



The Location of Anomalous flowers on flower-bearing stems of *Alchemilla monticola* Opiz (Rosaceae)

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ABSTRACT

Alchemilla monticola form multiflorous thyrse with a large number of orders of branching. Anomalous flowers with an increased number of elements are usually formed on the lower orders of branching. 2- and 3-membered flowers are formed more frequently at higher orders of branching. This distribution may be associated with a gradual decrease in the morphogenetic activity and sizes of floral apex at higher orders of branching.

Keywords: Teratology, reproductive structures, *Alchemilla monticola*, Rosaceae.

INTRODUCTION

A comprehensive analysis of plants with regular apomixis (Khokhlov 1967, Glazunova 1977, 1984, Rubtsova 1989, Batygina 2000, Kashin 2006, Janick 2010 *et al.*) contributes to the development of reproductive biology. The diversity of anomalous flowers of apomictic-plants (Khokhlov *et al.* 1978) is a very interesting object for morphogenetic studies. A detailed analysis of the developing anomalous configurations may contribute considerably to our understanding of normal morphogenesis and the mechanisms of its regulation and evolution. There are many publications devoted to the evaluation of the frequency of occurrence of anomalous flowers and their classification (see Andreeva & Notov 2011, Notov & Andreeva 2013 a, b, 2014). Special studies of the distribution of anomalous flowers in the inflorescence are necessary. Interesting object for such studies are species of the complex *Alchemilla vulgaris* L. s. l. They are characterized by regular apomixis (Murbeck 1901, Glazunova 1984). Apomixis determines a significant variability of anomalous flowers (Notov & Andreeva

2007, 2013b, 2014, Andreeva 2010). Also interesting are the multiflorous flower-bearing stems of *Alchemilla*, which have a large number of orders of branching (Notov 1993, Notov & Glazunova 1994, Tikhomirov *et al.* 1995). It is worth analyzing the occurrence of anomalous flowers on different orders of branching (Notov & Andreeva 2007, 2013b, Andreeva 2010).

MATERIALS & METHODS

We have studied the frequency of occurrence and morphological diversity using *Alchemilla monticola* Opiz. as an example. This is one of the most common micro-species of the *Alchemilla vulgaris* L. s. l. complex. Materials were collected in 1998-1999 near the Burashevo village in the Kalinin district of the Tver region (Russia) (Notov & Andreeva 2007). We found no sources of chemical or radiation contamination in the nearby areas. The studied plants were growing on a meadow with a domination of *Alchemilla* species and grass. All the specimens were in the middle-aged generative state (g_2) and the average vitality.

From every specimen one flower-bearing stem, the lowest on the yearly shoot, was studied. The flower-bearing stems were softened using a steam bath and the flower structure was studied using a binocular magnifying glass MBS-10 with an 18 and 63 zoom. A detailed branching diagram with the locations of all anomalous flowers was created for each flower-bearing stem (Notov & Andreeva 2007, 2013b). Features of anomalous flower structure were noted on the pictures. All together 45 flower-bearing stems and 10217 flowers were studied.

The typical structure of the *Alchemilla vulgaris* flower has been described in a number of studies (Glazunova & Mjatlev 1990, Tikhomirov *et al.* 1995, Notov & Glazunova 1994, Notov & Andreeva 2007, 2013 b). In previous works we developed an approach for classifying anomalous flowers (Notov & Glazunova 1994, Notov & Andreeva 2007, 2013 a, b, 2014, Andreeva & Notov 2011).

RESULTS & DISCUSSION

Structure of the flower-bearing stems of *Alchemilla monticola*—Flower-bearing stems of *Alchemilla* are multiflorous frondulose closed thyrseae (Notov 1993, Notov & Glazunova 1994, Tikhomirov *et al.* 1995). Stems are elongated, foliated, with 8-10 (13) nodes to a terminal flower. Axis I and II orders have the terminal flowers (Fig. 1). Under the terminal flowers are contiguous two nodes. The leaves on these nodes are fused. They form a bowl-shaped structure and are in the form of a notched cuff.

