Original article
UDC 582.57:581.331:502
doi: 10.17223/19988591/58/5

Andromonoecy and Floral Protandry of *Oenanthe aquatica* (Apiaceae)

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Summary. The paper studies the synflorescence structure and flowering biology of the biennial monocarpic wetland plant *Oenanthe aquatica*. This species has a set of adaptations to prevent self-pollination, including generation of a pseudanthium, andromonoecy, intra- and interfloral protandry, and a specific sequence of flowering within its umbels. The synflorescence of *O. aquatica* is a panicle of umbels. The size of perfect and male flowers consistently decreases as the order of synflorescence axes grows. The size-related differences between perfect and staminate flowers on axes of the same branching order consist only in the length of their calyx abaxial teeth, corolla diameter, width of abaxial petals, length and width of anthers, and length and height of stylopodium. We have identified two arrangements of staminate flowers in *O. aquatica*: male flowers are located at the periphery or in the center of the umbellets. The share of staminate flowers in umbels increases as an axis order grows. The female phase of flowers on axes of one order occurs simultaneously within entire synflorescences with the male phase of flowers on axes of the next order, which might mean geitonogamy. Perfect flowers live for five to seven days, while staminate flowers function for no more than one day.

The article contains 4 Figures, 2 Tables, 32 References.

Keywords: *Oenanthe aquatica*, synflorescence structure, andromonoecy, flowering sequence, protandry

Funding: The work is carried out in the framework of the State assignment of the Central Siberian Botanical Garden SB RAS № AAAA–A21–121011290026–9

For citation: Godin VN, Arkhipova TV, Vetlova MA, Kuranova NG. Andromonoecy and Floral Protandry of *Oenanthe aquatica* (Apiaceae). *Vestnik Tomskogo gosudarstvennogo universiteta. Biologiya = Tomsk State University Journal of Biology*. 2022;58:96-112. doi: 10.17223/19988591/58/5

Научная статья
УДК 582.57:581.331:502
doi: 10.17223/19988591/58/5

Андромоноэция и протандрия у *Oenanthe aquatica* (Apiaceae)

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Аннотация. Изучена структура синфлоресценции и биология цветения двухлетнего монокарпического водно-болотного растения *Oenanthe aquatica*. Этот вид обладает комплексом приспособлений для предотвращения самоопыления: образование псевданциев, андромоноэция, внутри- и межцветковая протандрия, а также специфическая последовательность цветения зонтиков синфлоресценции. Синфлоресценция *O. aquatica* представляет собой метелку из зонтиков. Размер обоеполых и тычиночных цветков последовательно уменьшается с увеличением порядка осей синфлоресценции. Размерные различия между обоеполыми и тычиночными цветками на оси одного и того же порядка ветвления проявляются только в длине абаксиальных зубцов чашечки, диаметре венчика, ширине абаксиальных лепестков, длине и ширине пыльников, длине и высоте стилоподия. Нами выявлено два варианта расположения тычиночных цветков у *O. aquatica*: по периферии зонтиков или в центре зонтиков. Доля тычиночных цветков в зонтиках увеличивается с увеличением порядка осей. Женская фаза цветения обоеполых цветков на осях одного порядка происходит одновременно с мужской фазой их цветения на осях следующего порядка, что не исключает гейтоногамии. Обоеполые цветки живут от пяти до семи дней, а тычиночные цветки функционируют не более одного дня.

Ключевые слова: *Oenanthe aquatica*; структура синфлоресценции; андромоноэция; последовательность цветения; протандрия

Для цитирования: Godin VN, Arkhipova TV, Vetlova MA, Kuranova NG. Andromonoecy and Floral Protandry of *Oenanthe aquatica* (Apiaceae) // Вестник Томского государственного университета. Биология. 2022. № 58. С. 96–112. doi: 10.17223/19988591/58/5

Introduction

The role of synflorescences in plant life consists of three main aspects: attracting pollinators, providing efficient pollination, and maintaining a breeding system [1–3]. A synflorescence is a compound inflorescence, composed of a terminal inflorescence and one or more lateral ones, or a group of inflorescences in a globose or subumbellate arrangement. In Apiaceae, the synflorescence typically consist of compact umbellets aggregated into the umbels. Many species of the Apioideae are self-compatible [4, 5], having the mechanisms to prevent or significantly decrease the risk of self-pollination, both within an individual flower and within a synflorescence. Such adaptations can be both structural (formation of pseudanthium, different types of flowers) and anthecological (dichogamy, a specific flowering sequence in a synflorescence) [3, 6–12].

In order to attract pollinators, umbellets and umbels in Apiaceae acquire the appearance of a pseudanthium. According to H. F. Froebe and G. Ulrich [13], Apioideae has three different ways of increasing in size of the petals of marginal umbel flowers. Thus, the corolla of the inner flowers is polysymmetric but the corolla of many outer ones becomes monosymmetric.

Synflorescences of many Apioideae include different types of flowers. The most common case is andromonoecy, the presence of perfect and staminate flowers in the same inflorescences [6, 7, 11, 14–16]. According to the surveys of T. Schmitz and R. Claßen-Bockhoff [17], there are at least four types of the arrangement for perfect and staminate flowers in Apioideae:

– The *Anthriscus sylvestris* type: perfect flowers occupy a marginal position in umbellets, where the terminal flower is absent.

