
Presence of two types of flowers with respect to nectar sugar in two gregariously flowering species

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Many species of animal-pollinated flowers are known to vary widely in the nectar content of flowers. Some proportion of flowers in many species is apparently nectarless, and such flowers are believed to be 'cheaters'. Cheating may explain a part of the variability in nectar content. If cheating exists as a qualitatively different strategy then we expect bimodality in the distribution of nectar content of flowers. It has been shown in a multispecies study that gregarious species have a higher proportion of cheater flowers. We studied the frequency distribution of total nectar sugar in two gregariously flowering species *Lantana camara* and *Utricularia purpurascens*, which differed in other floral and ecological characters. At the population level, both the species showed significant bimodality in the total sugar content of flowers. The obvious sources of heterogeneity in the data did not explain bimodality. In *Lantana camara*, bimodality was observed within flowers of some of the individual plants sampled. In *Utricularia purpurascens* the proportion of nectarless flowers was more in high-density patches, suggesting that the gregariousness hypothesis may work within a species as well. The results support the hypothesis of cheating as a distinct strategy since two distinct types of flowers were observed in both the species. The effect of density in *Utricularia purpurascens* also supports the gregariousness hypothesis.

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1. Introduction

Production of nectar involves substantial cost (Southwick 1987; Pyke 1991) and animal-pollinated plants incur this cost to enhance pollinator visits. However, in most species of flowers, pollinators may not be able to differentiate between nectar-containing and nectarless flowers before entering it and therefore some flowers are likely to 'cheat' pollinators by not making nectar (Bell 1986; Thakar *et al* 2003). There may be other advantages of making nectarless flowers, and the alternative benefits are not mutually exclusive. Empty flowers may compel pollinators to visit more flowers (Feinsinger 1978), enhance cross-pollination (Johnson 2000; Biernaskie and Cartar 2004) or induce deeper probing (Smithson and Gigord 2001). Thakar *et al* (2003) examined multispecies data to test differential testable predictions of

the alternative hypotheses and concluded that the cheater hypothesis predictions were the most widely supported. One of the important predictions of the cheater hypothesis is that gregarious species should show a greater proportion of nectarless flowers. This is because gregariousness makes individual identification difficult, making it difficult for pollinators to discriminate and avoid non-rewarding individuals. The prediction received strong support in multispecies analysis (Thakar *et al* 2003).

Thakar *et al* (2003) used qualitative sampling for nectar and their model is based on the assumption of two qualitatively different nectar strategies adopted by flowers. If two distinct strategies exist, we should expect a bimodal distribution when quantitative measurements are done. Although the variation in nectar production has been studied in many species (Biernaskie and Cartar 2004),

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the nature of frequency distribution has not been carefully examined. A significant bimodality is easier to detect for species in which there is a moderate-to-high proportion of nectarless flowers giving the first mode at zero. We therefore studied the frequency distribution of total nectar sugar in two gregariously flowering species *Lantana camara* and *Utricularia purpurascens*. Although both the species differ in other floral and ecological characteristics, they are gregarious and thus we expect a substantial proportion of nectarless flowers, following Thakar *et al*'s (2003) hypothesis. In *Utricularia purpurascens*, since an individual plant bears only one or a few flowers, frequency distribution within an individual cannot be studied. *Lantana camara*, on the other hand, has many multiflowered inflorescences and therefore in this species trends at the population as well as individual level can be studied. The study site had local density variations within the *Utricularia purpurascens* population. This made it possible to test whether the relationship between gregariousness and cheating was true within a species. Since both the species have small flowers and the nectar volume is often less than 1 μ l, we estimated the total sugar equivalent of sucrose w/w per flower (Corbet 2003), without measuring nectar volume.

