

Stylish lengths: Mate choice in flowers

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The styles of flowers may represent an arena for pollen competition in the race to fertilize ovules. Accordingly, selection should favour a longer 'race' to better discriminate among variable pollen by increasing style length. Sampling across a taxonomically diverse range of wild and outcrossed species, we found that the distribution of style lengths within plants were skewed towards longer styles, as predicted. In self-pollinated domesticated species, where discrimination among pollen is less important, we found no such pattern. We conclude that style length is under directional selection towards longer styles as a mechanism for mate choice among pollen of variable quality.

[Ramesha BT, Yetish MD, Ravikanth G, Ganeshaiah KN, Ghazoul J and Uma Shaanker R 2011 Stylish lengths: Mate choice in flowers. *J. Biosci.* 36 229–234] DOI 10.1007/s12038-011-9057-6

1. Introduction

In flowers the style separates the stigmatic surface, on which pollen is received, from the ovary, which contains the ovules. Pollen germinating on the stigma produces pollen tubes that must grow through the style in order to fertilize the ovules. The style length may therefore serve as a mate selection device by providing an arena for pollen competition (Grant 1995; Lankinen and Skogsmyr 2001). Pollen tubes that grow more slowly, or are not able to sustain growth through the length of the style, are less likely to fertilize the ovules. Viewed in this context, style length represents a sexually selected character in plants, allowing female parents to impose selection among variable pollen grains deposited on the stigmatic surface (Wilson and Burley 1983; Wilson *et al.* 1994). Accordingly, selection should, within the limits of the cost of style production and fertilization probability, favour longer styles in plants such that pollen grains are forced to run a longer 'race' for a more stringent selection (Mulcahy and Mulcahy 1975; Snow and Timothy 1991; Eberhard 1996).

A prediction emerging from this theory is that, within species, stylar lengths will be negatively skewed because of directional selection to increase style lengths. We offer a test of this prediction and conclude that styles might indeed have evolved as a sexually selected character to physically screen pollen grains.

2. Materials and methods

Style length was measured on 31 wild outcrossed species from 15 families occurring in the botanic gardens of the University of Agricultural Sciences, GKVK campus, Bangalore, India. For each species, about 25 to 30 opened flowers were sampled from five or six individuals of the species. The stylar length of individual flowers was accurately measured using digital vernier callipers from the stigmatic tip to the base of the ovary. For each species, median and skewness coefficient of style length were computed. We analysed the direction and the magnitude of skewness using a small-sample skewness test (<http://www.wessa.net/test.wasp>). Because our interest was to examine the directional selection on the style lengths, we

Keywords. Flowers; mate choice; outcrossed; skewness; style lengths

employed a one-tailed skewness test. To rule out the possibility that the distribution of style lengths could be an allometric feature of flower size equally affecting other floral structures and unrelated to mate choice, we examined the frequency distribution of the length of filaments (the structures bearing the anthers) in 21 of these species by using the same sampled flowers. Unlike styles, filaments are not expected to be subject to mate choice selection pressure, and hence a Gaussian distribution of filament lengths is predicted. As there is no *a priori* expectation on the direction of selection for filament

lengths, we analysed the significance of the distribution of their lengths using a two-tailed skewness test. For species for which both style and filament lengths were available, a sign test was used to analyse the magnitude of differences between the style and filament skewness.

Finally, as a test of prediction, we also examined the style length for three self-pollinated and domesticated species, namely, *Vigna unguiculata* (L.) Walp, *Cajanus cajan* (L.) Druce and *Lycopersicon esculentum* Mill. A congeneric species comparison of *Lycopersicon* used the highly self-compatible domesticated species *L. esculentum*

