no contamination of protein or carbohydrate. The yield was sufficiently good, 40–220 μg/ml per 300 mg of dried tissue. The $\lambda 260/\lambda 280$ ratio was greater than 1.8 (ranging from 1.55 to 2.51), showing the presence of protein and polysaccharide contamination. If the sample contained high amounts of polysaccharides or other metabolites, the quantity of DNA obtained was also reduced. For such plants, the time period for –80°C incubation was increased by 15–30 min. Certain antioxidants, e.g. β-mercaptoethanol and PVP helped remove the polyphenolic content and yielded more pure DNA (Figure 1). Increasing the time period of 60°C incubation from 2 to 4 min between the two –80°C incubation did not result in any significant change in DNA yield.

DNA extracted using this method was used for PCR amplification of the ITS region. The ITS regions of ten samples were successfully amplified and ranged between 680 and 850 bp (data not given).

When the amplified ITS product was digested with eight enzymes separately, *Bam*HI and *Hin*dIII had restriction sites only in the ITS of *Desmodium gigantiicum* and *Solanum indicum* respectively. The rest of the enzymes gave restriction fragments in all the plant species, except *Eco*RI which did not have sites for any of the ITS products (data not given).

Microsatellite fingerprinting showed uniform banding with all the three primers in some species and few variations in some, indicating intra-specific variations in species studied (data not given).

An alternate cold (–80°C) and heat (60°C) shock treatment was given to both fresh and dry tissues in order to break down the cell wall, instead of using liquid N_2. The DNA extracted by this method is suitable for PCR amplification of genes, PCR–RFLP, microsatellite fingerprinting and RAPD (data not shown), and gave a consistent result for all the plants used. It is also suitable for both dicotyledonous and monocotyledonous plants. In most of the earlier reports of DNA extraction without liquid nitrogen, fresh and young tissue or callus was used[8,9,12]. We have successfully extracted DNA with sufficient yield from both fresh as well as dry tissues. Since only 10–20 ng DNA is required for PCR amplification, several 100 reactions can be set up with such a yield. Another added advantage of this method is the use of –20°C freezer in case of non-availability of –80°C freezer. This would however require more time for extraction.

In conclusion, this method can be used for extraction of DNA of high-quality and high yield from any type of plant tissue and is suitable for all types of molecular biology experiments. The extracted DNA was stable and gave the same results in PCR, microsatellite fingerprinting, PCR–RFLP and RAPD after 2 years of storage at 4°C.

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Corolla elongation as an aid in self-pollination in *Rhamphicarpa longiflora* (Scrophulariaceae)

Rhamphicarpa Benth. is a small, Old World genus with only six species[1]. Like other members of the tribe Buchnereae of Scrophulariaceae, the genus *Rhamphicarpa* includes species that are root parasites on a wide range of hosts.

Rhamphicarpa longiflora Wight ex Benth. is the only species found in the Indian subcontinent restricted to peninsular India. It is a component species of herbaceous monsoon vegetation found on soils of rocky plateaus ranging in altitude from 30 to 1200 m, or on marshy grounds. According to Cisse *et al*.[2], moth pollination is known only in the genus *Cycnium* and *Rhamphicarpa* in the parasitic Scrophulariaceae, but very little is known about pollination in *R. longiflora*. We studied the pollination ecology of *R. longiflora* to answer the following questions: (1) What is the phenology? (2) Are moths the sole legitimate pollinators? (3) Is the species self-pollinated or cross-pollinated? (4) Does corolla play any role in self-pollination? (5) Why does the species adopt self-pollination?

Studies on pollination biology of the species were made during rainy months from August to October in 2007 and 2008. Flowering and fruiting periods

were observed weekly in plants tagged in wild populations in the Shivaji University Campus, Kolhapur (16°40′527″N, 074°15′791″E, altitude 618 m amsl). Two weeks were spent on the site during the flowering period (usually, 12 h between 1800 and 0600 h) when observations on pollinators, pollination and fruit-seed setting were made. With the onset of monsoon, the seeds of *R. longiflora* germinate and peak flowering of the species is seen in mid July–September. The flowers open in the evening as early as 1600 h during cloudy conditions and remain open throughout the night, whereas the closing time can be as late as 0800 h; however on days with heavy rain, flowers might open early and remain open even on the next day. The sweet-scented flowers have pure white spreading corolla lobes with a long and narrow slender tube. The nectary around the ovary starts secreting nectar at the time of flower opening. The nectar rises up to the middle of the corolla tube by midnight. In spite of continuous observations from dusk through night for several weeks, no moths or any other insects were found visiting the flowers, although they exhibited all the characteristic features of moth-pollinated flowers.