– The *Chaerophyllum bulbosum* type is similar to the previous, but there is a terminal perfect flower in umbellets.

– The *Oenanthe silaifolia* type: perfect flowers are only in inner umbellets. In *Echinophora spinosa*-type, the perfect flower is the only terminal flower in the umbellets.

Intrafloral protandry in Apioideae has long been known [6, 18, 19]. In the protandrous flowers, the anthers mature and open earlier than when the stigmas are ready to receive pollen. With a pronounced intrafloral protandry, the transfer of pollen within a flower is possible, but perception of pollen grains by stigma is not. However, not all Apioideae show such pronounced protandry. In some species, the male and female phases within the umbellets and umbels are hardly separated, and within and among umbel orders only partly synchronized. Thus, phase overlaps occur [6, 12].

In many Apioideae, the protandry occurs not only in the individual flowers, but also in entire umbels. In this case, the female stage occurs only when the stamens withered (and often fall off) not only in individual flowers, but also in all flowers of this umbel [6, 18]. The autogamy in such species is completely excluded but the geitonogamy, i.e., the transfer of pollen within one plant, is possible. Geitonogamy can occur if the anthers and stigmas of the nearest flowers come into contact (with a weak protandry), or if the pollen from flowers of an umbel falls on the stigmas of another umbel [6].

The protandry in Apioideae can be pronounced not only in individual umbels, but also in entire synflorescences [3, 12, 18, 20]. It is achieved due to the exact synchronization of the flowering in the umbels within the axes of the same branching order. As much as the lateral branches are synchronized, multicycle dichogamy is transferred to the whole plant. The opening of flowers, the passage and change of the male and female stages is carried out in all umbels on the axes of the same branching order strictly coordinated and simultaneously. Thus, there is a synchronous flowering of umbels on axes of the same order in the entire synflorescence. As a result, each individual appears sequentially and several times (depending on the degree of branching of the axis system) either in the male or female phase. The bisexual individuals become functionally dioecious and their protandry is an effective means of ensuring strictly cross-pollination between plants in different flowering phases. This flowering sequence is called duodichogamy [21, 22]. The presence of perfect and staminate flowers in Apioideae, in combination with their protandry and a fairly clear distinction between flowering phases in umbels on axes of different branching orders have adaptive value. When staminate flowers open in umbels on axes of higher branching orders, their pollen cannot pollinate flowers in umbels on axes of the previous branching order since the female phase is already completed. Consequently, only xenogamy and outbreeding can be carried out due to the pollen of staminate flowers.

The object of our study is the fineleaf water dropwort (*Oenanthe aquatica* L.), a biennial or perennial, monocarpic or oligocarpic wetland plant with semi-rosette axes. This species has a European-West Asian distribution [23, 24]. *O. aquatica* forms either exclusively perfect flowers, perfect and staminate flowers, or rarely pistillate flowers [6, 20, 25]. Its perfect flowers are protandrous, and during the female phase of umbels on axes of the n-order coincides with the male stage of umbels on axes of the next order [6, 20]. The inconsistency of information about its sexual polymorphism and flowering biology determined the aim of our work to identify the anthecological traits of *Oenanthe aquatica* in the Moscow region. To achieve this goal, we were solving the following tasks:

1. To examine the sexual polymorphism in the synflorescences of fineleaf water dropwort.
2. To identify the structure of umbellets and umbels as well as the architecture of its synflorescence.
3. To study the pollination biology, the flowering sequence of umbellets and umbels in a synflorescence of this species.

Materials and methods

Plant materials. Our observations on the synflorescence architecture and flowering biology in *O. aquatica* have been carried out in 2018–21 in the Moscow region (near the village Pavlovskaya Sloboda, Krasnogorsky district, Russian Federation). The populations of *O. aquatica* was studied in three different sites: swampy meadow (site 1), black alder sparse wetland forest (site 2), floodplain meadow (site 3).

Diagrams of the umbels and the synflorescence in *O. aquatica* were drawn up. Within its main axis, three zones have been distinguished following W. Troll [26]: the zone of inhibition, the flowering zone, and terminal internode below the terminal umbel (Fig. 1).

Analysis of morphological traits. The materials for the morphology of perfect and staminate flowers in umbels on axes of different orders was carried out during the entire flowering period of *O. aquatica* from late July to early August 2021.