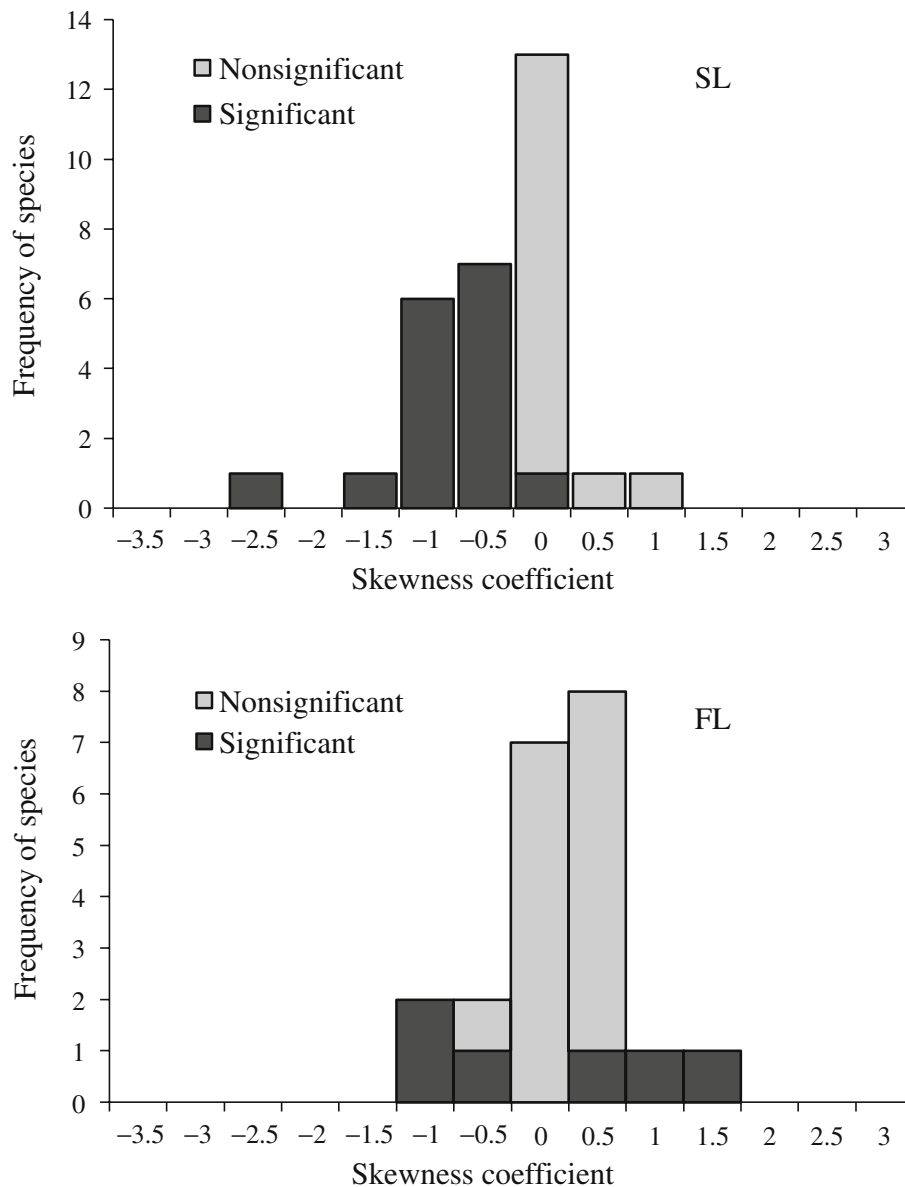


Figure 1. Frequency distribution of skewness coefficients of style lengths (SL) ($n=31$ species) and filament lengths (FL) ($n=21$ species). The significance of skewness coefficients of style lengths was determined using a one-tailed small-sample skewness test, and filament lengths skewness coefficient significance was tested using two-tailed small-sample skewness test (<http://www.wessa.net/test.wasp>).

Table 1. Descriptive statistics of style (S) and filament (F) lengths (in mm) for different plant species

