QUANTITATIVE CHARACTERISTICS OF THE GYNOECIUM IN *PRIMULA VULGARIS* HUDS

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Abstract

The examination of the quantitative characteristics of the gynoecium in a typical heterostylous species, *Primula vulgaris* points to several differences between the two morphs beyond the well-known ones that are closely related to the phenomenon of heterostyly. Stigma length, stigma width and ovule length differ significantly in the two morphs at $P \leq 0.001$, whereas ovary length, ovary width and ovule number per ovary do not differ in the two morphs. Standard deviation, range and frequency distribution diagrams of most examined traits prove that the pin morph is much more variable than the thrum one. Correlation studies show that the length of the gynoecium is determined only by the style length, and the sizes of the other parts of gynoecium are more or less independent of each other.

Key words: heterostyly, gynoecium, *Primula*

Introduction

Heterostyly is a special form of genetic polymorphisms. Heterostylous plant populations consist of two (distyly) or three (tristyly) morphs with reciprocal arrangement of stigmas and anthers (reciprocal herkogamy). *Primula* species are well-known and widely investigated examples of distyly. The two morphs are easily distinguishable: the pin form has long style and its anthers locate near the middle of the corolla tube, while the thrum form has short style and its anthers are near the top of the corolla tube.

The morphological manifestation of reciprocal herkogamy is connected to several ancillary polymorphisms of both gynoecium and androecium. The gynoe-

cium is built up from three parts: stigma, style and ovary, but most investigations of polymorphisms concern only the style and the stigma. Numerous general and particular instances can be found indicating quantitative and qualitative differences of these two parts of gynoecium.

In *Waltheria viscosissima* (Sterculiaceae) the style of the two morphs varies not only by length but also by shape. The long style is repeatedly curved and bears large verrucae and long hairs, whereas the short style is only slightly curved, has smaller verrucae and bears shorter hairs (KÖHLER, 1973, 1976). In *Primula obconica* (Primulaceae) the histological structure of the style differs in the two morphs: the transmitting tissue of the short style is much larger than that of the long one (DOWRICK, 1956). Difference in the size of the stigma is also a frequent feature in heterostylous species. The receptive surface of the pin morph is typically larger than that of the thrum one, this feature is conspicuous in *Plumbago capensis* (Plumbaginaceae) and *Linum grandiflorum* (Linaceae, DULBERGER, 1992). However there are species where an opposite ratio of stigma size occurs: in *Primula malacoides* (Primulaceae, PANDEY and TROUGHTON, 1974) and *Hedyotis caerulea* (Rubiaceae, ORNDUFF, 1980) the thrum stigma is larger than the pin one. The polymorphism of gynoecium can also be manifested by the shape of the stigmas. In *Rudgea jasminoides* (Rubiaceae) the thrum stigmas are long and narrow, while the pin ones are short and broad (BAKER, 1956). Regarding some *Primula* species (Primulaceae) small differences in the shape of stigmas are also mentioned, for example in *P. vulgaris* (HESLOP-HARRISON *et al.*, 1981), *P. elatior* (SCHOU, 1983) and *P. obconica* (BIR BAHADUR *et al.*, 1984).

In a previous study we examined the morphological differences of some perianth traits in the two morphs of *Primula vulgaris* and *P. veris*. The corolla-tube length proved to be applicable to distinguish the morphs by both means and frequency-distribution diagrams (KÁLMÁN and MIHALIK, 1996). Besides the features of the perianth we have also examined the gynoecium of *Primula* species and quantitative characteristics of stigma, style, ovary and ovules of *Primula vulgaris* are presented in this study.

Materials and methods

The object of our examination is a typical distylous species, *Primula vulgaris* HUDS. The examinations were carried out in a semi-natural population in the Botanical Garden of József Attila University. The plants were planted several years ago and now grow together with numerous herbaceous species constituting a steady community. We have investigated this semi-natural population since 1995 and established that the natural survival of the species is provided by both vegetative and generative reproduction.

