suggest that sex ratio will be adjusted such that there will be just enough pollen grains to fertilize the ovules<sup>7</sup>.

Rather, Charnov<sup>7</sup> argues that pollen grains exist as

an independent and equal means (to seeds) towards fitness gains but not simply as an end to the fertilization of females. Hence it is the relative gains through the males and females that decides the resource allocation between them and hence the P, O ratios.

Charnov<sup>7</sup> has attempted to mathematically relate the P, O ratio (as a rough indicator of the resource allocation ratio between the males and females) with the proportion of resource invested into the males ( $r$ ), females ( $1-r$ ), individual seed size ( $C_2$ ), and individual grain volume ( $C_1$ ), by the equation:

$$\log(P, O) = \log \frac{r}{1-r} + \log C_2 - \log C_1. \quad (1)$$

According to this equation, given a constant value for  $r$ , the log P, O ratio would be directly proportional to the log seed size if the pollen grain volume of the species is also constant.

In this paper, we offer an as yet first experimental verification of the above equation in the genus *Phyllanthus* (Euphorbiaceae). We also provide evidence suggestive of the role of sexual selection in shaping the P/O ratio in plants.

Ten species of *Phyllanthus* found in and around Bangalore (12° 58'N, 77° 35'E), India, were used for the study. All the species except one were herbs, monoecious and had different male to female flower ratios. The male and female flower numbers were counted and the sex ratio computed. Twenty anthers from randomly selected flowers were macerated, each on a slide in 1% acetocarmine stain and the pollen grains counted. These were expressed as P/O ratio. The pollen grain volume was measured using an ocular micrometer. Well-matured, randomly-selected fruits were oven-dried at 70° for 24 hr and the weight of seed was recorded to provide a rough estimate of the size of the seed.

Table 1 gives the list of species studied, their respective pollen grain volume, P/O ratio and the male to female flower ratio. The species did not differ significantly in their pollen grain volume. This facilitated a plot between the log P/O ratio and the log seed weight (figure 1). There is a strong positive correlation between the two ( $r = 0.8347$ ). The slope of the regression line was 0.8191 satisfying the relation between P, O ratio and seed weight as predicted by (1). Thus Charnov's<sup>7</sup> thesis that the P, O ratio depends more on the allocation of resources between the males and females than as an adjustment to fertilize the ovules seems valid.

Table 1 Data on sex ratio, pollen grain to ovule ratio and pollen grain volume of ten species of *Phyllanthus*.

Species	Male to female flower ratio	Pollen grain to ovule ratio	Pollen grain volume ( $\mu$ )
<i>Phyllanthus niruri</i>	1	100	27.81
<i>P. asperculatus</i>	1	133	25.90
<i>P. madraspatensis</i>	4	626	19.28
<i>P. virgatus</i>	3	353	27.91
<i>P. urinaria</i>	4.3	788	27.52
<i>P. rheedi</i>	3	316	28.00
<i>P. gardenaria</i>	3.05	313	28.54
<i>P. polyphyllus</i>	13	12000	26.07
<i>Phyllanthus</i> species <sup>a</sup>	1.5	258	19.28
<i>Phyllanthus</i> species <sup>b</sup>	1.5	98	30.42

<sup>a</sup> and <sup>b</sup> = The species are not yet identified but are different from others.