Species	S&F	N	Median	Skewness	Skewness (SE)	P
<i>Adathoda zeylanica</i> . Medic (Acanthaceae)	S	30	25.0	-0.36	0.43	0.200
	F	60	19.0	0.17	0.31	0.568
<i>Hippeastrum striatum</i> (Lam.) H.E.Moore (Amaryllidaceae)	S	30	34.0	-0.61	0.43	0.075
	F	60	24.0	-0.47	0.31	0.124
<i>Allamanda allicium</i> L. (Apocynaceae)	S	20	30.5	-2.77	0.51	0.000005
	F	80	19.3	0.31	0.27	0.238
<i>Allamanda cathartica</i> L. (Apocynaceae)	S	30	43.5	-0.55	0.43	0.100
<i>Bignonia venusta</i> Ker Gawl. (Bignoniaceae)	S	30	71.0	-1.87	0.43	0.00005
	F	60	46.3	-1.11	0.31	0.0004
<i>Jacaranda mimosifolia</i> D.Don (Bignoniaceae)	S	30	17.8	-0.15	0.43	0.363
	F	60	9.0	-0.21	0.31	0.502
<i>Spathodea campanulata</i> Buch.Ham ex DC. (Bignoniaceae)	S	26	80.9	-0.63	0.46	0.082
	F	103	53.7	0.04	0.24	0.865
<i>Tabebuia argentea</i> Britton (Bignoniaceae)	S	30	24.0	-1.39	0.43	0.0005
	F	60	20.0	-1.17	0.31	0.0002
<i>Tecoma stans</i> Juss. (Bignoniaceae)	S	30	22.5	-0.31	0.43	0.230
	F	60	21.0	0.79	0.31	0.010
<i>Bixa orellana</i> L. (Bixaceae)	S	30	16.1	0.65	0.43	0.064
<i>Brassica oleracea</i> L. (Brassicaceae)	S	19	3.84	-0.10	0.52	0.421
	F	48	5.6	-0.56	0.34	0.101
<i>Capparis zeylanica</i> Roxb. (Capparaceae)	S	30	39.5	-0.75	0.43	0.038
<i>Mesua ferrea</i> L. (Clusiaceae)	S	25	74.9	-0.28	0.46	0.274
	F	125	53.9	0.44	0.22	0.042
<i>Gloriosa superba</i> L. (Colchicaceae)	S	30	55.9	-1.40	0.43	0.0005
	F	113	37.8	1.15	0.22	0.0001
<i>Ipomoea carnea</i> G.Forst. (Convolvulaceae)	S	30	25.0	-0.92	0.43	0.015
	F	60	21.8	-0.16	0.31	0.61
<i>Bauhinia variegata</i> L. (Fabaceae)	S	50	11.0	-1.02	0.34	0.0012
	F	125	34.0	-0.20	0.22	0.352
<i>Butea monosperma</i> Kuntze (Fabaceae)	S	60	52.0	-0.14	0.31	0.323
<i>Cassia roxburghii</i> Steud. (Fabaceae)	S	20	17.0	-1.43	0.51	0.002
	F	125	5.9	0.34	0.24	0.167
<i>Cassia</i> sp. L. (Fabaceae)	S	25	11.3	0.34	0.46	0.233
	F	55	9.3	-0.19	0.32	0.555
<i>Crotalaria juncea</i> L. (Fabaceae)	S	25	15.0	-1.27	0.46	0.003
	F	125	13.1	-0.28	0.22	0.187
<i>Delonix regia</i> (Bojer) Raf. (Fabaceae)	S	30	98.3	-0.11	0.43	0.394
	F	60	72.4	-0.66	0.31	0.032
<i>Erythrina suberosa</i> Roxb. (Fabaceae)	S	30	8.58	-0.42	0.43	0.164
<i>Orthosiphon stamineus</i> Benth. (Lamiaceae)	S	30	66.8	-0.80	0.43	0.06
<i>Clerodendrum inerme</i> R.Br. (Lamiaceae)	S	25	46.2	-0.22	0.46	0.312
	F	75	27.2	-0.08	0.28	0.779
<i>Ixora pudica</i> Baker (Rubiaceae)	S	80	35.3	-0.45	0.26	0.093
<i>Santalum album</i> L. (Santalaceae)	S	25	2.3	-0.82	0.46	0.038
<i>Lycopersicon cheesmanii</i> Riley (Solanaceae)	S	15	9.5	-1.04	0.58	0.037
<i>Lycopersicum persium</i> (Solanaceae)	S	22	9.8	-0.59	0.49	0.113
<i>Lycopersicum pimpinellifolium</i> (Jusl.) Mill. (Solanaceae)	S	18	6.4	-0.24	0.54	0.330

Table 1. (continued)

Species	S&F	N	Median	Skewness	Skewness (SE)	P
<i>Solanum seaforthianum</i> Andrews (Solanaceae)	F	54	2.9	0.31	0.32	0.342
	S	25	11.1	-0.11	0.46	0.409
<i>Solanum torvum</i> Sw. (Solanaceae)	F	124	6.2	0.12	0.22	0.582
	S	25	8.9	-0.32	0.46	0.242
* <i>Vigna unguiculata</i> (L.) Walp. (Fabaceae)	F	125	7.8	0.35	0.22	0.101
	S	20	14.75	0.81	0.51	0.056
* <i>Cajanus cajan</i> (L.) Druce (Fabaceae)	F	100	9.05	0.57	0.24	0.017
	S	20	12.57	0.28	0.51	0.288
* <i>Lycopersicon esculentum</i> Mill. (Solanaceae)	F	100	6.3	1.29	0.24	0.0005
	S	18	6.92	0.19	0.54	0.359