Under the terminal flowers of axes I and II orders of flower-bearing stems usually develop two dichasium branches. These branches are characterized by differences in their branching style. Each branch order under the terminal flower also forms two branches (Fig. 1). The first branch is a compact monochasium. The second larger branch is dichasium (Notov 1993, Notov & Glazunova 1994, Tikhomirov *et al.* 1995). The total number of orders of branching varies from 8 to 14. In plants with high vitality sometimes you can see up to 20 orders of branching.

Morphological diversity of anomalous flowers—When classifying atypical flower patterns we identify six major groups (Notov & Glazunova 1994, Notov &

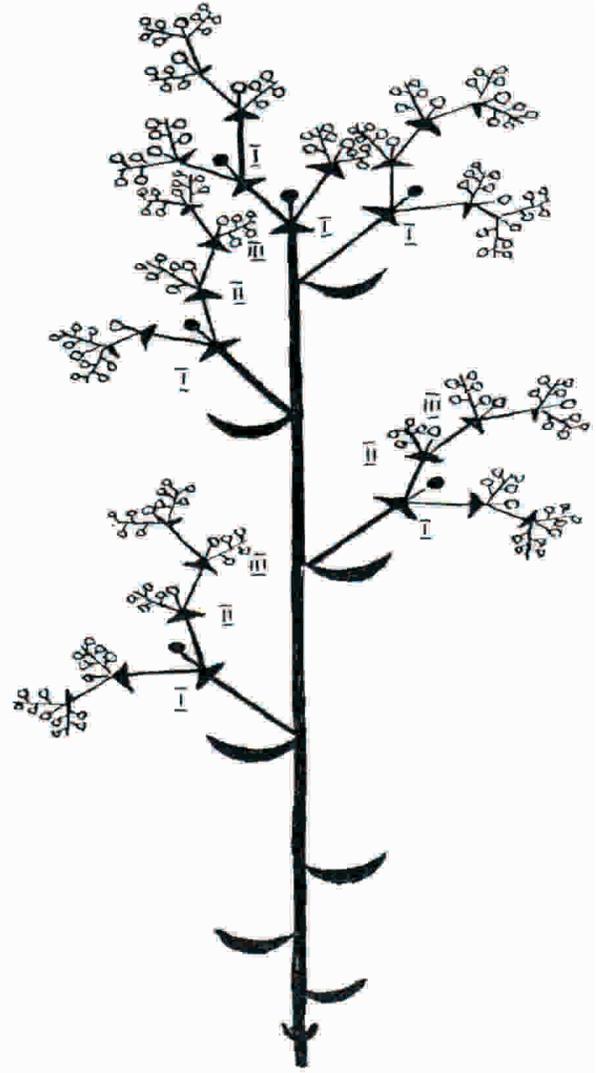


Fig. 1— Structure of *Alchemilla monticola* flower-bearing stem (scheme)

Andreeva 2007, 2013 a, b, 2014 Andreeva & Notov 2011). The characteristics and conventions used are given in earlier papers (Notov & Andreeva 2013 a, b, 2014). In some groups we applied additional symbols: 1) N – altered number of circles (Nk), elements of one (two) circles (Ne); combined changes to the number of elements (Nek); 2) S – bidental and tridental or bipartite and tripartite leaves of the epicalyx and calyx, stamens with a flat stamen filament; 3) T – these have been noted only in connection with other alterations; 4) Fe – concrescence (fusion) of elements of one circle or of different circles (stamens, carpels), as a rule combined with other alterations; 5) occurrence of a new element

(structure) – presence of a spathe-like structure on the hypanthium; 6) combination of two, three or four types.

Using the approach mentioned above we were able to connect each anomalous flower with one of the groups described. There are 7 groups of combined anomalies in *Alchemilla monticola*. They are: a) flowers with alterations in the number and structure of elements (NeS); b) flowers with altered numbers of elements and concrescence of elements (NeFe); c) flowers with altered structure and atypical location of elements (ST); d) flowers with altered structure and concrescence of elements (SFe); e) flowers with altered number of elements, circles and atypical element structure (NekS); f) flowers with altered number and structure of elements and concrescence of elements (NeSFe); g) flowers with alterations in the number, structure and location of elements (NeST) (see Notov & Andreeva 2014).