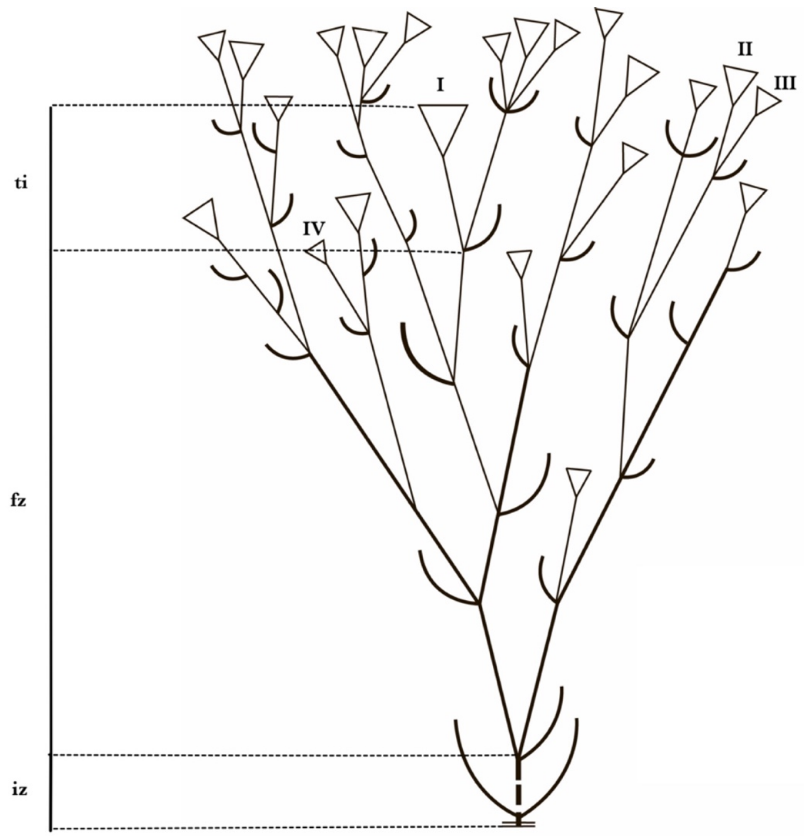

Fig. 1. Structural and functional zones of monocarpic axes in *O. aquatica*.
Symbols: iz – zone of inhibition, fz – flowering zone, ti – main inflorescence.
I – a terminal umbel, II – an umbel on axis of order II, III – an umbel on axis of order III,
IV – an umbel on axis of order IV

As perfect flowers are characterized by marked protandry (separation of the time of functioning of male and female generative structures within a single flower), it was necessary to collect and fix flowers from the same umbel in different phases of blossoming. For that, the first collection of flowers from any umbel was carried out during its male phase when the stamens function in the flowers. The second collection of flowers from the same umbel was carried out in the female phase when the stamens are already completely absent in the flowers and the female generative organs reach their definitive size. Flowers in different flowering phases of umbels were fixed in 70 % ethanol. From each umbel, 50 flowers were collected in different flowering phases. The same was done with flowers in umbels on axes of different orders (I–IV) within each plant. As a result, flowers from 20 plants in the population were included in our analysis.

In all three sites, from late July to early August in 2021, when the vast majority of flowers and umbels had faded on different individuals, we studied the ratio of plants with different degrees branching. For that, the flowers and umbels at least in 100 flowering plants have been examined along a transect of 1 m wide. counted. In each plant, the number of umbels on axes of II–IV branching orders was counted. In each site, 10 plants were randomly selected from the transect, differing in the degree branching, to study the parameters of umbels. Eight traits were estimated in each umbel: the umbel diameter, the length of all rays of the umbels, the number of umbellets in the umbels, the diameters of each umbellet, the lengths of pedicels of all flowers of umbellets, the total number of flowers in each umbellet, and the proportion of staminate flowers. Furthermore, the obtained data was averaged depending on the position of the umbels in synflorescence and the degree of branching of the axis system of plants to obtain a generalized characteristic of umbels and umbellets in *O. aquatica*.

The morphological traits of 100 perfect flowers in terminal umbels, 200 perfect flowers in umbels on axes of the second and third order and 300 staminate flowers in the umbels on axes of the second, third, and fourth orders of branching per 20 different individuals were analyzed. The following morphological traits of the *O. aquatica* flowers were measured: length and width of abaxial calyx teeth, length and width of adaxial calyx teeth, the corolla diameter, length and width of abaxial petals, length and width of transversal petals, length and width of adaxial petals, the stamen length, length and width of anthers, length and height of abaxial stylopodium, the ovary length, the style length, and the stigma diameter. The measurements were carried out using a Biomed MS-1 stereoscopic microscope with an eyepiece micrometer at a magnification of 20 or 40, depending on the value of the measured characters.

Morphology and fertility of pollen grains of perfect and staminate flowers in umbels on axes of different orders have been studied. For this, the same fixed material was used as in the study of the size of flowers separately for each type of flower and the position of the umbel in the synflorescence of a plant. Using a Biomed-5 microscope at a magnification of 16x10, the lengths of polar axis and equatorial diameter of pollen grains were measured. In total, we studied pollen of 100 perfect flowers in terminal umbels, 200 perfect flowers in umbels on axes of the second and third order of branching and 300 staminate flowers in umbels on axes of the second, third, and fourth orders of branching in 20 different individuals. Subsequently, the obtained data were averaged over flower types and their positions in umbels on axes of different orders. To determine the degree of pollen fertility, temporary preparations were stained with acetocarmine and the numbers of fertile and sterile pollen grains was counted in 20 fields of view at a magnification of 16x10.