2. Materials and methods

2.1 Species sampled

2.1.1. *Lantana camara*: An exotic shrub that spreads as a weed in India, it has now established itself throughout peninsular India. The shrub has multiple inflorescences with 20–25 flowers per inflorescence placed in whorls. Flowers in two whorls are open at a given time. The plants generally grow close to each other, often forming impenetrable thickets. The flowers have a short life span of approximately 2–3 days. The plant flowers throughout the year, but the amount of nectar obtained is high during the monsoon. Thakar *et al* (2003) found a high proportion of nectarless flowers in this species. We sampled flowers in an urban locality of Pune, India. Inflorescences were bagged at the bud stage and sampled after blooming.

2.1.2. *Utricularia purpurascens*: It is a seasonal small herb, growing during the monsoon from July to October. It grows gregariously on wet rocky patches. Each plant has 1–2 flowers at a given time, which have a long life span. The flowers have nectar till pollination occurs. Fruits develop rapidly after pollination. Since it is a gregariously flowering species we expect a high proportion of nectarless flowers. Flowers were sampled from a lateritic plateau at Kaas, south of Pune. Bagging of individual inflorescences from the bud stage as in *Lantana camara* was not possible in this species

owing to the small size of the plant, high density and long floral life. Instead, patches containing 20–60 plants were covered together overnight to prevent nectarivory before sampling. Flowers obviously pollinated before sampling were avoided.

2.2 Sampling nectar sugar.

In both the species, the nectar volume was very small (often $<0.5 \mu$ l) and accurate measurements of nectar volume could not be done. Therefore, we expressed the total nectar sugar equivalents of sucrose per flower (Corbet 2003). Nectar was extracted from individual flowers by flushing the nectary with 3–10 μ l distilled water using a micropipette with a least count of 0.5 μ l. The entire content was taken on a hand refractometer (Erma A-contrast 11-520-0, range 0–32%, least count of 0.2%) to measure the per cent sugar equivalent of sucrose w/w.

The percent sugar we obtained was for a diluted sample. To obtain the total sugar content, the product of per cent sugar and (volume flushed + volume of nectar) should be taken. The volume of nectar being negligible compared to the volume flushed, the total sugar was calculated as a product of the refractometric per cent sugar and the volume flushed.

Since while flushing and squeezing the nectar some tissue fluid from the nectar tube was likely to have contaminated the contents (Corbet 2003), squeezed tissue fluid from previously washed nectar tubes diluted similarly was placed on the refractometer. This was repeated on 20 flowers for both the species. The mean tissue fluid reading was subtracted from the nectar reading. On some occasions the subtraction was marginally negative and it was treated as zero.

2.3 Sampling design.

In *Lantana camara*, a total of 155 flowers from 24 randomly selected inflorescences of 10 individual plants were sampled and pooled to plot the frequency distribution. Three of the individuals were sampled extensively and nectar from 332 flowers of 61 inflorescences was measured.

For *Utricularia purpurascens*, nine gregarious patches 2000 cm² each were marked and the standing density of flowers was recorded. Three of the patches were designated low density (100–150/m²), three as medium density (200–300/m²) and three as high density (>400 /m²). One patch from each density was sampled in the evening between 16.30 and 18.00 h. One patch each was covered overnight to prevent pollinator visits and one each was kept open. Both the covered and open sets were sampled the next morning between 7.00 and 8.30 h.

3. Results

Both the species showed a high percentage of nectarless flowers, which is compatible with the gregariousness hypothesis (Thakar *et al* 2003). In the pooled data, *Lantana camara* had 44.14% nectarless flowers whereas *Utricularia purpurascens* had 46%. Both the species showed two distinct modes of distribution of total nectar sugar with pooled data on all flowers as assessed visually (figure 1a, b). In order to test the statistical significance of bimodality, we attempted to fit unimodal distributions to the data. Since the distributions were highly positively skewed with a mode at zero, it was necessary to test the possibility that the mode at zero resulted from our inability to detect nectar below a threshold. Therefore, we attempted to fit truncated normal and log-normal distributions but they did not offer good fits. A geometric distribution, in which the frequency in each class was a constant fraction of the previous class, offered a better fit than normal or log-normal. Taking the best-fit unimodal distribution as the expected distribution, Chi-square test for goodness of fit was applied. Distributions for both the species differed significantly from the best-fit unimodal distribution (figure 1). Further cutting down the second mode to the expected value and repeating the fitting exercise with changed parameters gave a good fit in both the cases, showing that deviation from the unimodal distribution was caused by the second peak alone (figure 1).

