COLOUR CHANGE OF PETALS IN MALVAVISCUS ARBOREUS FLOWERS

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SUMMARY

Colour changes in the petals of two varieties of the tropical plant species *Malvaviscus arboreus* were investigated. The light absorbance of living petals and the absorbance of petal-extracts were measured. A separation of pigments by means of paper chromatography was attempted. Older stages of living petals show a low absorbance at all wavelengths compared with younger ones, and also a shift of the minimum at 440 nm and the maximum at 520 nm towards higher wavelengths. Petal extracts of different stages also have a different absorption spectrum; non-nectar producing flowers show a maximum at about 515 nm while nectar producing ones have merely a shoulder at 515 nm which may be due to a loss of red pigments. This loss is clearly demonstrated by paper chromatography and coincides with the beginning of nectar production. Hummingbirds, the main visitors of *Malvaviscus*, probably use this colour-shift for recognizing nectar producing flowers.

INTRODUCTION

The purpose of the present study was to investigate the change in composition of the pigments in petals of two varieties of the tropical plant species *Malvaviscus arboreus* Cav. during anthesis. *Malvaviscus arboreus* var. *arboreus* was described as a hummingbird flower by PORSCH (1939:79). The present author (GOTTSBERGER 1967) has made extensive observations on bird-visitors to flowers of *Malvaviscus arboreus* var. *penduliflorus* in the São Paulo region in Brazil. His final conclusions will be discussed again briefly to show the ecological background of this report.

Malvaviscus arboreus var. *penduliflorus* has flowers which are specialized for hummingbirds. The scarlet red petals though not sympetalous form a long flower tube which at its base contains a large amount of nectar. During several months the behaviour of hummingbirds visiting the flowers was studied. It was observed that hummingbirds from the São Paulo region with relatively short bills cannot reach the nectar legitimately via the deep flower tube, but get it only by making short cuts through the corolla. This boring or cutting by subtropical short-billed hummingbirds into a long-tubed tropical flower presented an excellent opportunity to start experiments on flower visits. Every single flower visit by such a bird was easily established by simply counting bill-marks, viz. holes in the petals. The first observation was that the hummingbirds visited only flowers which already had started with nectar production and neglected younger flowers without nectar. They are certainly not able to see the

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nectar in the pendent, long-tubed flowers. When the nectar production starts, the stigma plus staminal column grows out of the flower tube. As soon as the stigma plus staminal column becomes visible the birds can recognize a nectar-producing flower. However, this is not the only characteristic used by the bird to choose the nectar containing flowers. Nectar producing flowers with cut-off staminal columns or an otherwise modified appearance were still discriminated. On the other hand, placing nectar in non-producing flowers was without effect; flowers treated in such a manner were still not visited. The observation that nectar producing flowers always bear a less intense colour than non-producing ones – the petal colour becomes progressively lighter from the bud stage onwards during anthesis until the flower wilts – was pointed out and this brought the author to the consideration that the trained bird is probably able to distinguish a non-producing flower from a producing one by observing a difference in the colour intensity.

The question whether the slight difference in colour intensity is a dilution effect (the petals grow continuously) or the result of a modification of the pigments themselves remained unanswered up till now. In the present communication a first contribution is related concerning the phenomenon of flower-colour change of *Malvaviscus arboreus* during anthesis and the author has tried to give a provisional answer to the question how sharply flowerbirds can discriminate.

MATERIAL AND METHODS

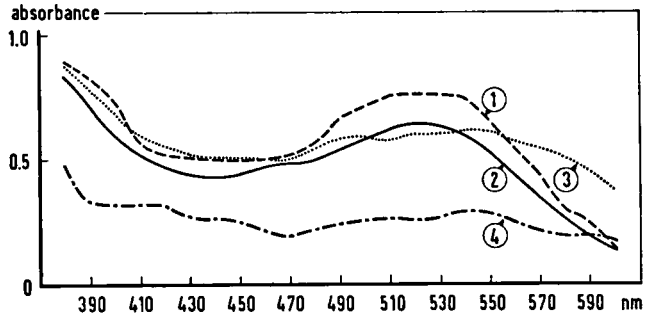
Flowers of two varieties of *Malvaviscus arboreus* were studied; dried flowers of var. *penduliflorus* from plants cultivated in São Paulo, and fresh flowers of var. *arboreus* obtained from the Botanical Gardens at Haren, Netherlands, and Mainz, Germany.