Flowering biology. Flowering biology of *O. aquatica* has been examined while the mass blooming. Five plants of *O. aquatica* were labeled prior to their flowering in the budding phase of the flowers in the terminal umbel. The following algorithm was used to study the flowering sequence of both individual flowers and umbellets and umbels and the entire synflorescence. From the beginning of the opening of the first flowers, the flowering phase was noted every day, depending on their sexual status and the location in umbellets of the terminal umbel. The same was done to examine the flowering in umbels on axes of higher orders. In the perfect flowers, the developmental phases were noted from the buds to the postanthetic phase. In staminate flowers, the developmental phases were noted until the end of the male stage when stamens dry out and fall off. The duration of the staminate phase was determined from the moment of the opening of the anthers of the very first stamens to the appearance of dryed stamens. The readiness of stigma for receiving pollen was determined using Robinsohn's method with incubation in a weak solution of potassium permanganate [27]: immature stigmas are not stained by this reagent whereas the mature ones are stained brown.

Statistical analysis. All obtained morphometric data was processed by the variation statistics methods using parameters such as the mean (M) and its standard error (m) [28]. Comparison of the arithmetic means of perfect and staminate flowers was carried out using the Student's t-test. Analysis was carried out by Microsoft Excel for Mac (version 16.63.1).

Results

Flower morphology. Perfect flowers are actinomorphic (the inner flowers in umbellets) or zygomorphic (the outer flowers in umbellets), epigynous. The perianth and androecium are pentameric. Calyx teeth are lanceolate, finely pointed, retained and enlarged as the fruit ripens. The abaxial teeth are larger than the adaxial ones (Table 1). The petals are white, free, flat, obovate, deeply notched at the apex, and have an inwardly curved segment. The petals of the outer and part of the inner flowers in umbellets differ in size: there is an increase in the entire abaxial petals and the adjoining halves of two adjacent transversal petals.

Five stamens are alternating with the petals. Anthers with two pollen locules are attached dorsally to the filament, oblong, open longitudinally. The filaments thin, long, of the same length in all stamens.

Pollen grains are 3-tricolporate, mostly ellipsoidal, rounded-triangular in outline from the pole. The lengths of polar axes vary from 27.2 to 27.4 μm; their equatorial diameter varies from 14.3 to 14.6 μm. Pollen grains have a high fertility (94.1–95.6%).

Gynoecium is syncarpous, ovary is inferior, bilocular. Styles are two, free, sticking up. The stylopodia are conical, develop in the upper part of the carpels at the base of the styles and function as nectaries. Abaxial and adaxial stylopodia differ in size. The former is larger ($t > 4.158$, $p < 0.000$).

No differences between perfect and staminate flowers in the structure of perianth, androecium and pollen grains were found. In staminate flowers, the ovaries are completely absent and their stylopodia are much smaller than in perfect ones. However, the two sexual types of flowers differ in a number of parameters of the perianth, androecium, and gynoecium.

Differences in size and arrangement between perfect and staminate flowers. Perfect flowers are formed in umbels on axes of orders I–III, while only staminate flowers produce in umbels on axes of order IV. The sizes of perfect flowers parts depend on their position in the synflorescence (Table 1), however, the differences are not always significant. Instead, the size of pollen and their fertility do not depend on the location of perfect flowers on axes of different orders. However, the most significant changes in the size of perfect flowers are observed between umbels on axes of II and III orders.

Table 1

Morphological parameters of perfect (n = 300) and staminate (n = 300) flowers in umbels on axes of different order in *Oenanthe aquatic*

Signs	Flower	Terminal umbel	Umbels on axes II order	Umbels on axes III order	Umbels on axes IV order
Length of abaxial teeth of calyx, mm	p	1.1±0.08	0.9±0.08	0.8±0.02	–
	s	–	0.7±0.06	0.6±0.07	0.6±0.02
Width of abaxial teeth of calyx, mm	p	0.3±0.01	0.2±0.02	0.2±0.03	–
	s	–	0.2±0.01	0.2±0.01	0.1±0.01
Length of adaxial teeth of calyx, mm	p	0.6±0.02	0.6±0.02	0.5±0.05	–
	s	–	0.5±0.03	0.4±0.04	0.3±0.03
Width of adaxial teeth of calyx, mm	p	0.1±0.01	0.1±0.01	0.1±0.01	–
	s	–	0.1±0.01	0.1±0.01	0.1±0.01
Diameter of corolla, mm	p	3.5±0.11	3.4±0.15	2.9±0.12	–
	s	–	3.0±0.12	2.9±0.09	2.6±0.09
Length of abaxial petals, mm	p	1.7±0.13	1.6±0.08	1.5±0.03	–
	s	–	1.5±0.07	1.4±0.12	1.2±0.07
Width of abaxial petals, mm	p	1.6±0.15	1.4±0.07	1.4±0.04	–
	s	–	1.2±0.08	1.1±0.09	1.1±0.09
Length of transversal petals, mm	p	1.5±0.14	1.4±0.09	1.3±0.05	–
	s	–	1.3±0.08	1.2±0.10	1.1±0.03
Width of transversal petals, mm	p	1.4±0.06	1.4±0.12	1.2±0.06	–
	s	–	1.1±0.10	1.0±0.07	0.9±0.03
Length of adaxial petals, mm	p	1.2±0.08	1.1±0.03	1.0±0.06	–
	s	–	1.0±0.03	1.0±0.03	1.0±0.03
Width of adaxial petals, mm	p	1.2±0.05	0.9±0.04	0.9±0.03	–
	s	–	0.9±0.04	0.9±0.07	0.8±0.06
Stamen length, mm	p	1.7±0.06	1.5±0.10	1.2±0.10	–
	s	–	1.2±0.11	1.0±0.03	0.9±0.02
Anther length, mm	p	0.7±0.01	0.5±0.01	0.4±0.01	–
	s	–	0.4±0.01	0.4±0.01	0.4±0.01
Anther width, mm	p	0.4±0.01	0.4±0.01	0.4±0.01	–
	s	–	0.4±0.01	0.3±0.01	0.2±0.02
Length of polar axis of pollen grains, μm	p	27.2±0.16	27.3±0.12	27.1±0.16	–
	s	–	27.1±0.18	27.3±0.12	27.3±0.12
Equatorial diameter of pollen grains, μm	p	14.6±0.18	14.4±0.20	14.3±0.19	–
	s	–	14.5±0.19	14.6±0.18	14.7±0.16
Pollen fertility, %	p	95.6±2.4	94.9±1.9	94.1±2.2	–
	s	–	95.8±2.1	95.1±2.0	96.1±1.8
Abaxial stylopodium length, mm	p	0.5±0.01	0.5±0.02	0.4±0.02	–
	s	–	0.4±0.02	0.4±0.01	0.4±0.02
Abaxial stylopodium height, mm	p	0.8±0.03	0.8±0.02	0.6±0.02	–
	s	–	0.7±0.02	0.6±0.01	0.6±0.02
Ovary length, mm	p	1.1±0.02	1.1±0.05	0.5±0.01	–
Style length, mm	p	1.0±0.02	1.0±0.01	0.3±0.03	–
Stigma diameter, mm	p	0.2±0.01	0.2±0.01	0.1±0.01	–

