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Morphological characteristics of *Vaccinium ×intermedium* Ruthe

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Abstract: *Vaccinium ×intermedium* Ruthe, the hybrid of *V. myrtillus* and *V. vitis-idaea*, by contrast with the parental species is very rare plant and has a discontinuous geographical range in north-western Europe. This paper describes the morphology of *V. ×intermedium* from Polish populations occurring in part of Pomeranian Lakeland in comparison with both parent species. The results presented here confirm the suggestions of earlier research that *V. ×intermedium* displays intermediate characteristics of leaf and floral morphology concordant with hybrid status. Also, is discussed the problem of the isolating mechanisms between parent species and hybrid's ecology.

Additional key words: hybrid bilberry, hybridisation, morphology of leaves and flowers, taxonomical status, Poland

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Introduction

Vaccinium ×intermedium Ruthe is a natural interspecific hybrid between *Vaccinium myrtillus* L. and *Vaccinium vitis-idaea* L. discovered by J. R. Ruthe in North Germany in 1826. The discovery of several populations of the putative hybrid in Staffordshire, U.K in the latter half of the 19th century (Garner 1871) stimulated both interest in the plant and some controversy about its status. However, by the end of the 19th century it was generally regarded as an interspecific hybrid (*V. myrtillus* × *V. vitis-idaea*).

The results of experimental cross-breedings performed by Ritchie (1955b) showed that *V. ×intermedium* is a hybrid plant between *V. myrtillus* and *V. vitis-idaea*, with the first being a female plant. However, while reverse cross-breeding was not demonstrated, seedlings from the backcross *V. ×intermedium* × *V. vitis-idaea* were produced experimentally. The reproductive capacity of *V. ×intermedium* is significantly less than that of both parent species – it produces fewer flowers and the production of fruit is approximately one tenth of that of the parent species (Ritchie 1955b; Majewski and Majewski 1984). Ritchie (1955b) emphasizes that the number of flowers produced by the hybrid in certain area cannot be treated as representative sample, because the region of flower production is confined to older shoots near the centre of the origin of the clone. Investigations by Rousi (1967) suggest that the partial hybrid sterility in *V. ×intermedium* is the result of chromosomal irregularities at meiosis resulting in low pollen fertility, as observed by Ritchie (1955b). The other kind of barrier is low ability of seedling germination in dense undergrowth. Therefore, spread and durability of hybrid clones depends on vegetative reproduction and higher vigor than the parental species. However, developing the second generation of hybrid is unlikely.

Higher vegetative activity is an advantage for *V.* ×*intermedium* over parental species. The hybrid spreads faster than clones of *V. myrtillus* and *V. vitis-idaea* due to higher number of rhizomes (Majewski and Majewski 1986; personal observation). Research conducted in the area of England showed, besides higher vigor, higher tolerance for wider range of soil-moisture conditions (Ritchie 1955a).

By contrast with the parental species whose distribution in north-western Europe is continuous over large areas, *V.* ×*intermedium* has a discontinuous geographical range composed of localized sites. The biggest confirmed population is in England, especially in Staffordshire, with over 60 known sites (Cavalôt 1996). There are several sites in southern and central Sweden (Småland, Uppland, Dalarna) and in south-western Finland, mainly in Pori (information from the Herbaria at the Universities of Turku, Helsinki and Uppsala). In Germany sites were found in Saxony, Mecklenburg, Brandenburg and South Württemberg. The presence of the taxon was also confirmed in Denmark (Jutland), in Russia, near Kaliningrad (Ritchie 1955a; Avrorin 1958; Hansen 1972), and the Netherlands (Arts et. al. 1986).

In Poland confirmed sites were found in Forest Districts of: Trzebież, Trzcianka, Podanin, Żmigród, in Tuchola Forest National Park and in Drawsko Forest. The size of populations varies from several plants in Tuchola (Rutkowski 2000, personal communication) to 100 m^2 in Żmigród (Danielewicz and Maliński 2002). Reports of sites in Poland published in late 19th and early 20th century by German authors lack specific details and have not been confirmed. In "Flora von Ost- und Westpreussen" Abromeit (1898) reports the presence of the hybrid near Oliwa, Wejherowo and Człuchów. Hegi (1966) reports on the north-western part of Pomeranian Lakeland (Międzyzdroje, Świnoujście, Czarnków) and the western part of Wielkopolska Lowland (area of Czerwińsk, Nowogród Bobrzański, Szprotawa and Chojna). There were also sites of Lower Silesia, eg: Dobra near Bolesławiec, Małomice, Nowy Dwór, Osiecznica and Brzeźnica quoted (Fiek 1881; Schube 1903).