Since bimodality can arise due to any kind of heterogeneity in the population, we tested for the obvious sources of heterogeneity. In the case of *Lantana camara* flowers, two whorls are open at a time and the ones in the outer whorl are older than those in the inner. This can be a potential source of bimodality. The two whorls did not differ significantly in their nectar content (Mann–Whitney $U = -0.0735$, NS, mean rank for inner whorl = 166.9107, outer = 166.9842). Bimodality was observed even after separating the data for the inner and outer whorls (figure 2). This suggests that the difference in whorl was not the source of bimodality. Whorls are also associated with colour. Flowers of *L. camara* are known to change colour after pollination (MohanRam and Mathur 1984). Since the flowers sampled were bagged, they were unlikely to have been pollinated. However, many flowers of the outer whorl had changed in colour. Separating the data by colour did not change the nature of the distribution.

In the *Utricularia purpurascens* data, heterogeneity could potentially result from the two different timings of sampling or from variable densities of plants. The method also involved covering the flowers overnight before sampling. If the covered and uncovered flowers differed significantly in nectar content, bimodality could result in the pooled data. It was found that the proportion of nectarless flowers was not significantly different in the morning and evening samples (Chi-square: 1.501; $P >$

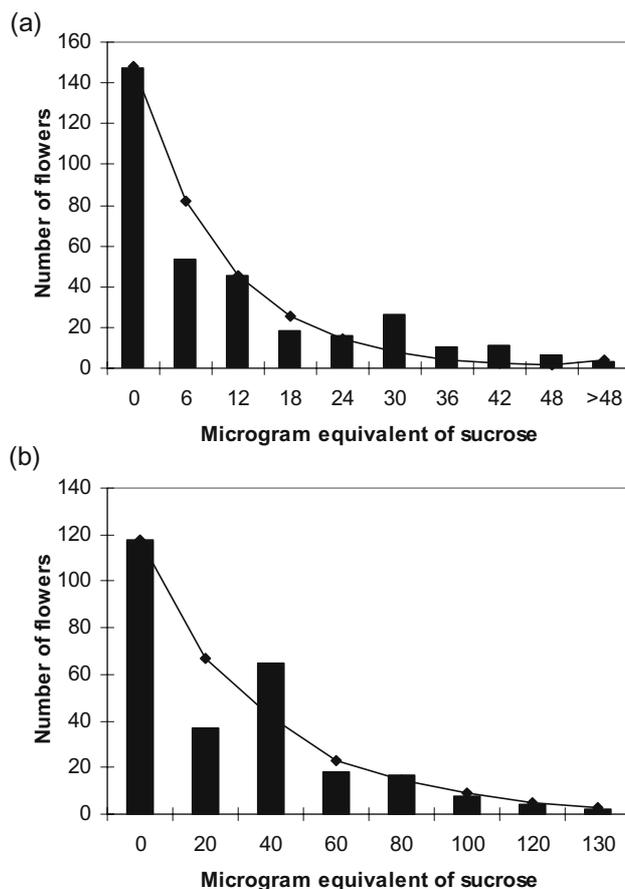


Figure 1. Frequency distribution of total nectar sugar in pooled data. The line represents the best-fit unimodal distribution. We take the bimodality to be significant if the observed distribution deviates significantly from it. **(a)** Significant bimodality in pooled data for *Lantana camara* (Chi-square = 101.4473; df = 6; $P < 0.05$). **(b)** Significant bimodality in pooled data for *Utricularia purpurascens* (Chi-square = 32.29; df = 10; $P < 0.05$).

