Flower sex expression in cucurbit crops of Kerala: implications for pollination and fruitset

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Cucurbits are monoecious in general; the female flowers occur lower than the male flowers in fields. Often farmers are not aware of this type of sex expression, and are concerned about the low fruit set despite 'profuse' flowering and abundant pollinators in the crop fields. We assessed flower sex expression during the peak (female) flowering and fruiting period in three widely grown cucurbit crops, *Cucurbita maxima*, *Benincasa hispida* and *Momordica charantia* in a village ecosystem of northern Kerala. Sex expression was male-biased in two species. In *M. charantia*, 97.65% of the flowers produced were staminate. In this condition, the farmers should ensure that effective pollinators are available in plenty to achieve maximum fruit set from the low number of pistillate flowers. *C. maxima* produced an average of 75.39% of staminate flowers, which varied across the fields; three fields consistently produced 100% pistillate flowers in all the 14 days of observations. The fruit set in fields with only pistillate flowers is likely to be affected by pollen limitation, rather than pollinator limitation. These fields may require supplementary manual pollination to enhance the fruit set. *B. hispida* produced more or less equal proportion of staminate and pistillate flowers across the fields and days studied. This kind of information may help the farmers to manage pollination services in their fields as well as to predict a realistic yield.

Keywords: *Benincasa hispida*, *Cucurbita maxima*, flower sex expression, *Momordica charantia*, pollination.

GLOBALLY, the plant family Cucurbitaceae comprises of 118 genera and 825 species. The Asia-Pacific region is the centre of origin of many edible cucurbit species. A number of them are cultivated in both the developing and developed parts of the world. FAO estimates show that about 6% of the total vegetables produced in India come from eight species of cucurbit plants[1]. Since they produce unisexual flowers, cross-pollination by insects is essential for fruit and seed set. Studies have been made to record the insect visitors and to understand the pollinators of some of the important cucurbit crops in the Indian subcontinent[2–7]. More critical studies conducted in other parts of the world have examined pollination efficiency of important pollinators, and analysed the factors influencing pollinator diversity and fruit set in cucurbit crops[8–11]. Since cucurbit plants are monoecious, the proportion of both staminate and pistillate flowers in the population is likely to directly influence pollination efficiency under field conditions. Studies investigating pollination ecology in cucurbit crops have not looked into the details of sex expression (proportion of staminate and pistillate flowers), and have not related this feature to explain fruit set (but see Hoehn *et al*.[9]).

This study was conducted to characterize the flower sex expression in three most widely cultivated cucurbit vegetable crop plants of South India, *Cucurbita maxima* (pumpkin), *Benincasa hispida* (ash gourd) and *Momordica charantia* (bitter gourd). The study asked the following questions: (1) what is the general pattern of flower sex expression in the cucurbit crop species selected in this study in northern Kerala? (2) Does the flower sex expression seen in a particular crop vary across fields and days?

The study was conducted in a typical tropical coastal village ecosystem of Kasaragod district, Kerala, South India (12°08.141′N and 12°18.681′N; 75°09.392′E and 75°18.956′E, between 2.45 and 8.6 m amsl). Kasaragod district has 7986 ha of cultivable paddy land that is used to cultivate rice during the *khariff* season (June–October). During the *rabi* season (November–April), the land is used to cultivate vegetables on a small scale by farmers, mostly for their own consumption and to sell the excess in local markets. According to district statistics, about 40% (3,126 ha) of the paddy land is traditionally used to cultivate vegetables, such as gourd, pumpkin, cucumber, melon, tomato, chilly, amaranthus, brinjal, ladies-finger, beans and other tuber crops, such as tapioca and sweet potato. Kasaragod district is the first organic district declared by the Government of Kerala, owing to the tragic incidence of endosulfan-related health issues in the district. Presently, vegetable farmers are using only organic manure and to a certain extent biofertilizers, such as trichoderma-enriched fertilizers for crop production.