The absorbance of light within a wavelength range from 380 to 600 nm of fresh *Malvaviscus arboreus* var. *arboreus* petals was measured with a Bausch & Lomb Spectrophotometer, model Spectronic 20 with colour analyzer reflectance attachment. Four subsequent stages were investigated: Flower 1 just starting with nectar production; flower 2 in full anthesis; flower 3 starting to wither, and flower 4 wilted. Comparable results were obtained by always measuring petal pieces of the same size (8 mm diam.)

The petals of young flowers of both varieties in a not yet nectar producing stage (called bud) and of already nectar producing ones (called flower) were weighed and extracted during several hours with methanol containing 0.01% HCl. The solvent was renewed several times to give sufficient extraction. The extracts were centrifuged and concentrated on a water bath.

The extracts of flowers of var. *penduliflorus* were diluted and the absorbance of light within a wavelength range of 250 to 550 nm was measured with a Bausch & Lomb Spectrophotometer, model Spectronic 505.

The different pigments of the petals of both varieties were separated by paper chromatography and subsequent analysis of the separated compounds was attempted. The concentrated extracts were applied as small spots to sheets of



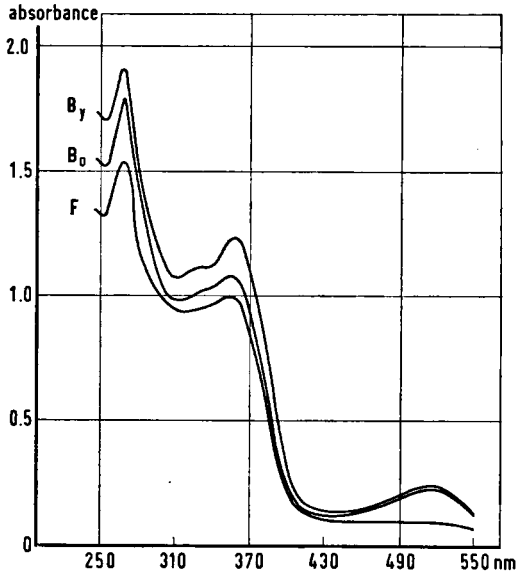
Graph 1: *Malvaviscus arboreus* var. *arboreus*. Absorbance within a wavelength range of 380 to 600 nm of four subsequent stages of living petals.

carefully washed Whatman No 3MM paper (method by ISHERWOOD and HANES, SCHWEPPE 1959:112), cut according to LINSKENS (1959:14, *fig. 13*) and subsequently developed in the alcoholic phase of n-butanol/acetic acid/water (4:1:5) by means of ascending chromatography. This method gave sufficiently good separation and enabled us to draw some conclusions about the presence or absence of the determined petal pigments at the different stages of anthesis.

RESULTS

The light absorbance of petals of the 4 subsequent stages of *Malvaviscus arboreus* var. *arboreus* flowers is shown in *graph 1*. It demonstrates instructively that young flowers have a strong absorbance at a wavelength of about 520 nm and that in older flowers the light absorbance gradually diminishes. The petals become progressively lighter during anthesis. The minimum of absorbance lies in younger stages at about 440 nm and the maximum at about 520 nm. In older stages a slight shift of the minimum and maximum of absorbance towards higher wavelengths is seen. The oldest stage already has a minimum at 470 nm and a maximum at 540 nm. From these results it can be concluded that not only the colour intensity changes, but that the composition of pigments themselves is probably modified during anthesis. The results do not necessarily reflect the processes observed in flowers of var. *penduliflorus* where (presumably) a difference of absorbance of different flower stages may be even more striking than in var. *arboreus*.