In order to understand the mechanism of pollination, 10 plants were randomly selected for each treatment (mentioned below) from wild populations and from each plant a single flower was bagged. For establishing the breeding system the following treatments were given:

(i) Autogamy: Ten flowers were bagged with muslin cloth before anthesis until senescence to exclude pollinators and hence any cross-pollination.

(ii) Apomixis: Ten flowers with undehisced anthers were emasculated and bagged until senescence.

(iii) Open pollination: Ten flowers were tagged and allowed to develop capsule on their own under natural conditions.

All of the flowers bagged for autogamy showed capsule and seed formation. None of the emasculated flowers showed any capsule or seed formation, excluding any chances of apomixis operating in the species. Flowers left under natural conditions showed capsule and seed setting. The fruit set in autogamous and open-pollination treatments was 100% and 80% respectively. The number of seeds

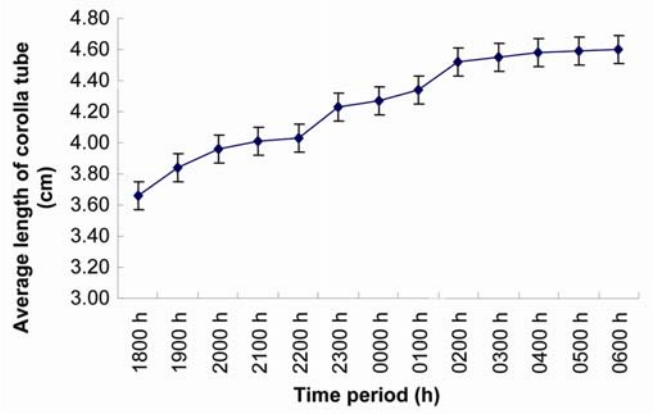

Figure 1. Graph showing changes in the length of corolla tube of *Rhamphicarpa longiflora* from the time of opening till it withers the next day.