0.05). Among the flowers sampled in the morning, the covered and uncovered flowers did not differ significantly in the proportion of nectarless flowers (Chi-square: 1.3903; $P > 0.05$). The proportion of nectarless flowers was significantly different in the low- (100–150 plants/m²), medium- (200–300 plants/m²) and high-density (> 400 plants/m²) patches (Chi-square: 5.419; $P < 0.05$). Using quantitative data, and considering population density and covered–uncovered sampling together, we performed the non-parametric Gore test (Deshpande *et al* 1995), which showed that the low-, medium- and high-density patches differed significantly in their nectar content, with the low-density patches having the maximum nectar content ($S = 13.98$; $P < 0.05$).

When the data were separated according to density, the low- and medium-density flowers showed significant bimodality, whereas the high-density patch had only a minor second peak which did not cause significant distortion of the

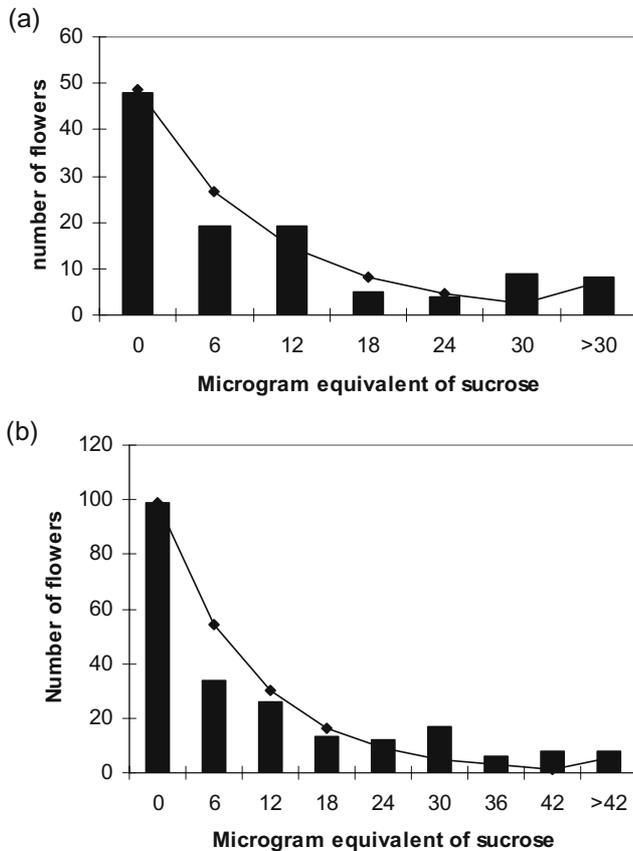


Figure 2. Frequency distribution of total nectar sugar in *Lantana camara*. (a) Inner whorl (Chi-square = 22.43013; df = 6; $P < 0.05$). (b) Outer whorl (Chi-square = 68.75771; df = 8; $P < 0.05$).

best-fit unimodal distribution (figure 3). Thus, although the density affected nectar content, it did not explain bimodality. Since none of the obvious sources of heterogeneity were able to explain bimodality in both the species, it was likely to be due to two distinct strategies of nectar production. It can also be seen that as the frequency of empty flowers increased with density, the frequency of the second mode decreased without substantially affecting the frequencies of the other classes. If density affected the nectar contents of all flowers, we would expect the second peak to move leftwards. But instead, the frequency of this class reduced and that in the zero class increased without affecting the frequency of the intermediate class. This shows that the proportion of the two types of flowers changed without changing the average nectar content within the two types. This pattern strongly supports the hypothesis that there are two distinct types of flowers.