Graph 2 shows the absorbance of petal extracts of var. *penduliflorus*, of non-nectar producing states (B=bud, By=young bud, Bo=older bud, just before the nectar production starts) and of a nectar producing stage (F=flower). Both stages (B and F) show their maxima and minima at about the same wave lengths. The position of the maxima and minima of absorbance indicates that the extracts contain a mixture of flavonoid pigments. We know that anthocyanin absorption spectra in general show a maximum at 270–280 nm and another

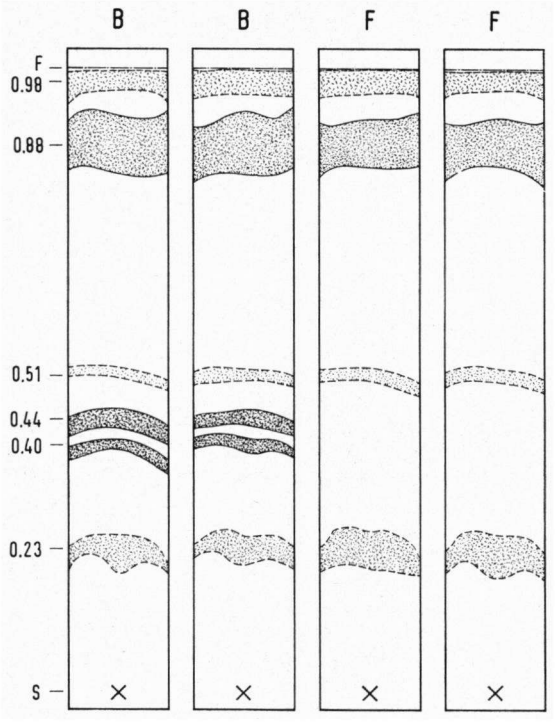


Graph 2: *Malvaviscus arboreus* var. *penduliflorus*. Absorbance of petal-extracts within a wavelength range of 250 to 550 nm of bud stages (B) and a flower in full anthesis (F).

one at about 520 nm; the yellow flavones and flavonols have two regions of high-intensity absorption, one region at about 240–260 nm and another one at about 330–375 nm according to GEISSMAN (1955: 486–488). However, the "peak" of B and the "shoulder" of F at 515 nm demonstrates that the non-producing flowers (B) contain more anthocyanins than the older nectar producing ones (F). The yellow compounds have also diminished in older flowers, but much less, which is probably due to the slight dilution effect. The results suggested that the amount of red anthocyanin compounds have decreased relatively more in comparison with the amount of yellow compounds. This drop in red flower pigments seems to coincide with the beginning of nectar production. It is notable that the flower which nearly produces nectar (Bo) shows only a slightly decreased maximum at 515 nm compared with the young bud stage (By) whereas the nectar producing flower (F) shows a strong decrease at this maximum.

The colour compounds were separated by means of paper chromatography. Chromatograms of non-nectar producing flowers (B) and nectar producing ones (F) of *Malvaviscus arboreus* var. *penduliflorus* are shown in *graph 3*. Six main compounds are distinguishable (some under U.V. light) in extracts of the non-producing flowers. In nectar producing flowers two red compounds (R_F values 0.40 and 0.44) have completely disappeared.

The young flowers (B) of *Malvaviscus arboreus* var. *arboreus* (*graph 4*) show nine clearly distinguishable compounds (*table 2*). In later, nectar producing stages (F1 and F2), the lilac compound No 5 has nearly disappeared. Old, withered flowers (F3) contain even fewer compounds: Compound No 5 is now completely absent, the red compound No 3 is also lacking and instead of the yellow pigment No 7 we find a bluish one with a slightly lower R_F value.