Among the vegetable crops, gourds (bitter gourd, ridge gourd and ash gourd), pumpkin and cucumber are grown on sizeable acreage of land. All cucurbit plants are grown in a mixed-crop system, where the vines of ash gourd, ridge gourd and pumpkin are grown together, but trailing on the ground. The bitter-gourd vines are grown on the same land on platforms at a height of about 1.5 m from the ground. Plants in all the fields (8–10 cents) were planted from a single stock of seeds of native varieties of the respective crops by the respective farmers under our supervision. All planting was done in November 2014, and received manual or pump irrigation once or twice daily. Cow dung compost was the major source of manure. Observations on the flowering pattern were carried out at peak flowering period of each crop during January–February 2015. We selected 18 (number of plants = 18–24/field; total observations = 8–15 days/field), 10 (number of plants = 4–16/field; total observations = 5–10 days/

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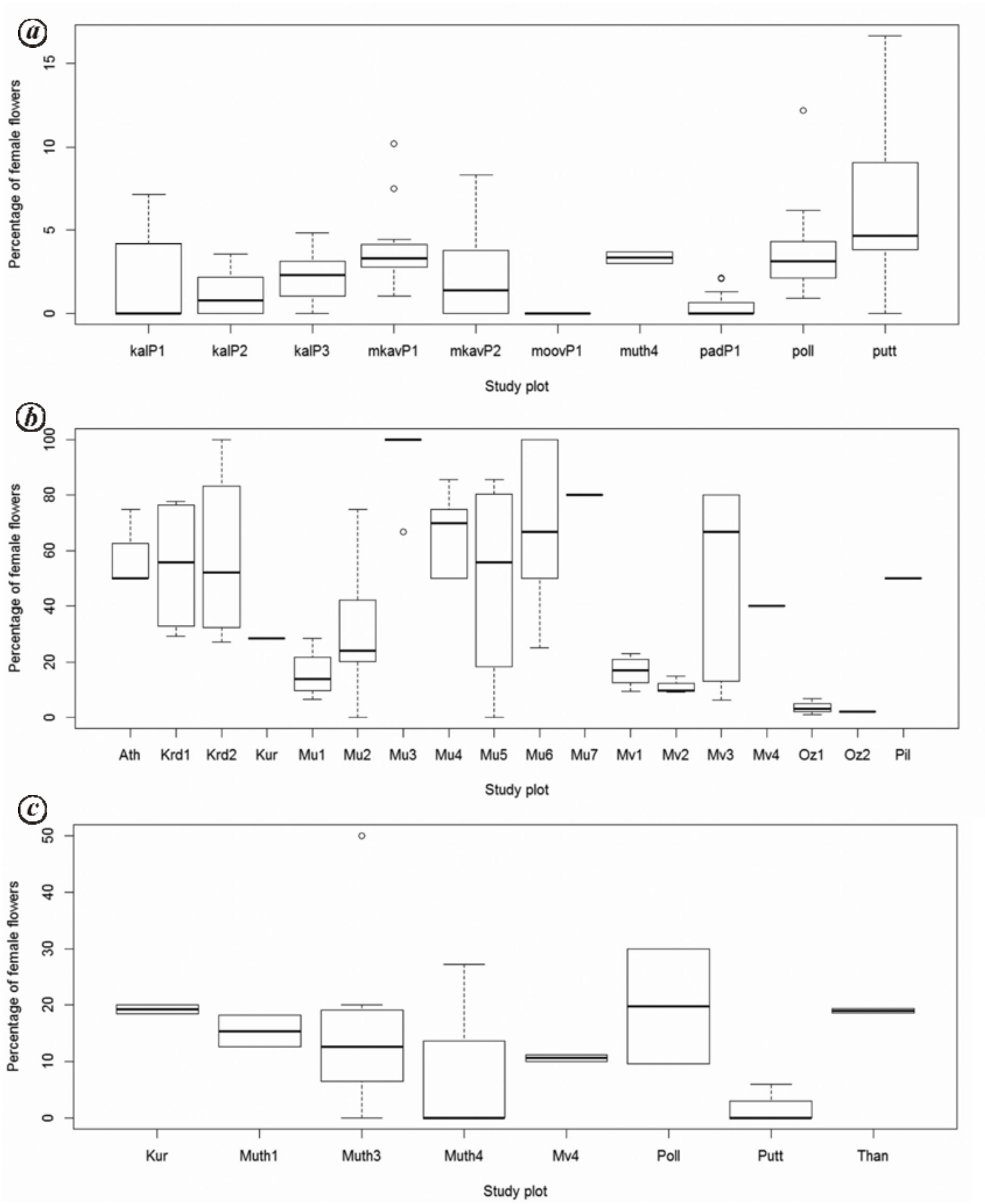