P-values refer to the likelihood of the null expectation that style skewness is equal or greater than 0 (one-tailed test, critical value = 0.1) and the likelihood the null expectation that filament skewness is equal to 0 (two-tailed test, critical value = 0.05). Self-pollinated and domesticated plant species are indicated with an asterisk.

and its outcrossed wild congeners, *Lycopersicon persium* Mill., *Lycopersicon cheesmanii* Riley and *Lycopersicon pimpinellifolium* (Jusl.) Mill. (Rick 1982). Seeds of these

species were procured from the Indian Institute of Horticultural Research, Bangalore, and grown under potted conditions at the University of Agricultural Sciences,

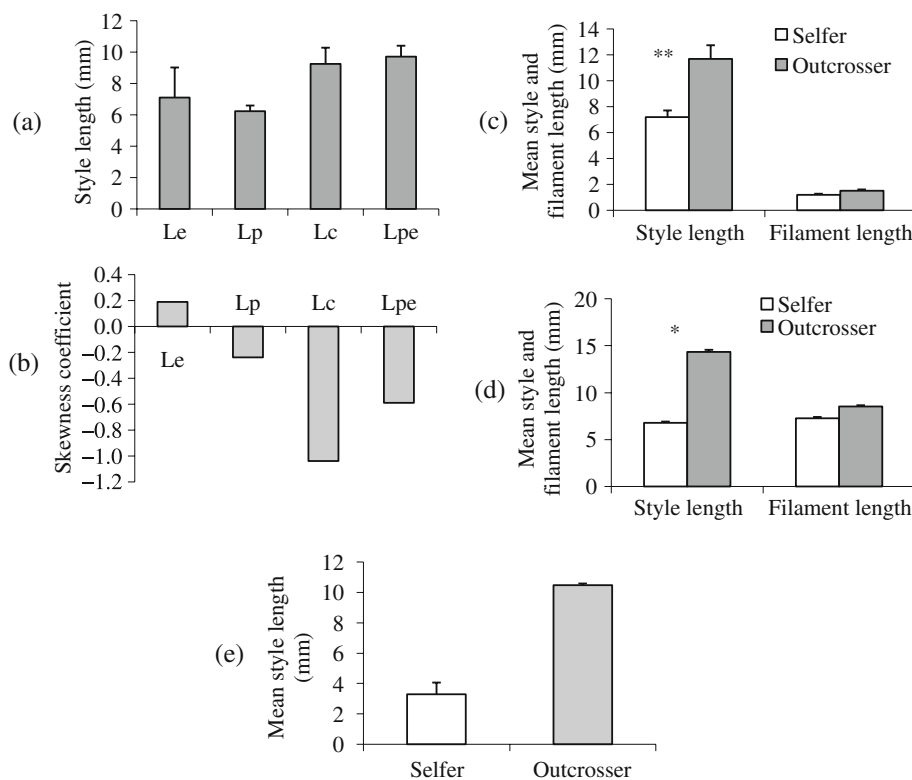


Figure 2. Mean style length (a) and skewness (b) of *L. esculentum* (Le; $n=18$), *L. pimpinellifolium* (Lp; $n=18$), *L. persium* (Lpe; $n=22$) and *L. cheesmanii* (Lc; $n=15$). (c) Lp self-pollinated population, $n=45$; its outcrossing population, $n=65$. (d) Self-pollinated species (*Clarkia parviflora*, $n=48$; its congeneric outcrossing species *Clarkia xantiana* $n=48$). (e) Self-pollinated species (*Mimulus nasutus*, $n=40$); its congeneric outcrossing species (*Mimulus guttatus*, $n=40$). Graphs c, d and e were redrawn using the data from Georgiady and Elizabeth (2002), Runions and Monica (2000), Diaz and Macnair (1999). **Significant difference at $P<0.01$, *significant difference at $P<0.05$ following the two-tailed Student's *t*-test.

Bangalore. For each species, based on availability, fully opened flowers were randomly collected and transferred to the laboratory in an icebox. The style lengths of individual flowers were measured as described above. We also sourced literature to locate data on style and filament length for selfed and outcrossed congeneric species. The search was limited to studies in which style and filament lengths were measured in wild populations.