Notes. The data are presented in the format M ± m, where M is the arithmetic mean of the feature, m is its error. Flower types: p – perfect, t – staminate flowers. The sign "– " means the absence of such flowers in umbels.

Staminate flowers are formed in umbels on axes of II–IV branching orders. The sizes of staminate flowers and their parts also depend on the position in the synflorescence (Table 1). However, the size differences of staminate flowers on axes of different orders differs from that considered in perfect flowers. Significant differences between staminate flowers on axes of II and III orders were not revealed for any of the studied traits. There is a decrease in the size of parts of flowers between umbels on axes of II and III branching orders. Staminate flowers on axes of III and IV orders differ significantly in width of adaxial teeth of the calyx, diameter of flower, width of transversal petals, length of stamens, as well as length and width of anthers. Other size parameters of staminate flowers also decrease with an increase in the order of axis branching, however, these differences are not statistically significant.

Our studies show that there are slight differences in the size of flowers and their parts between perfect and staminate flowers located in the same umbel. In umbels on the axes of II order, the following studied parameters of perfect flowers are significantly greater than those of staminate flowers: length of the calyx abaxial teeth, corolla diameter, width of abaxial petals, length and width of anthers, length and height of stylopodium (Table 1). On axes of the third order, the values of a small part of the morphological features of perfect flowers are statistically greater than the similar parameters of staminate flowers: the length of the abaxial and adaxial teeth of the calyx, the width of the abaxial petals, and the width of the anthers. The parameters of pollen grains and pollen fertility do not significantly differ in perfect and staminate flowers.

Synflorescence structure. All studied plant individuals were biennials with orthotropic semirosette monocarpic axes. In *O. aquatica* the flowering axis system is subdivided into three structural and functional zones (Fig. 1): 1) the zone of inhibition (axillary buds here do not generate axes, *sensu* W. Troll [26]) includes six to 10 internodes of the basal part of the axis; 2) flowering zone consists of three to six metamers, with vegetative-generative reiterated axes; 3) the main inflorescence.

The main axis ends with the main inflorescence, a terminal umbel. Lateral vegetative-generative axes of the second order develop in the axils of alternate leaves. They also have a zone of inhibition and end with umbels, thus, they are paracladia of the first order (*sensu* W. Troll [26]). In *O. aquatica* the terminal umbels are usually not dominant and over- topped by axes of higher order.

Two kinds of the synflorescence branching are clearly distinguished in *O. aquatica*. In some plants, the umbels are located on axes of I, II, and III branching orders. Paracladia of the first order in such plants are racemes of umbels. In more branched plants, umbels are formed on axes of I, II, III, and IV branching orders (Table 2). In this case, paracladia of the first order are represented by panicles of umbels. In both variants, the synflorescence can be classified as a panicle of umbels. Different environments affect, however, the incidence of plants with different degrees of synflorescence branching (Fig. 2). In the light sites 1 and 3, the plants with umbels on axes of I–IV branching orders predominate, whereas, the plants with a lesser branching (umbels only on axes of I–III orders) are more common under the forest canopy (site 2).