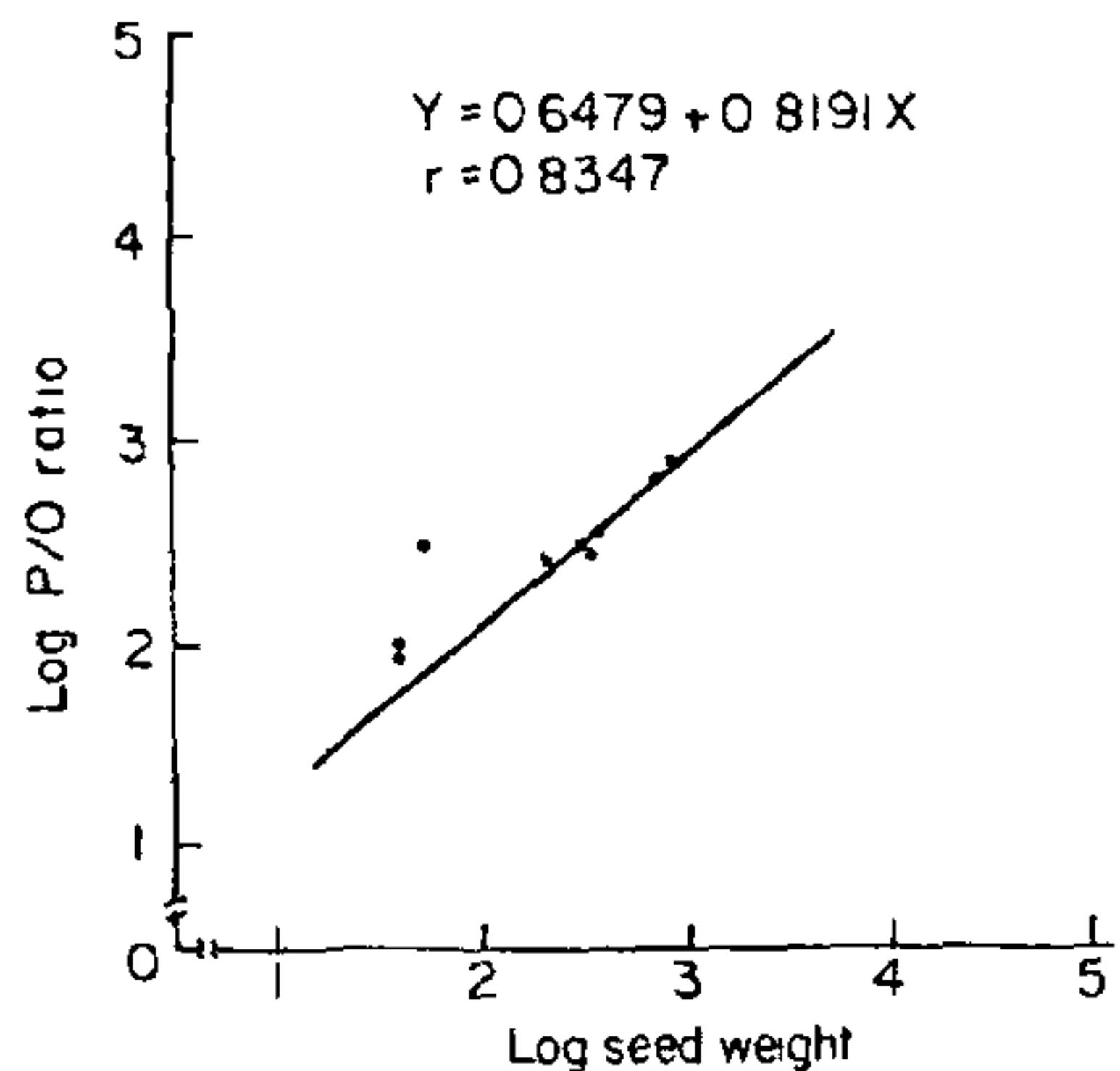


Figure 1. Relationship between log pollen to ovule ratio and log seed weight.

Further, if variations in P/O ratios merely reflect the variations in the success of pollination and fertilization as claimed by Cruden, then one should expect the number of pollen grains deposited on the stigma (as an indicator of the efficiency of pollination system) to be independent of the P/O ratio. But our data revealed a strong positive correlation ( $r = 0.7316$ ) between the number of pollen grains on the stigma and the P/O ratio (table 2). This led us to re-evaluate the claims of Cruden and conclude that Cruden's explanation of pollinator efficiency in shaping P/O ratios



**Table 2** Correlation co-efficient (*r*) between the different variables.

Variables	Values
Log P/O and log seed weight	0.8347**
Number of pollen grains per stigma and P/O ratio	0.7316**
Seed set percentage and log P/O ratio	-0.0070NS
Seed set percentage and number of pollen grains per stigma	-0.2107NS

\*\* = Significant at 1%; NS = Non-significant

may not be justified in the case of *Phyllanthus*. Rather, the variations in the P/O ratios may be explained on the basis of sexual selection as follows.

In the genus *Phyllanthus*, seed set appears to be independent of the P/O ratio ( $r = -0.007$ ) and of the number of pollen grains deposited on the stigma ( $r = -0.2107$ ) (table 2). Thus, pollen grain availability clearly is not a factor limiting seed set in *Phyllanthus*. This substantiates the earlier assumptions made to advance the theory of local mate competition or sexual selection<sup>7-12</sup>. Our results also corroborate, though for the first time across species, the recent report of Queller<sup>13</sup>, who observed that pollination did not limit fruit (seed) set in *Asclepias exaltata*.

The increase in the P/O ratio from herb to shrub habitat might be explained if we consider that shrubs, which are spatially more separated than herbs, are predisposed to greater pollen grain exchange between genetically diverse individuals and hence provide a natural advantage to male-male competition. This is perhaps a reason why we find an increase in the number of pollen grains on the stigma from *P. niruri*, an herb on one extreme, to *P. polyphyllus*, a shrub on the other. This aspect of sexual selection may be brought about by various ways like increased pollen grain number per anther or very simply by increase in the male to female flower ratio. In *Phyllanthus*, it seems to occur mainly through increase in the latter, the pollen grain number per anther remaining almost constant.

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#### SEXUALITY AND OXIDASE TESTS OF *HETEROPORUS BIENNIS* (BULL. EX FR.) LAZ.

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NOBLES<sup>1</sup> divided the family Polyporaceae into two major groups—a primitive group consisting of species which are bipolar heterothallic and do not produce extracellular oxidases in culture, and the other, the more advanced group, including species which are tetrapolar heterothallic and liberate extracellular oxidase enzymes. Review of the literature<sup>2,3</sup> shows that in Polyporaceae, with exceptions, the species which are bipolar, cause brown rots in wood and exhibit negative oxidase reactions in culture; while the species which are tetrapolar, cause white rots and give positive oxidase reactions. The present paper gives the result of investigation on the sexuality and oxidase tests of *Heteroporus biennis* (Bull. ex Fr.) Láz., a fungus which is reported to cause white rots<sup>4</sup>.

The fungus was collected at Dehra Dun growing from the stump of *Dalbergia sissoo* Roxb. The hymenial layers of the basidiomes collapsed very quickly and did not shed basidiospores. But the culture established from context tissue of basidiome, when grown on 2.5% malt-agar media in a 10 cm Petridish and kept in light at (20 ± 2) C were found to fruit readily within 2-3 weeks producing abundant ba-