In *Lantana camara*, three plants were extensively sampled for studying the within-plant frequency distribution. All the three plants showed a major and a minor peak but bimodality was not significant in two of them. Two of the plants had the

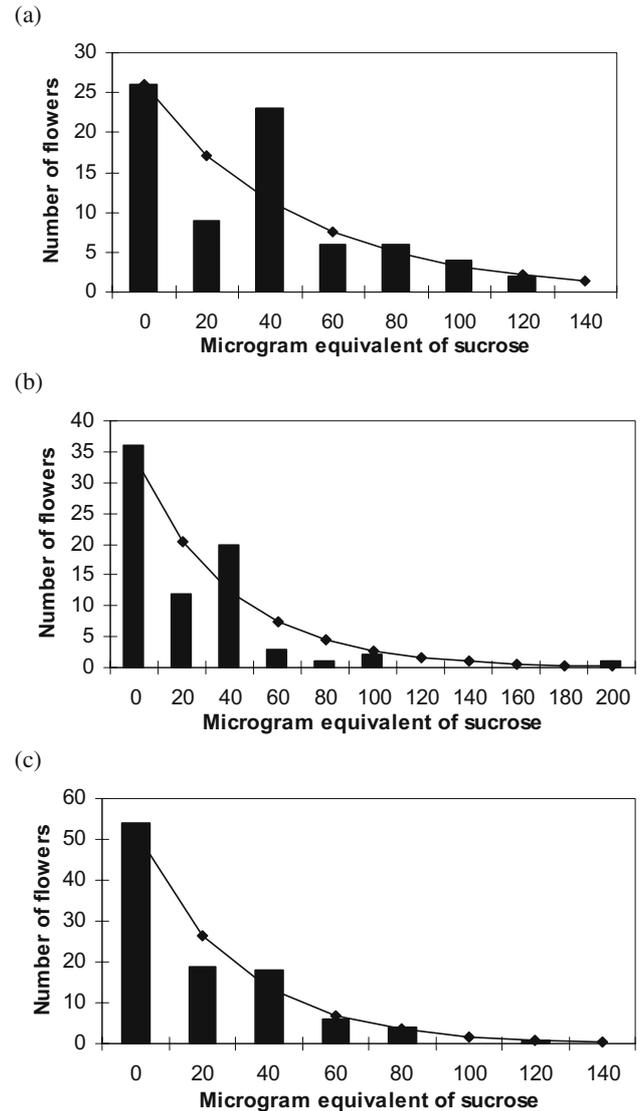


Figure 3. Frequency distribution of total nectar sugar in *Utricularia purpurascens*. (a) In low-density patch (Chi-square = 18.03; df = 7; $P < 0.05$). (b) In medium-density patch (Chi-square = 20.38; df = 10; $P < 0.05$). (c) In high-density patch (Chi-square = 6.14; df = 7; NS).

major mode at zero (figures 4a, b), and one of them showed significant bimodality (figure 4b). The third plant had a non-zero mode and bimodality was not significant (figure 4c).

4. Discussion

The frequency distribution of nectar sugar supports the hypothesis that there are two types of flowers in each of the species sampled. The two strategies may exist within a plant as well. The models by Bell (1986) as well as by Thakar *et al* (2003) indicate that plants will employ a mixed strategy and