The frequency of anomalous flowers on different orders of branching—The high frequency of anomalies in *Alchemilla monticola* allowed to obtain a large sampling size of anomalous flowers with different orders of branching (Notov & Andreeva 2013b).

The frequency of the main types of anomalies at different orders of branching of the flower-bearing stems of *Alchemilla monticola* is shown in Table 1. Of the most interest are the Ne, S, NeS types. These variations of anomalies occur most frequently.

The frequency of anomalies in different orders of branching varies from 5.0 to 16.8 % (group Ne), 3.4 to 12.2% (group S) and from 2.5 to 7.1% (for the group NeS) (Table 1). The maximum frequency for these groups of anomalies was registered on 8th and 9th orders of branching.

The complex combined variations of anomalies are often confined to the lower orders of branching (Table 1). Anomalous flowers combining all of the selected types of changes were observed only on the third and fourth orders. Features of the structure of the flower-bearing stems (see Table 1) define a smaller sampling for first and second order branch.

Many observations on different species of *Alchemilla* confirm that the most unusual variations of anomalous flowers are usually found on the first or second orders of branching (Notov & Glazunova 1994,

Tikhomirov *et al.* 1995, Notov & Andreeva 2007, 2013b, Andreeva 2010). All this confirms a special morphogenetic status of basal areas of the dichasium-branches. At this level it is more likely a violation of typical hormonal gradients, which play a significant role in the differentiation of different types of shoots from monopodial-rosette plants (Andreeva & Notov 2011, Notov & Andreeva 2013b). An increase in the frequency of anomalies in high orders of branching occurs due to the increase in the share of three-membered flowers and more frequent changes in the structure of four-membered flowers at this level.

Table 2 shows the frequency flowers with a different number of elements of the perianth in different orders of branching. There are no two- and three-membered flowers on the first order branch. Their frequency on the second and third orders is very low. The frequency of two- and three-membered flowers increases with the increase in orders of branching. The maximum frequency of two- and three-membered flowers is found at the eleventh and twelfth orders (Table 1, Fig. 2).

5-membered flowers are often formed on the first-order branch. At higher orders their frequency decreases (Table 1, Fig. 2). Our observations indicate the confinement of six- and seven-membered flowers only to the first and second orders of branching. The smaller sample size for the flowers on the first order branch doesn't permit us to confirm this pattern statistically. If the size of the total sample is increased the revealed correlation will be seen even more clearly and accurately.

Data on the frequency of anomalous flowers in dichasium and monochasium is given in Table 3. There is a marked increase of the most common variations of anomalous flowers in *Alchemilla monticola* within the monochasium. It can be explained by the increase in the number of three-membered flowers at high orders of branching. Three-membered flowers were noted only in the monochasium.

The complex combined variations of anomalous flowers (NeSTFe) found only in dichasium. This can be explained by the localization of these variations only on the first and second orders of branching. In the first and second orders of branching there is only dichasium.

Table 1–The frequency of anomalous flower groups in different orders of branching of flower-bearing stems of *Alchemilla monticola* : * – number of anomalous flowers of this group in this order of branching; ** – frequency in % has been derived from the total number of anomalous flowers at this order of branching.