Figure 1. Box and whisker plots showing the percentages of pistillate flowers in different fields during the study (***a***) bitter gourd, (***b***) pumpkin, and (***c***) ash gourd. Legend for locations. Ath, Athiyadam; kalP1–P3, Kalichamaram population 1–3; Krd1-3, Kurunthur Road; Kur: Kurunthur; mkav, muth and Mu, Muthappanarkavu; moov and mv, Moovarikundu; oz, ozhinjavalappu; pad, Padannakad; Pil, Pilathara; poll, Pollakada; putt, Puttlott; Than, Thannott.

field) and 8 (number of plants = 18–24/field; total observations = 5–7 days/field) fields to record the flowers of pumpkin, bitter gourd and ash gourd respectively. Since plants trail the ground and the area cultivated was slightly different, it was difficult to assess the number of flowers per plant. Therefore, we have used the percentage of staminate and pistillate flowers per field as a measure for the comparisons. The proportion data, wherever required, were arcsine-transformed before comparing the averages. One-way ANOVA was used to determine the significance of the difference in the percentage of open pistillate flowers between fields. Student *t*-test was applied to test whether the mean percentage of staminate flowers opened per day was significantly different from that of pistillate flowers in each crop species. All the analyses were performed in R (R Core team 2007)[12].

A total of 6750 flowers of bitter gourd were counted from 10 fields; 6591 flowers were staminate and 159

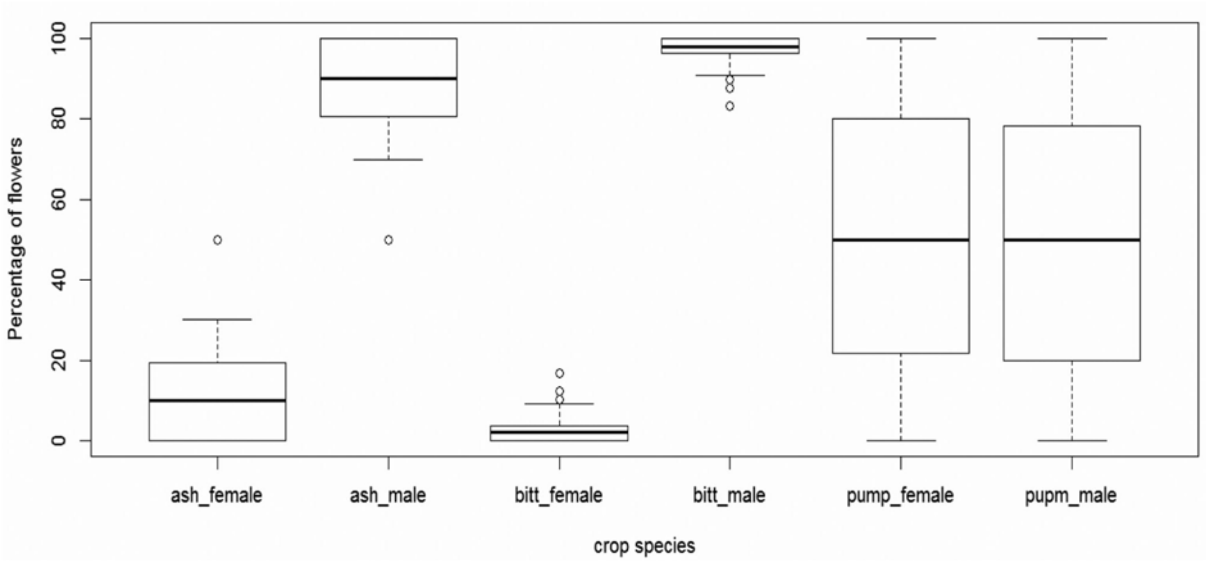

Figure 2. Box and whisker plots showing the difference in percentage of staminate and pistillate flowers open/day in different cucurbit species. ash, Ash-gourd; bitt, Bitter gourd; pump, Pumpkin.