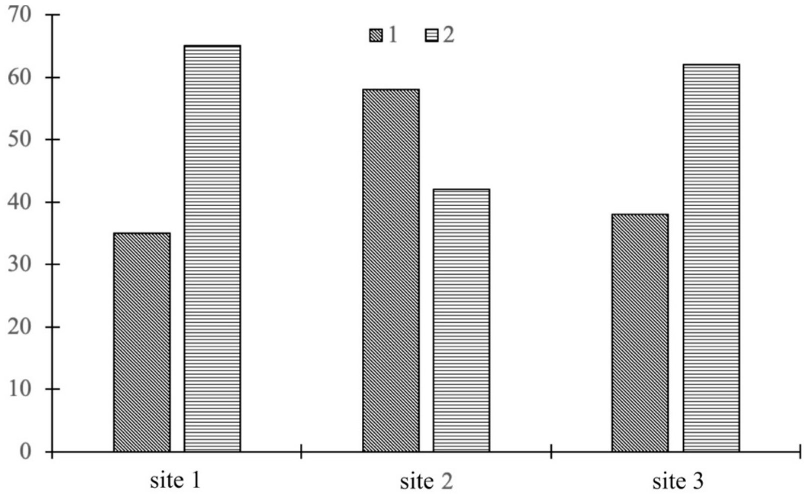

Fig. 2. Incidences of *Oenanthe aquatica* plants with different degree of branching of synflorescences in the plants from the studied sites.
Legend: The X axis, the studied sites of the population (site 1, 2, and 3); the Y axis, the proportion (in %) of plants with umbels on axes of different orders; 1 – plants with umbels on axes of I–III orders, 2 – plants with umbels on axes of I–IV orders.

Table 2

Quantitative parameters (n=20) of umbellets and umbels on axes of different orders in *Oenanthe aquatica*

Sign	Min–max	M±m
Plants with umbels on axes of I–III orders		
Terminal umbels		
Umbel diameter, cm	5.0–5.8	5.4±0.32
Length of rays of umbels, cm	1.3–2.3	1.8±0.21
Number of umbels, pcs.	1	1
Number of umbellets in umbels, pcs.	5–8	6.5±1.13
Diameter of umbellets, cm	1.3–1.5	1.4±0.04
Pedicel length, cm	0.1–0.6	0.34±0.06
Number of flowers in umbellets, pcs.	16–25	20.5±1.82
Portion of staminate flowers in umbellets, %	0	0
Umbels on axes II order		
Umbel diameter, cm	4.4–5.3	4.9±0.22
Length of rays of umbels, cm	1.3–2.5	1.8±1.13
Number of umbels, pcs.	4–9	6.6±0.52
Number of umbellets in umbels, pcs.	10–12	11.0±0.41
Diameter of umbellets, cm	0.7–1.3	1.0±0.12
Pedicel length, cm	0.1–0.4	0.28±0.05
Number of flowers in umbellets, pcs.	12–26	19.9±1.73
Portion of staminate flowers in umbellets, %	0–52.6	15.1±6.51
Umbels on axes III order		
Umbel diameter, cm	2.1–3.2	2.6±0.22
Length of the rays of umbels, cm	0.6–1.2	0.9±0.11
Number of umbels, pcs.	5–18	11.8±1.62
Number of umbellets in an umbel, pcs.	9–12	10.8±0.64

Sign	Min–max	M±m
Diameter of umbellets, cm	0.5–0.8	0.6±0.04
Pedicel length, cm	0.1–0.3	0.18±0.03
Number of flowers in umbellets, pcs.	8–23	14.5±1.71
Portion of staminate flowers in umbellets, %	100	100
Plants with umbels on axes of I–IV orders		
Terminal umbels		
Umbel diameter, cm	5.5–9.0	8.0±0.71
Length of the rays of umbels, cm	1.6–4.5	3.2±0.32
Number of umbels, pcs.	1	1
Number of umbellets in umbels, pcs.	4–8	6.3±0.72
Diameter of umbellets, cm	1.2–1.7	1.4±0.06
Pedicel length, cm	0.2–0.7	0.4±0.06
Number of flowers in umbellets, pcs.	13–35	23.0±2.61
Portion of staminate flowers in umbellets, %	0	0
Umbels on axes II order		
Umbel diameter, cm	7.0–10.0	8.9±0.41
Length of the rays of umbels, cm	1.8–4.3	3.2±0.22
Number of umbels, pcs.	6–10	8.1±0.51
Number of umbellets in an umbel, pcs.	12–16	14.2±0.62
Diameter of a umbellet, cm	0.8–1.8	1.4±0.11
Pedicel length, cm	0.1–0.5	0.3±0.04
Number of flowers in umbellets, pcs.	16–35	26.2±2.01
Portion of staminate flowers in umbellets, %	0.0–38.2	7.1±4.74
Umbels on axes III order		
Umbel diameter, cm	5.4–7.5	6.3±0.41
Length of the rays of umbels, cm	1.3–3.3	2.3±0.22
Number of umbels, pcs.	9–30	21.5±2.61
Number of umbellets in umbels, pcs.	12–18	14.5±0.82
Diameter of umbellets, cm	0.7–1.4	1.1±0.06
Pedicel length, cm	0.1–0.5	0.3±0.05
Number of flowers in umbellets, pcs.	16–33	24.3±1.82
Portion of staminate flowers in umbellets, %	0.0–100.0	71.5±12.13
Umbels on axes IV order		
Umbel diameter, cm	1.1–4.0	2.6±0.52
Length of the rays of umbels, cm	0.3–1.8	0.9±0.11
Number of umbels, pcs.	1–40	18.1±4.42
Number of umbellets in umbels, pcs.	4–14	9.8±1.71
Diameter of umbellets, cm	0.3–1.0	0.6±0.06
Pedicel length, cm	0.1–0.4	0.2±0.02
Number of flowers in umbellets, pcs.	2–23	12.7±2.03
Portion of staminate flowers in umbellets, %	100	100

Notes. min–max is minimum and maximum values of the feature, M is arithmetic mean value of the feature, m is its error.