Groups	Order of branching												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Ne	2*	12	34	93	142	196	209	228	159	100	48	14	2
	5,0**	4,5	5,3	8,1	9,0	10,3	12,9	16,8	15,1	14,4	13,6	12,6	7,1
Nk	–	–	–	–	–	–	–	2	–	–	1	–	–
	–	–	–	–	–	–	–	0,2	–	–	0,3	–	–
Nek	–	–	1	–	–	2	–	1	1	–	–	–	–
	–	–	0,2	–	–	0,1	–	0,1	0,1	–	–	–	–
S	2	9	22	71	78	117	154	135	128	72	32	13	–
	5,0	3,4	3,4	6,2	4,9	6,2	9,5	9,9	12,2	10,4	9,1	11,7	–
T	–	–	–	–	–	–	–	–	–	–	–	–	–
Fe	–	–	–	–	–	–	–	–	–	–	–	–	–
NeS	1	7	18	34	66	88	96	80	75	32	13	15	1
	2,5	2,6	2,8	3,0	4,2	4,6	5,9	5,9	7,1	4,6	3,7	4,5	3,6
NekS	–	–	–	–	1	1	1	–	–	–	–	–	–
	–	–	–	–	0,1	0,1	0,1	–	–	–	–	–	–
NeFe	–	1	1	1	–	1	1	–	–	–	–	–	–
	–	0,4	0,2	0,1	–	0,1	0,1	–	–	–	–	–	–
ST	–	–	–	–	1	–	–	–	–	–	–	–	–
	–	–	–	–	0,06	–	–	–	–	–	–	–	–
SFe	–	1	4	2	4	8	4	9	4	3	–	1	–
	–	0,4	0,6	0,2	0,3	0,4	0,3	0,7	0,4	0,4	–	0,9	–
NeSFe	–	1	1	2	6	3	4	4	3	2	1	1	–
	–	0,4	0,2	0,2	0,4	0,2	0,3	0,3	0,3	0,3	0,3	0,9	–
NeST	–	–	–	–	–	–	–	–	1	–	–	–	–
	–	–	–	–	–	–	–	–	0,1	–	–	–	–
STFe	–	–	–	–	–	1	–	–	–	–	–	–	–
	–	–	–	–	–	0,1	–	–	–	–	–	–	–
NeTFe	–	–	–	–	–	–	–	–	–	1	–	–	–
	–	–	–	–	–	–	–	–	–	0,1	–	–	–
NeSTFe	–	–	1	1	–	–	–	–	–	–	–	–	–
	–	–	0,2	0,1	–	–	–	–	–	–	–	–	–
F	–	–	–	2	1	1	2	4	–	–	–	–	–
	–	–	–	0,2	0,1	0,1	0,1	0,3	–	–	–	–	–
Total	5	31	82	206	299	418	471	463	371	210	95	34	3
	12,5	11,5	12,7	18,0	18,9	22,0	29,1	34,1	35,3	30,3	27,0	30,6	10,7

Table 2—The frequency of anomalous flowers with different number of elements on different orders of branching of flower-bearing stem of *Alchemilla monticola* : * - number of anomalous flowers of this group in this order of branching; ** - frequency in % has been derived from the total number of flowers at this order of branching.

Order of branching	Number of elements			
	2-membered	3-membered	4-membered	5-membered
1	–	–	4*(10,0**)	1(2,5)
2	–	7 (2,6)	18 (6,7)	6 (2,2)
3	–	16 (2,5)	52 (8,1)	11 (1,7)
4	–	43 (3,8)	148 (12,9)	16 (1,4)
5	1 (0,1)	84 (5,3)	207 (13,1)	9 (0,6)
6	1 (0,1)	128 (6,7)	279 (14,7)	10 (0,5)
7	–	114 (7,1)	352 (21,8)	6 (0,4)
8	1 (0,1)	122 (9,0)	324 (23,8)	10 (0,7)
9	1 (0,1)	90 (8,6)	266 (25,3)	4 (0,4)
10	–	67 (9,7)	145 (20,9)	2 (0,3)
11	1 (0,3)	29 (8,2)	66 (18,8)	–
12	–	18 (16,2)	26 (23,4)	–
13	–	1 (8,6)	2 (7,1)	–