3. Results and discussion

Style lengths were significantly ($P < 0.1$, one-tailed test) negatively skewed in 16 species. Of the total 31 species, only two had a (nonsignificant) positive skew, a highly unlikely distribution based on a null expectation of no directionality (sign test: $P < 0.0001$, figure 1, table 1). Of the 21 species for which filament lengths were available, 3 were negatively skewed and 3 were positively skewed (based on $P < 0.05$, two-tailed test), indicating no pattern in the distribution of filament lengths. Style and filament skewness coefficients were significantly different (paired t -test, $P < 0.001$).

Where there is less opportunity or necessity to select among pollen genotypes, as in self-pollinated plants, we further expect no significant skewness in either direction. Interestingly, we obtained such a result in three domesticated self-pollinated species that we tested, *V. unguiculata*, *C. cajan* and *L. esculentum* (table 1). Among congeneric *Lycopersicum* species, the style length was shortest in the domesticated self-compatible *L. esculentum* (figure 2a). Style length was positively skewed in *L. esculentum* and negatively skewed in the 3 wild species, although significantly so only for *L. cheesmanii* (figure 2b).

Previous studies (Diaz and Macnair 1999; Runions and Monica 2000) have also shown that congeneric outcrossing species had longer styles compared to their selfing counterparts. In two out of three independent studies, mean style length was significantly longer for outcrossers as compared to selfing species (figure 2c–e). Similarly, Georgiady and Elizabeth (2002) identified two populations of *L. pimpinellifolium*, one of which was strictly self-pollinated and other cross-pollinated. The mean style length of cross-pollinated population was significantly higher (11.7 mm) than that of the self-pollinated population (7.2 mm); however, the two populations did not differ with respect to their filament lengths (figure 2c).

Maximum style lengths may be defined by trade-offs between benefits of enhanced paternal vigour and energetic cost of investment in additional stylar tissue, or probability of fertilization (even vigorous pollen tubes may fail to reach ovules at the end of very long styles). In the latter case, the predictability and abundance of

pollen deposition on the stigmatic surface is likely to affect the trade-off, as ovules at the base of short styles are more likely to be fertilized, and therefore contribute to fitness as seed, even by pollen of comparatively poor quality. Seed-limited recruitment is also likely to act against selection for long styles, whereas seed competition would favour selection for mate choice mechanisms.

Apart from mate choice, style length distribution in plants could be shaped by several other factors including selection for optimal stigma placement and pollen deposition and herkogamy. However, none of these latter factors explain the observed negative skewness of stylar lengths. If style lengths were shaped purely by the selection for optimal stigma placement and pollen deposition, then a normal distribution of style lengths centred on the optimal style length would be expected, and not a skewed distribution. Further, if selection for pollen deposition was an overdriving pressure, it could have been manifested in several other features including elongation of flower stalks and placement of ovary on stalks (as in superior ovaries), and not necessarily through selection of longer styles. If herkogamy is present, then one would expect a positive skew in styles and negative skew in filaments and vice versa, depending on which type of herkogamy is present (if any), a pattern clearly in contrast to what has been obtained.

In summary, negatively skewed style lengths appear to be a consistent feature among taxonomically diverse plant species, suggesting that selection acting through mate choice favours investment in longer style lengths. Where there is less opportunity or necessity to select among pollen genotypes (as expected among predominantly selfed plants), there is no apparent negative skewness of style lengths. These results offer an evolutionary perspective on style length distributions in plants (Mulcahy and Mulcahy 1975; Chasan 1992). Viewed in this context, styles in plants are not merely ‘sterile’ botanical tissues, but seem to play an important role in sieving among variable pollen grains, just as marathon race tracks sieve among variable and competing athletes.

Acknowledgements

The work was partly supported by grants from the Department of Biotechnology, Government of India, and ATREE, Bangalore. Dr Dipanwita Banik, Botanical Survey of India, India, helped with the taxonomic identification of the plants. Thanks are due to Dr KV Ravishankar, Indian Institute of Horticultural Research, for providing the seed material of the congeneric species of *Lycopersicum*.

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MS received 10 December 2010; accepted 25 February 2011

ePublication: 16 May 2011

Corresponding editor: IMRAM SIDDIQI