For the examinations the flowers were collected from older plants with at least 15 leaves. All the flowers were from different plants and were collected 2–3 days after opening. Gynoeciums were dissected from the flowers and immediately placed onto a wet filter-paper. The gynoeciums were divided

into three parts: stigma, style and ovary and the ovules were removed from the ovaries. By using a binocular stereo-microscope connected to a video camera we took pictures about the stigmas, ovaries and ovules and the pictures were processed by an image-analysing software (Image Pro-Plus 3.0).

The length of the whole gynoecium, the stigma and the ovary were measured along the longitudinal axis of the gynoecium and the width of the stigma and the ovary were measured perpendicularly to the length. The length of the style was calculated by subtracting the sum of stigma and ovary length from the whole length of the gynoecium. The number and the largest length of ovules was also determined. 100–120 gynoeciums and about 500 ovules removed from 10 ovaries were measured in each morph.

In the first step of data processing the frequency-distribution diagrams of the measured characters were created and data which did not fit into the normal distribution were left out. It occurred only in the case of pin stigma length where there were three extremely large values. By using modified data the mean, standard deviation and range (the difference between the largest and smallest values) of each character were calculated. Means of each character in the two morphs were compared by Student's t-test. We calculated the ratio of data if it seemed to be informative and compared the behaviour of the two morphs. Correlations were also checked among the measured features and the equations of the regression lines were calculated.

Results

Characterisation of style, stigma and ovary

The mean, standard deviation and range of measured features in thrum and pin morphs are given in Table 1. and Table 2., respectively. The most striking manifestation of reciprocal herkogamy is the difference in the length of style between the two morphs. The pin style is about twice as long as the thrum one (pin:thrum ratio is 2.17:1). There is no overlap between the two morphs, the longest thrum style is 7. 88 mm, while the shortest pin one is 10. 33 mm. The standard deviation of the pin style length is about twice as big as that of the thrum one and the range of the pin data is half as big again as that of the thrum one. The frequency distribution diagrams are typical of the morphs: in the diagram of thrum data there is a characteristic peak with 50% of all the cases in the category of 6–7 mm, while the diagram of pin data is much flatter, in the middle of the range there are four categories (from 12 mm to 16 mm) with 15–30% of all the cases.

Apart from the difference of style length the difference in stigma size is also mentioned in some heterostylous species. According to our results in *Primula vulgaris* both the length and the width of stigma are different in the two morphs: the pin stigma is longer and wider than that of the thrum one. Comparison of the means by Student's t-test shows that the difference is significant ($P \leq 0.001$). The standard deviations and the ranges are slightly larger in the pin morph than in the thrum one. The frequency distribution diagrams of the stigma length show unambiguous differences between the two morphs. The peaks of the thrum and the pin data are separated sharply: the thrum peak appears in the category of 0.9–1.0 mm, while the pin one is in the category of 1.2–1.3 mm. The frequencies at the

peaks are about 30% of all the cases in both morphs. The separation of thrum and pin peaks is not so clear in the frequency distribution diagrams of stigma width. There is a common peak in the category of 1.3–1.4 mm, but the frequency of pin data in the peak is higher than that of thrum ones (37% of all the cases for pin and 29% of all the cases for thrum morph). In the categories smaller than 1.3 the frequency of thrum data is higher than that of pin ones, while in the categories bigger than 1.4 the arrangement of frequencies is opposite.

Table 1. Mean, standard deviation and range of measured features in thrum morph (St. dev. = standard deviation, N= number of cases)

	length of style (mm)	length of stigma (mm)	width of stigma (mm)	length of ovary (mm)	width of ovary (mm)
Mean	5.87	0.92	1.20	1.95	1.95
St. dev.	0.72	0.13	0.14	0.27	0.19
Range	4.18	0.59	0.79	1.29	0.97
N	117	118	118	121	121