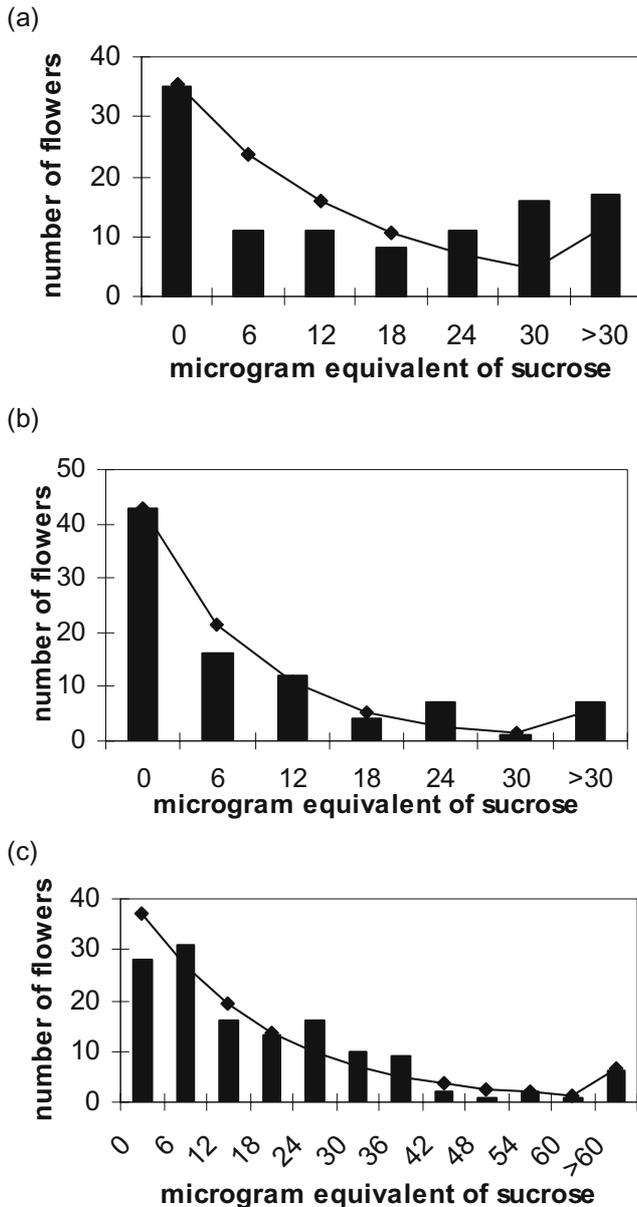


Figure 4. Frequency distribution of total nectar sugar within individual *Lantana* plants. (a) Plant 1: Chi-square = 39.89429; df = 6; $P < 0.05$. (b) Plant 2: Chi-square = 9.309968; df = 6; NS. (c) Plant 3: Chi-square = 11.63984; df = 11; NS.

make nectar in some flowers but not in others. If this is true, then even within a plant one should observe two distinct types of flowers with respect to nectar sugar. This could not be tested in *Utricularia purpurascens* since only a few flowers are borne by a plant. In *Lantana camara*, out of the three plants sampled for studying the within-plant frequency distribution, one showed significant bimodality.

The results of quantitative sampling are in general agreement with the qualitative sampling of Thakar *et al*

(2003) for *Lantana camara*. *Utricularia purpurascens* was expected to show a high proportion of nectarless flowers according to the cheater hypothesis and our data are in agreement with this. Thakar *et al* (2003) showed an interspecific association between gregariousness and cheating. The *Utricularia purpurascens* data show that the association is true even within a species. The significant difference caused by density suggests that individuals in high-density patches are more likely to be cheaters than those in low-density patches. This is compatible with and supportive of the gregariousness hypothesis. Further, the expected bimodality was observed in both the species at the population level and in some plants at the individual level.

Factors affecting nectar production can be many and complex. We restricted our sampling to specific seasonal and climatic conditions to reduce some of the possible sources of variation. Other obvious sources of heterogeneity do not seem to explain the observed bimodality and therefore bimodality is likely to reflect two distinct strategies of nectar production. Other factors can nevertheless affect the distribution and mask the bimodality if sampled among varied conditions. Therefore, although a large variation in nectar production is reported (Biernaskie and Cartar 2004, Biernaskie *et al* 2002), bimodality has not been reported earlier. It would be interesting to know whether the distribution in other species is similar. This will throw light on whether the current model of two strategies (Bell 1986; Thakar *et al* 2003) is sufficient or the nectar production decision is more complex, demanding higher complexity models.

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