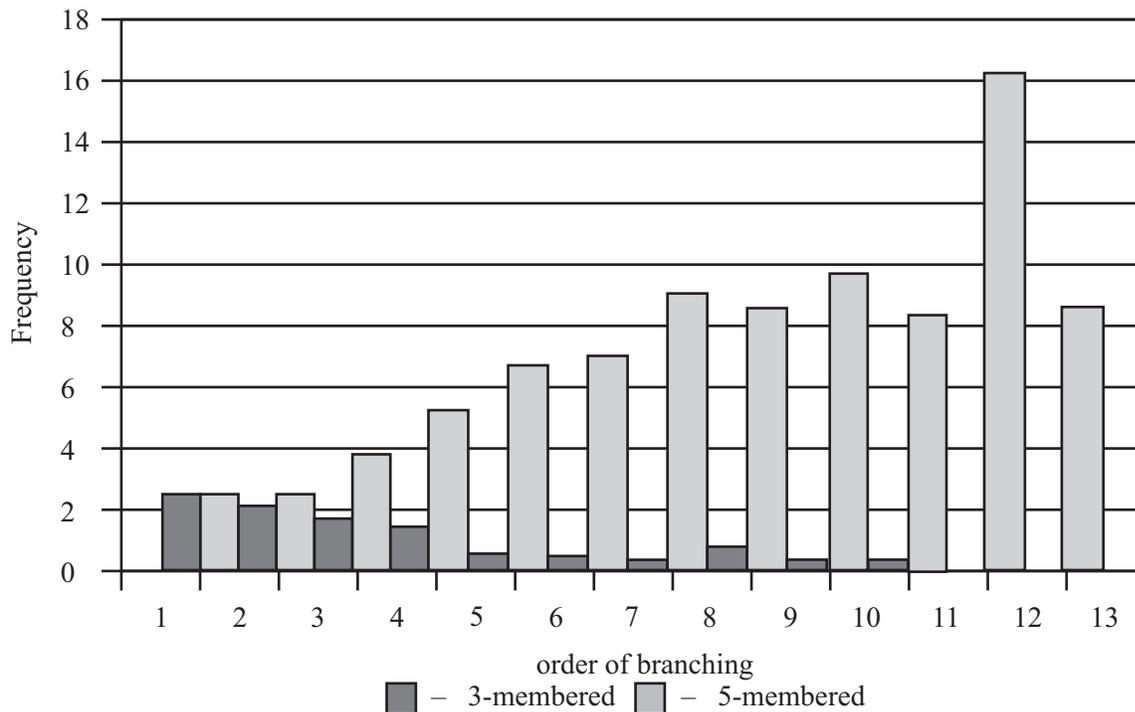


Fig. 2— The frequency of 3- and 5-membered flowers on different orders of branching of the *Alchemilla monticola* flower-bearing stem (%)

Table 3–The frequency of 3- and 5-membered flowers of *Alchemilla monticola* in dichasium and monochasium: frequency in % has been derived from the total number of anomalous flowers in the elements of inflorescences.

Groups	Frequency			
	number		in %	
	dichasium	monochasium	dichasium	monochasium
Ne	53	1186	4,0	12,5
Nk	–	3	–	0,03
NeS	1	4	0,1	0,04
S	38	797	2,9	8,4
T	–	–	–	–
Fe	–	–	–	–
NeS	22	494	1,7	5,2
NekS	1	2	0,1	0,02
NeFe	2	3	0,2	0,03
ST	1	–	0,1	–
SFe	2	38	0,2	0,4
NeSFe	1	27	0,1	0,3
NeTS	–	1	–	0,01
STFe	1	–	0,1	–
NeTFe	–	1	–	0,01
NeSTFe	2	–	0,2	–
F	–	10	–	0,1
2-membered	–	5	–	0,1
3-membered	15	704	1,1	7,4
4-membered	83	1806	6,3	19,1
5-membered	19	56	1,4	0,6
Total	124	2564	9,4	27,1

CONCLUSION

Thus, *Alchemilla monticola* form multiflorous thyrse with a large number of orders of branching. Anomalous flowers with an increased number of elements (5-membered) are usually formed on the lower orders of branching. 2- and 3-membered flowers are formed more frequently at higher orders of branching. This distribution may be associated with a gradual decrease in the morphogenetic activity and sizes of floral apex at higher orders of branching. The complex combined variants of anomalous flowers found only in the first and second orders of branching. At this level it is

more likely to be a violation of typical hormonal gradients, which plays a significant role in the differentiation of different types of shoots from monopodial-rosette plants.

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