Table 2. Mean, standard deviation and range of measured features in pin morph (St. dev. = standard deviation, N= number of cases)

	length of style (mm)	length of stigma (mm)	width of stigma (mm)	length of ovary (mm)	width of ovary (mm)
Mean	12.76	1.19	1.33	1.90	2.02
St. dev.	1.34	0.16	0.16	0.26	0.22
Range	5.98	0.86	0.83	1.11	1.21
N	102	99	102	120	119

The third part of the gynoecium is the ovary. In *Primula vulgaris* the length and the width of ovary are similar in both morphs, so according to the means the ovary seems to be spherical. Comparison of the means shows that these traits are very similar to each other in the two morphs: there is no significant difference for the ovary length and the difference is very small for the ovary width ($P \leq 0.05$). The standard deviations, the ranges and the frequency distribution diagrams are also almost the same in the two morphs. The only one difference which can be found is that in the middle of the ranges (between 1.8 and 2.4 mm) the frequencies of thrum data are higher than the frequencies of pin ones, whereas at the edges of the ranges the frequencies of the pin data are higher than that of the thrum ones.

Ratios of the measured features

Some ratios seemed to be informative in the exact description of the characteristics of gynoecium. We examined the length:width ratios of stigma and ovary and the length ratios of each part of the gynoecium. The mean, standard deviation and range of the calculated ratios are summarised in Table 3.

The length:width ratio of the stigma is larger in the pin morph than in the thrum one so the thrum stigmas seem to be shorter and/or wider than the pin ones. With respect to the standard deviation and range there is no difference between the two morphs at all, but in the frequency distribution diagrams the data of the two morphs are separated sharply. The peak of the thrum data is in the categories of 0.7–0.9 mm with 60% of all the cases (the pin frequency is 23% of all the cases there), the peak of the pin data is in the categories of 0.9–1.1 mm with 55% of all the cases (the thrum frequency is 30% of all the cases there). The most important difference between the two morphs can be seen when the distributions of frequencies are compared to the 1.0 value, where the length and width are equal, so the stigma is almost spherical. 82% of all the thrum proportions is smaller than 1.0, while 50% of all the pin proportions is equal with or bigger than 1.0.

Table 3. Mean, standard deviation and range of the calculated ratios
(St. dev. = standard deviation, N= number of cases)

	stigma length: stigma width		ovary length: ovary width		stigma length: ovary length		style length: stigma length		style length: ovary length	
	thrum	pin	thrum	pin	thrum	pin	thrum	pin	thrum	pin
Mean	0.77	0.90	1.00	0.94	0.48	0.64	6.53	10.81	3.07	6.89
St. dev.	0.12	0.12	0.11	0.10	0.09	0.12	1.28	2.01	0.59	1.23
Range	0.62	0.62	0.54	0.64	0.48	0.61	7.57	9.71	3.18	5.90
N	118	97	118	120	117	99	117	102	117	102

The length:width ratio of the ovary is obtained opposite to that of the stigma: the thrum ratio is larger than the pin one. Standard deviations and ranges are similar in the two morphs and there is no significant difference between the frequency distribution diagrams either. The peaks are in the category of 1.0–1.1 mm in both morphs, so most of the ovaries are really spherical. In the categories under 1.0 the pin frequencies are higher, while in the categories above 1.0 the thrum ones are higher.

The length ratios of the parts of the gynoecium, namely stigma length:ovary length, style length:stigma length and style length:ovary length ratios were also

calculated. Strikingly in all the three examined ratios the standard deviations and the ranges of pin data are larger than those of thrum ones and the data of the two morphs are separated sharply in the frequency distribution diagrams. In the diagram of stigma length:ovary length ratio the peak of the thrum data is in the category of 0.5–0.6 mm, while the pin peak appears in the category of 0.7–0.8 mm with 40% of all the cases in both morphs. The diagrams of style length:stigma length and style length:ovary length ratios show clearly the general difference between the two morphs: in the diagrams of thrum data there is a definite peak with a high frequency, while the diagrams of the pin data are much flatter, there are more categories with similar frequencies in them.