Quantitative parameters of umbels change with an increase in the order of the axis on plants with umbels on axes of I–III branching orders (Table 2). Dimensional parameters from terminal umbels to umbels on axes of the III order generally decrease. Quantitative parameters behave differently. In the same row, there is a consistent increase in the number of staminate flowers, the proportion of which in umbels on axes of order III reaches 100%.

The quantitative parameters of umbels decrease with an increase in the order of axis on plants with umbels on axes of I–IV branching orders. Some exceptions are found only in the umbels on axes of the second order, which are not statistically different in size from terminal umbels. The most significant differences are observed between umbels on axes of II and III, III and IV branching orders, respectively. With increasing branch order the number of perfect flowers declines and the proportion of male flowers increases leading to completely male umbels in the highest branch order.

There are two positions of staminate flowers in umbellets (Fig. 3): male flowers are located at the periphery of the umbellets or they are located in the centre of the umbellets. Sometimes, umbellets with both arrangements of staminate flowers are found as part of one umbel.

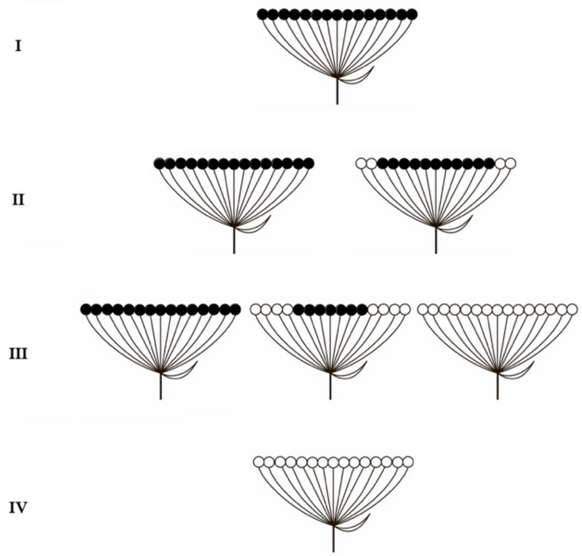

Fig. 3. Distribution of the floral sexual types within the umbellets located on the axes of different orders in the synflorescences of *O. aquatica*.
Symbols: umbellets on axes of I, II, III and IV orders. Black circles are perfect flowers, white circles are staminate flowers

Sequence of flower opening within the umbels. The perfect flowers of *O. aquatica* are characterized by a pronounced protandry, when the anthers mature and exhibit much earlier than the stigmas become receptive. In perfect flowers, the duration of the female phase is longer than the male phase. The female phase lasts from two to three days, while the male one is only one day. The perfect flowers function from five to seven days. The lifespan of the staminate flowers is one day (Fig. 4). In this case, the lifespan of a flower is determined by its position in an umbellet.

The opening of flowers within umbellets and umbels occurs in a certain sequence. Blossom begins with peripheral staminate flowers of marginal umbellets. The next day, peripheral staminate flowers open in almost all umbellets of umbel.

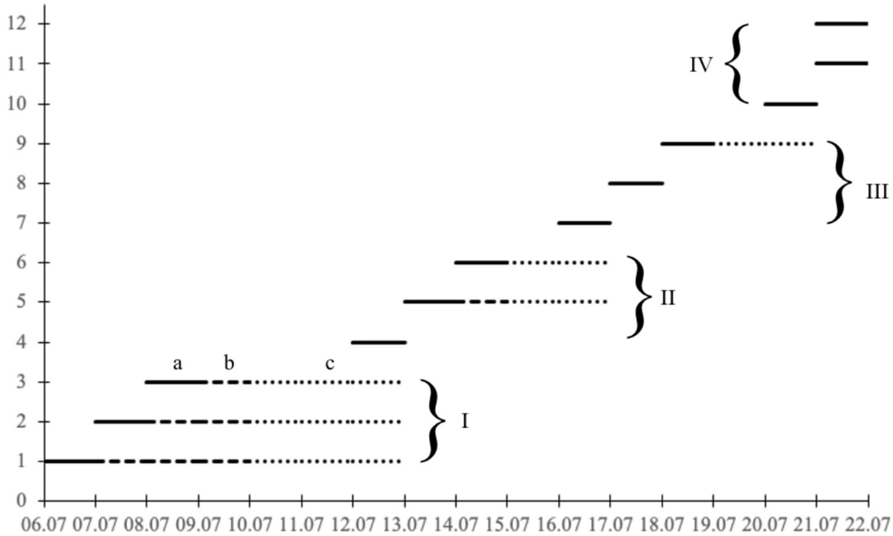

Fig. 4. Flowering time (in 2021) in umbellets and umbels on axes of different orders. Marginal perfect (1), median perfect (2) and central perfect flowers (3) of umbellets on the main axis (I), marginal staminate (4), median perfect (5) and central perfect (6) on axes II order, marginal staminate (7), median staminate (8) and central perfect (9) flowers on axes of order III, marginal (10), median (11) and central (12) staminate flowers on axes of order IV. I – a terminal umbel, II – an umbel on axis of order II, III – an umbel on axis of order III, IV – an umbel on axis of order IV. a – male phase, b – sterile phase, c – female phase