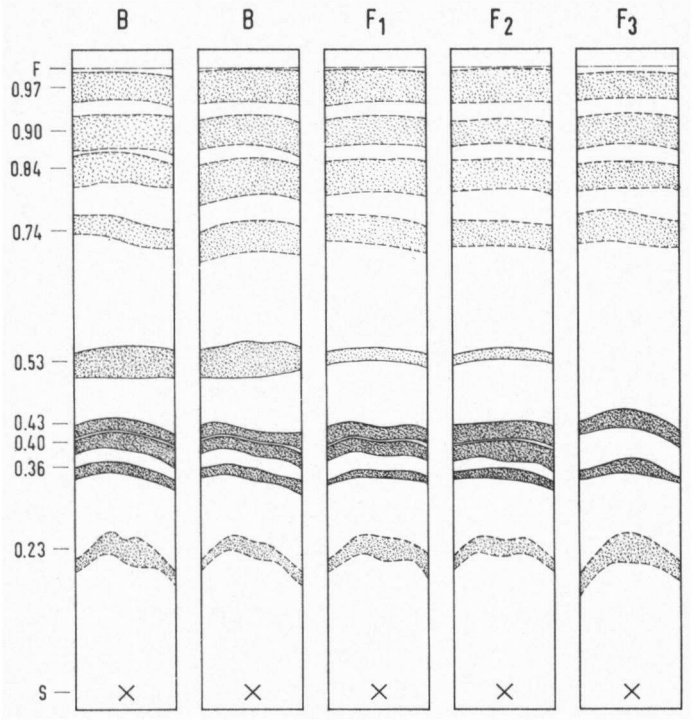


Graph 3: *Malvaviscus arboreus* var. *penduliflorus*. Paper chromatograms of petal extracts of bud stages (B) and flowers in full anthesis (F).

An attempt at identification of the separated pigments failed. The bands of pigment No 2 and No 4 of var. *arboreus* were cut off from the chromatogram and diluted in methanol containing 0.01 % HCl. By measuring the absorbance at the wavelengths between 250 and 550 nm it was found that the compounds thus separated are still mixtures of at least two pigments with the same or nearly the same R_F value. A further separation and identification of the pigments may be the subject of a future study.

Table 1. Pigments, pigment colour and R_F values in order of appearance in flower extracts of *Malvaviscus arboreus* var. *penduliflorus*.

Pigments	Pigment colour	R_F value
Pigment 1	violet (UV)	0.23
Pigment 2	red	0.40
Pigment 3	red	0.44
Pigment 4	bluish (UV)	0.51
Pigment 5	yellow	0.88
Pigment 6	light blue (UV)	0.98



Graph 4: *Malvaviscus arboreus* var. *arboreus*. Paper chromatograms of petal extracts of bud stages (B), flowers in full anthesis (F1, F2) and withered flowers (F3).

Table 2. Pigments, pigment colour and R_F values in order of appearance in flower-extracts of *Malvaviscus arboreus* var. *arboreus*.

Pigments	Pigment colour	R_F value
Pigment 1	violet (UV)	0.23
Pigment 2	red	0.36
Pigment 3	red	0.40
Pigment 4	red	0.43
Pigment 5	lilac	0.53
Pigment 6	blue (UV)	0.74
Pigment 7	yellow (UV)	0.84
Pigment 8	blue (UV)	0.90
Pigment 9	light blue (UV)	0.97

DISCUSSION

A colour change in flowers, fruits, seeds, and leaves, due to a change of water-soluble pigments, is a well-known phenomenon in plants. SEYBOLD has summarized our knowledge in 1954. The colour change is probably a purely physiological effect and reflects the actual stage of the cells. KUGLER (1936, 1952, etc.) and VOGEL (1950) proved that such a colour change enables insects to locate flowers in full anthesis immediately, so that they can neglect the younger and older ones. About insects and their ability to see and distinguish even subtle colour differences we are quite well informed by the work of VON FRISCH (1915, 1953, 1960 etc.) and his school, KUGLER (1947, 1950, 1951a, 1951b, 1966, 1970), KNOLL (1921–1926), SCHREMMER (1941a, 1941b), and others.

Similar studies are rare for flowerbirds (POLEY 1968, WINKEL 1969, and others) and questions regarding the ability of flowerbirds to discriminate even very small colour differences are still open. We know that flowerbirds are attracted by showy, bright colours, probably due to the general vision of birds, which is thought to be similar to that of man. Red flowers may be even more important in extra-tropical regions, through which flowerbirds only migrate quickly (GRANT & GRANT 1966, 1967a, 1967b). There red flowers are easily recognizable signals to the migrating bird to locate flowers rich in nectar in as brief a time as possible. The few investigations about the vision of birds in general indicate that their eyes are less sensitive to blue and violet, about as sensitive to yellow, and much more sensitive to red than the human eye (KNOLL 1956:139, FAEGRI & VAN DER PIJL 1966:110). Should this be true for flowerbirds too, which has not been investigated yet in this respect, then they should be able to detect a colour change in the red of petals much more readily than the human eye. For them flowers of *Malvaviscus arboreus* starting with nectar production should look quite distinct from others which are not yet producing. In fact, two red anthocyanin compounds are disappearing in the petals of the var. *penduliflorus* at the beginning of nectar production.

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