Correlation and regression

The correlation coefficients of the compared traits are given in Table 4., the most important ones are enhanced in bold. In the first step the correlation between the length of the whole gynoecium and the length of each part of the gynoecium were examined. According to our results there is a strong connection between the whole gynoecium length and the style length, in the diagram representing the style length against the gynoecium length the points fit tightly to the regression lines (Fig. 1.). The slope of the regression line (r) is 0.86 for the thrum data and 0.96 for the pin ones. In contrast to the style length there is no correlation in the case of stigma length and ovary length, so the length of these parts of the gynoecium seems to be independent of the whole gynoecium length.

Table 4. Correlation coefficients in thrum and pin morphs

	length of gynoecium		length of stigma		length of ovary	
	thrum	pin	thrum	pin	thrum	pin
length of style	**0.91**	**0.97**				
length of stigma	0.28	0.10				
length of ovary	0.26	0.07	0.08	–0.07		
width of stigma			0.32	0.31		
width of ovary					**0.63**	**0.64**

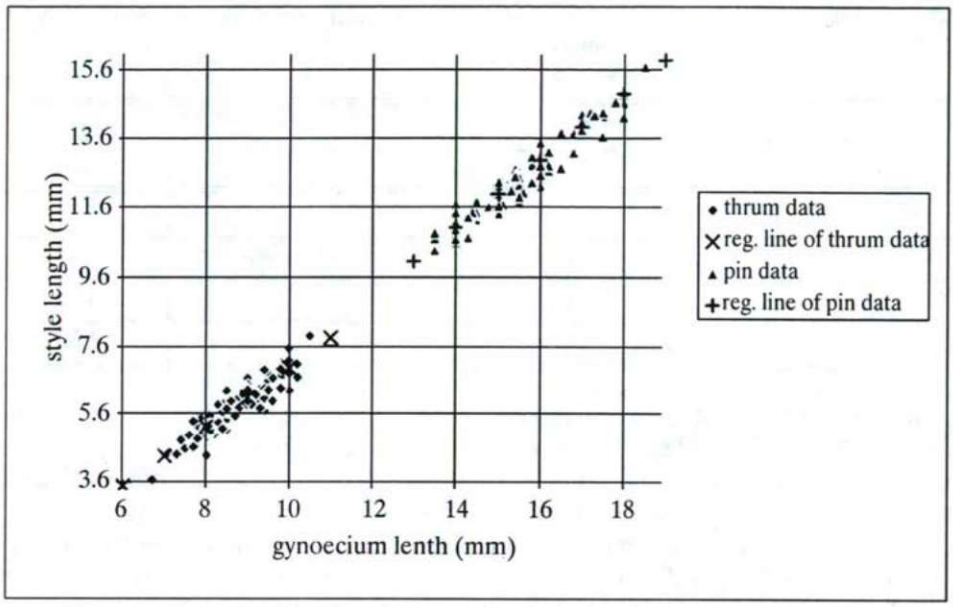

Figure 1. Gynoecium length as function of style lenght (reg. line = regression line)

There is no correlation between the stigma length and the ovary length at all, the correlation coefficients are practically zero, and the regression lines are almost parallel with the X-axis. But a slight connection can be revealed between the length and the width of the stigma and the ovary. In the case of ovary length and width the relation is stronger: the correlation coefficients are about 0.6 and the regression lines are quite steep.

Number and size of ovules

Mean, standard deviation and range of number and size of ovules are summarised in Table 5. The mean and the standard deviation of the number of ovules per ovary are equal in the two morphs, there is no significant difference between them. The frequency distribution diagrams of the morphs are also very similar, the peaks of the thrum and pin data are in the category of 60–70 pieces with 31% and 38% of all the cases, respectively. The number of ovules does not correlate with the ovary length at all, but it seems to be slightly connected with the ovary width (thrum correlation coefficient is 0.35 and pin one is 0.39).