Thus, in marginal umbellets, the male stage as a whole usually takes about four days. At the beginning of flowering of the middle and central perfect flowers of the central umbellets, the perfect flowers of the peripheral umbellets are already in the sterile phase, when their androecium has completely finished to function. On the fifth day, the marginal and central perfect flowers shift simultaneously to the female phase. Similar processes take place in all umbellets of an umbel; flowering sequence is acropetal (starting from the proximal branches). Therefore, such an umbel is entirely in the female stage since the stigmas of the perfect flowers are already ripe to receive pollen. The lifespan of flowers and the sterile phase in perfect flowers shorten toward the center of the umbel. The female phase occurs synchronously not only in all perfect flowers in umbellets, but in all perfect flowers of an umbel (Fig. 4). Therefore, from now on, all flowers in the umbel are in the female phase and can capture pollen for pollination and seed setting.

The opening of flowers in umbellets and umbels on axes of higher orders occurs in a similar way (Fig. 4). The staminate phase in umbellets and umbels on axes of the second order coincides with the female phase in umbellets of the terminal umbel (Fig. 4). In turn, the female phase in umbellets and umbels on axes of the second order coincides with the male phase in umbellets and umbels on axes of the third order. Similarly, flowers bloom on axes of III and IV orders.

Discussion

Our studies discovered a set of adaptations in *O. aquatica* that can reduce the probability of self-pollination and promote cross-pollination. Such adaptations include the presence of two sexual types of flowers, intrafloral and interfloral protandry, and a specific sequence of flowering of umbels in synflorescence. Thus, these adaptations involve four different architectural levels, i.e. the flower, umbellet, umbel and umbel order.

We confirmed the information [6, 25] that this species, like many other Apiaceae, is characterized by the andromonoecy. We found that the perfect flowers in *O. aquatica* differ from staminate flowers in structural traits. In many Apiaceae, staminate flowers are located in the center of umbellets [6, 12]. That is why they are typically much smaller than marginal perfect flowers. However, in *O. aquatica*, the staminate flowers in umbels occupy the same position as the perfect flowers, marginal in umbellets. The spatial arrangement of staminate flowers determines the almost complete absence of their size differences from perfect flowers. Such spatial arrangement of perfect and staminate flowers is extremely rare among other Apiaceae [3, 6, 12]. According to a few surveys [3, 6], in the genus *Oenanthe* as well as in *Pleurospermum austriacum* male flowers are outers and perfect ones are inners. In many other andromonoecious Apiaceae, there is a size gradient [3, 6–8, 12] within umbellets from larger marginal to central staminate flowers, which is most likely due to a gradient in the availability of plastic substances. Marginal flowers in umbellets get more plastic substances than central flowers.

The presence of petals of various sizes in many flowers in umbellets is the manifestation for the zygomorphy of *O. aquatica* flowers. The zygomorphy of *O. aquatica* flowers is also displayed by spatial arrangement of five stamens and two carpels and the differences in the parameters of the adaxial and abaxial stylopodia. Many Apiaceae have zygomorphic flowers, which is not always associated with enlarged petals at marginal flowers in umbels, but precisely because of the spatial arrangement of parts of the androecium and gynoecium, through which only one plane of symmetry can be drawn [30, 31].

Five patterns floral protandry in temperate Apioideae ("*Libanotis intermedia*", "*Chaerophyllum prescottii*", "*Seseli ledebourii*", "*Laser trilobum*", and "*Peucedanum lubimenkoanum*") have been described by A.N. Ponomarev [18]. These five patterns differ in the flowering sequence of umbellets and umbels in the synflorescences. We showed that in *O. aquatica,* the female phase on axes of one order occurs simultaneously with the male phase of flowers on axes of the next order. These are features characteristic of the flowering model "*Chaerophyllum prescottii*" appear. Consequently, even within the entire synflorescence, there is a coincidence of the male and female stages on axes of different orders (Fig. 4), which should not exclude the transfer of pollen between these flowers and the existence of seed setting as a result of geitonogamous pollination.

The presence of staminate flowers in the synflorescence as part of umbels on axes of a high branching order is used to increase the probability of xenogamy [22, 32]. Typically, staminate flowers consist (in whole or in part) of umbels on axes of sufficiently high branching orders (sometimes starting from the second). This trait in Apiaceae, in combination with interfloral protandry and the differentiation of flowering phases in umbels on axes of different branching orders, acquires an adaptive value [3, 12, 22, 31]. When staminate flowers in umbels open along axes of higher orders, their pollen cannot pollinate flowers on axes of the previous order, so the female phase is already over. Therefore, only cross-pollination can be carried out due to the pollen. In *O. aquatica*, the synchrony of flowering of umbels on axes of the same order, in combination with intrafloral and interfloral protandry, is an advantageous adaptation.

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The article was submitted 25.03.2022;
approved after reviewing 31.07.2022; accepted for publication 03.10.2022.

Статья поступила в редакцию 25.03.2022;
одобрена после рецензирования 31.07.2022; принята к публикации 03.10.2022.