flowers were pistillate, which is 97.65% and 2.35% of the total flowers respectively. The difference in the mean percentage of pistillate flowers observed and accounted among fields (2.41% ± 0.28; range = 0–16.7%) was significant (one-way ANOVA: $F_{9,90} = 7.13$, $P = 0$; Figure 1 *a*). In pumpkin, a total of 1284 flowers were counted from 18 fields; 968 (75.39%) flowers were staminate and the remaining 316 flowers were pistillate (24.61%). The difference in the mean percentage of pistillate flowers among the fields (51.31% ± 3.58; range = 0–100%) was significant (One-way ANOVA: $F_{17,66} = 5.03$, $P = 0$; Figure 1 *b*). A total of 468 ash-gourd flowers were estimated from eight fields; 412 (88.03%) and 56 (11.96%) flowers were staminate and pistillate respectively. The difference in the mean percentage of the pistillate flowers of ash gourd between fields (13.08% ± 2.44; range = 0–50%) was not significant (one-way ANOVA: $F_{7,16} = 2.15$, $P = 0.10$; Figure 1 *c*).

When all the fields were taken together, differences in the mean percentage of staminate and pistillate flowers observed and accounted per day in bitter gourd (98.71% versus 1.29%; Student *t*-test: $t_{99.17} = 40.03$, $P = 0$) and ash gourd (87.31% versus 12.69%: Student *t*-test: $t_{40} = -19.02$, $P = 0$) were significant, but it was not significant for pumpkin (47.69% versus 52.31%: Student *t*-test: $t_{166} = 0.90$, $P = 0.36$) (Figure 2).

Overall, the study found that the natural cultivated populations of the three cucurbit species were dominated by staminate flowers in two species, which is in agreement with similar studies in other related cucurbit species[13,14]. Among the three cucurbit species, bitter gourd produced maximum staminate flowers; an average of about 98% of the flowers produced per field was staminate. The result was consistent in all the 10 fields. This indicates that roughly 2% of the flowers produced in bitter gourd are only pistillate and available for plausible fruit set. In 38 days of observations, only staminate flowers were recorded. Under such conditions, farmers should ensure that the fields have sufficient effective pollinators to facilitate cross-pollination and to maximize fruit set out from the low percentage of pistillate flowers. Some supplemental manual pollination may be taken up by farmers if visits of effective pollinators are inadequate to the female flowers as wind-pollination efficiency is low[6]. However, a single visit of a given bee visitor itself is sufficient for fruit set in bitter gourd[6]. Pumpkin showed great variation in flower sex expression among the fields. Against an overall average of 75.39% of staminate flowers, three fields were consistently producing 100% female flowers in 14 days of observations. Although the same cultivars were used in all the fields and the age of the plants was also similar, this inconsistency is likely due to the physiological traits of the plants rather than spatial factors such as the average distance between the fields (which was only 1.4 km). This inconsistency in flower sex expression among the fields (Figure 1 *b*) explains why the average percentage of staminate flowers is not significantly different from that of pistillate flowers in pumpkin (Figure 2). Pistillate flowers skewed fields are likely to be under greater pollen limitation rather than pollinator limitation. In fact, this situation is more critical than the staminate flower-dominated fields, and necessitates supplementary manual pollination for fruit set. Ash gourd, on the other hand, maintains a balance in the production of staminate and pistillate flowers in the fields.

Pollination efficiency in general and particularly in the monoecious plants is directly affected by the pollinator diversity (both species and abundance), functional diversity of pollinators, variation in the reward quality and quantity, diversity of floral type (staminate or pistillate), preference of key pollinators for foraging, and foraging time and visitation frequency of the pollinators with respect to pollen and stigma viability and receptivity[8,9,15–19]. Floral traits also affect the pollination efficiency of flower visitors[20–23]. With the recent concern about the pollination crisis in crop plants[24,25], the highly skewed flower sex expression may further affect crop production in monoecious plant species. We have assessed the flower sex expression during the peak flowering (pistillate flowering and fruiting) period of the respective crop species, which is likely to vary with different phases of flowering, seasons and plant maturity[26,27]. Studies that have investigated variables affecting yield in cucurbit plants have rarely incorporated flower sex expression in their models[4,6,9,11]. The present study indicates that flower expression may be an important predictive variable of a realistic yield in cucurbits. This information also aids the farmers to schedule fertilization programmes and predict an 'acceptable' and realistic yield.

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