Table 5. Mean, standard deviation and range of ovule number and ovule length
(St. dev. = standard deviation, N= number of cases)

	Number of ovules per ovary		Length of ovules (μm)	
	thrum	pin	thrum	pin
Mean	55	54	399	432
St. dev.	13	12	46	67
Range	56	75	271	365
N	120	117	525	479

In contrast to the number of ovules the length of them is significantly different in the two morphs at $P \leq 0.001$: the pin ovules are slightly larger than the thrum ones. The standard deviation and the range are also bigger in the pin morph than in the thrum one, similarly to most of the examined features. The frequency distribution diagrams also show the most general behaviour of the two morphs: the thrum data have a characteristic peak, while the pin diagram is much flatter (Fig. 2).

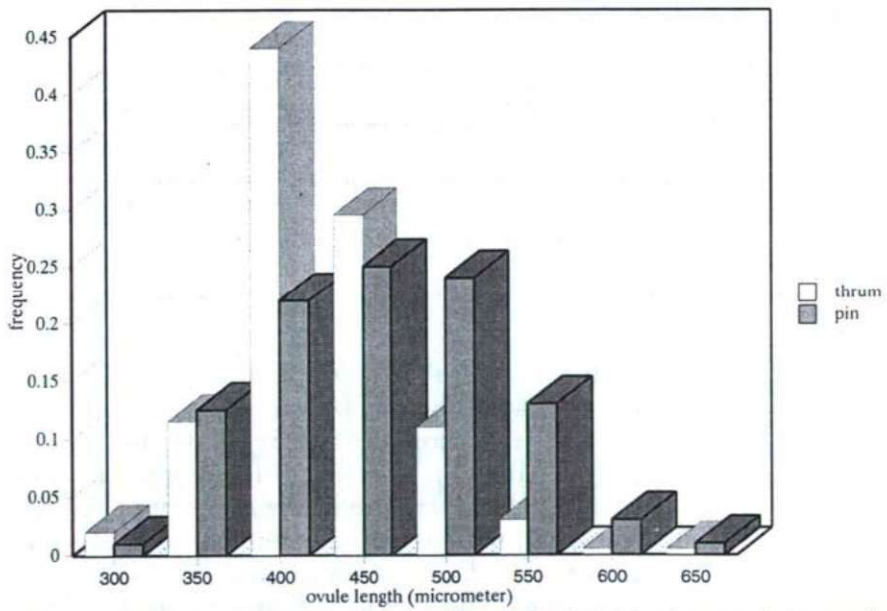

Figure 2. Frequency distribution diagram of ovule lenght in the thrum and pin morphs

Conclusions

The most characteristic manifestation of reciprocal herkogamy is the occurence of two types of gynoecium: thrum one with short style and pin one with long style. According to our results the gynoeciums can distinguish unambiguously in this respect and the pin:thrum ratio is 2:1.

Difference in the stigma size between the two morphs is reported in some *Primula* species and two types have been described: in some species the pin stigma is larger than the thrum one (HESLOP-HARRISON *et al.*, 1981), whereas in other species the ratio is opposite (PANDEY and TROUGHTON, 1974). Our results revealed that pin and thrum stigma is significantly different at $P \leq 0.001$ by both length and width confirming that the pin stigma is longer and wider than the thrum one.

The ovule size is a rarely examined feature of heterostylous species, so our observation that the largest length of the pin and thrum ovules is significantly different at $P \leq 0.001$ is a new result.

For style length, stigma size and ovule length where significant difference was found between the morphs the standard deviation and the range of values are larger in the pin morph than in the thrum one which means that variability of these pin traits is more considerable than that of thrum ones. For these features the frequency distribution diagrams also reveal characteristic differences between the morphs: in thrum diagrams there is a definite peak with a high frequency, while the pin diagrams are flat with several similar frequencies in the middle of the range.

Ovary length, ovary width and ovule number per ovary do not differ between the two morphs, their variability is low and the frequency distribution diagrams of the pin and thrum data are almost the same, so these characters seem to be enormously conservative.

Correlation studies show that the length of the gynoecium is strictly determined by the style length, but apart from this strong connection there is no correlation between the size of the gynoecium parts.

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