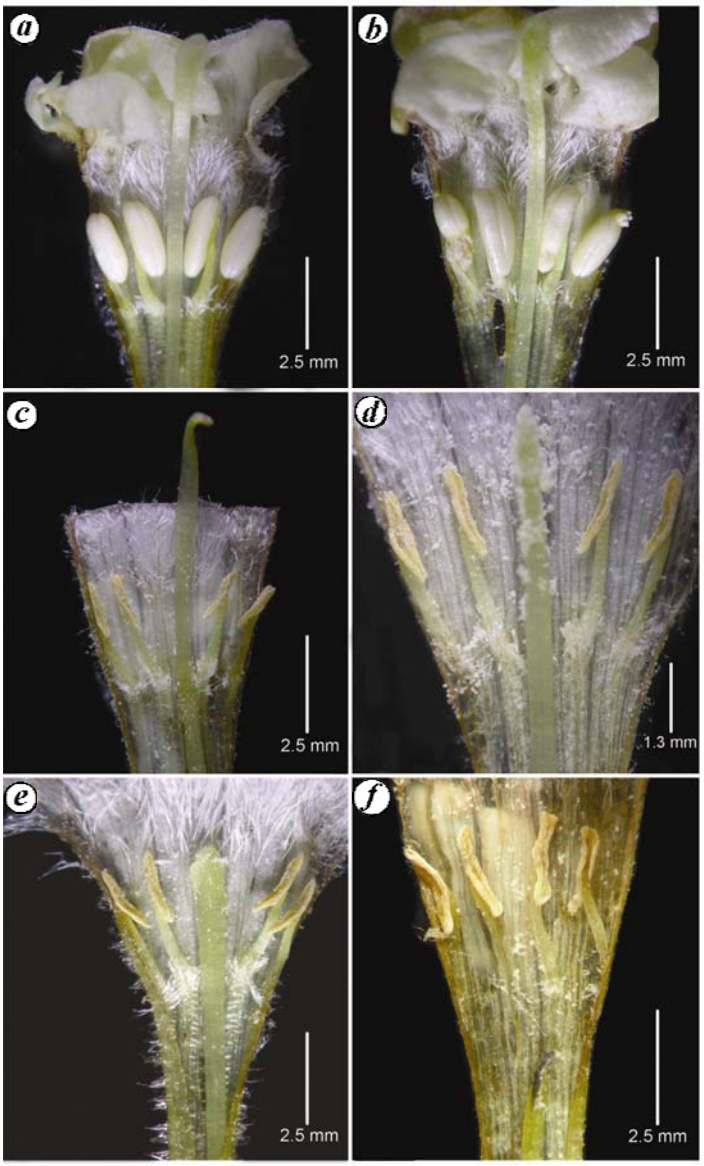

Figure 2. Position of anthers with respect to stigma. ***a***, Flower a day before opening. ***b***, Flower before opening at 0600–0700 h. ***c***, Flower just after opening at 1700–1800 h (extended part of corolla removed). ***d***, Anthers slightly below stigma at 2000–2200 h. ***e***, Anthers at stigma level at 2300–0100 h. ***f***, Stigma overtopped by anthers at 0200–0400 h.

per capsule in open-pollinated individuals was found to be 813.33 ± 2.52.

To understand the mechanism of self-pollination, observations were made on flower and corolla tube length at intervals during the night. Corolla tube length was measured for 10 flowers from 10 individuals using thread and ruler. This was done after every 1 h from the evening time of blooming (1800 h) till 0600 h in the morning. Observations were repeated weekly on different sets of flowers (10 in number). The data are shown graphically in Figure 1 and as photographs in Figure 2.

The stigma of the flower lies about 5–7 mm above the anthers at the time of flower opening during 1700–1800 h. The corolla tube length gradually increases with time, raising the level of the anthers. However, the length of the style does not increase. Between 2300 and 0100 h at midnight, the dehisced anthers come in contact with the stigma resulting in autogamy (Figure 2). The corolla tube length continues to increase till morning when the stigma comes to lie 2–4 mm below the anther level. By morning the flowers start withering and the corolla fades. By 0800 h, the corolla lobes close and flower colour changes from white to dull brown. The flowers of *R. longiflora* with long, narrow corolla tube are showy enough to advertise themselves and provide reward by being nectariferous. The corolla tube bears stipitate glandular hairs on the outer surface and a narrow neck with dense white hairs inside. Nectary is located at the base of the corolla tube. Only Lepidoptera members, particularly moths (since majority of butterflies are diurnal) with their long mouth-organs modified into proboscides can have access to the nectar. This specificity restricts the pollinators to moths only. Species-specific, pollination-dependent plants are at risk of pollination failure owing to pollinator limitation as a result of environmental change or disturbance[3]. Also, autogamy is more reliable when compared with cross-pollination[4]. In the absence of pollinators (for whatever reason), the species has adopted to self-pollination through corolla tube elongation bringing the anthers at the level of the stigma, thus leading to autogamy. Some species of *Myosotis* (Boraginaceae) show similar phenomenon and they are termed as initially herkogamous, i.e. the stigma protrudes but the corolla extension lifts the anthers above the stigma during anthesis[5]. There are two hypotheses for the evolution of self-pollination. One is 'automatic selection', whereas the other is 'reproductive assurance'[6]. Self-pollination in this species seems to have evolved in the absence of pollinators for reproductive assurance. Like *R. longiflora*, there are many examples of evolution of self-pollination in plants[7,8]. Indeed, the adoption of self-pollination is one of the most common trends in the evolutionary history of angiosperms[9].

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Cosmic rays and global warming

In this paper we discuss the paper 'Contribution of the changing galactic cosmic ray to global warming' by Rao[1]. It is well known that the saturation vapour pressure over a curved liquid surface is larger than that over a flat one. This inhibits the formation of condensation nuclei at sizes of nanometres in clouds. In the standard picture such nuclei form on atmospheric impurities such as sulphates, or on microscopic dust or salt particles. Yu[2] has shown that ionization can also act to overcome this inhibition. The crucial question is the extent to which cosmic rays (CR) can provide enough ionization to change significantly the rate of droplet growth compared to that provided by the standard known processes.

In his article, Rao[1] relies heavily on the work of the geologist Veizer[3], who uses the work of Svensmark *et al.*[4]. These works imply that CR provide a significant effect in helping such condensation nuclei to form. In this way changing CR can influence cloud cover and hence the climate. We examine this implication below.

Rao[1] asserts that the correlation between CR and low cloud cover (LCC) is well established, citing Veizer[3]. Veizer has shown some impressive correlations between temperature proxies and CR proxies on geological timescales. These correlations could be explained by a strong connection between the earth's temperature and CR, but several other processes were happening on these timescales, many of which were correlated. The observation of a correlation between two processes does not prove that one causes the other, if each is correlated to