V. ×*intermedium* is restricted to forest plantations and heathland. It occurs rarely over 400 m above sea level. The majority of populations develop on the sides of forest roads and paths in half-shaded areas, and it is always accompanied by the parental species plants. The common feature of the habitat of the hybrid populations is the influence of anthropogenic factors. It is known that these factors were operative as early as 700–800 years ago, and on a wider scale in the middle ages. A consequence of this habitat disturbance has been to disrupt the natural community structure, producing more open conditions with reduced competition, probably conducive to the establishment and spread of the hybrid.

The aim of the present work is to confirm the hybrid status of individuals occurring in Trzcianka District. The previous investigation (Majewski and Majewski 1984) did not give detailed description and analysis of morphological characters. We will also discuss the problem of introgression in hybrid population.

Materials and methods

Populations of *V.* ×*intermedium* and the parent species were investigated at a site near Smolarnia in Trzcianka District, part of Pomeranian Lakeland (53°02'N 16°20'E). The hybrid is located in an area of direct human influence – in a young pine forest, near a forest road, enclosing an area of 50 m^2. The population of *V.* ×*intermedium* contains several single shoots of *V. myrtillus* and *V. vitis-idaea* and is clearly separated from the parental plants. It is possible that the population in the young pine forest is a younger part of a small clone (about 10 m^2), located on the edge of a pine forest (*Molinio-Pinetum*) about 50 m from the younger clone, and has been moved to the present location as a result of pine (*Pinus sylvestris* L.) cutting and forest clearing. The investigated parental populations are 50 m apart from the hybrid population and are situated in a pine forest (*Molinio-Pinetum*).

For a clonal species, all individuals arising from a single seed are collectively referred to as genet and each vegetatively produced individual of the genet that is actually or potentially independent is referred to as a ramet. In this study we use the term ramet for each raised shoot of the investigated species for a few reasons. Firstly, in closed populations, it is usually impossible to know whether one independent individual originated from sexual or asexual reproduction. Secondly, in populations of the investigated species, siblings are very rare (personal observation) so it is more suitable to assume each separated shoot as ramet.

The leaf material was collected on 10 October 1996. One leaf from the middle of the shoot of 118 ramets of *V. vitis-idea*, 101 ramets of *V. myrtillus* and 106 ramets of hybrid was taken. The leaves of the parental species were gathered from the location situated 50 m away from hybrid's population in order to include the whole range of variability of the leaf characters. Each leaf was analysed separately for 8 characters (Table 1) on dry material. Characters number 6 and 7 were recorded under a stereoscopic microscope at magnification of 4×.

The data were analysed statistically by Statistica PL for Windows software. The arithmetic means, standard deviations and coefficients of variation were calculated and analysed. A discriminant analysis was

Table 1. Leaves characters analysed

No.	Character	Precision
1	Blade length	1 mm
2	Blade width	1 mm
3	Position of the broadest part of leaf (distance from the base to the broadest part of leaf / trait 1 × 100 %)	
4	Basal angle of leaf	1°
5	Apical angle of leaf	1°
6	Number of veins on the half part of blade	
7	Number of teeth on the leaf edge of the half of blade	
8	Petiole length	0.5 mm

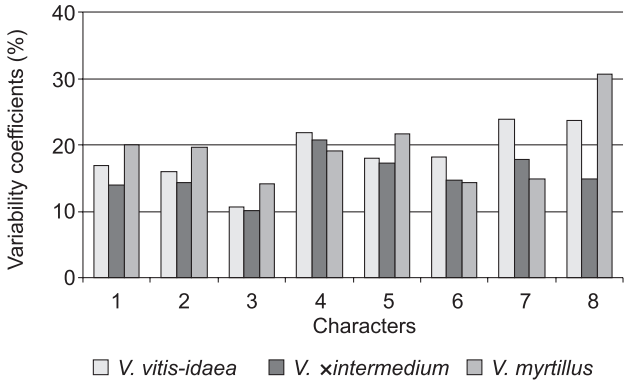

Fig. 1. Coefficients of variation of leaf characters in analysed populations of *V. myrtillus*, *V. intermedium* and *V. vitis-idaea*

performed and the position of the specimens was examined along the first two discriminant variables to find interspecific and intraspecific variation.

For the purpose of the morphological analysis of the generative and vegetative structures the fresh material was collected on 10 May 2000 in the above mentioned populations. The morphology of reproductive structures of the three taxa was made using a stereoscopic microscope. This analysis provided a qualitative description of morphological characters of the hybrid and the parental species.

Results

Statistical characteristics of the 8 analysed leaf characters are presented in Table 2. The population of *V. myrtillus* appeared to be more variable than the other species, with coefficient of variation higher for most characters (Fig. 1). The basal angle of leaf (character 4) and the apical angle of leaf (character 5) were the most variable characters in all populations. The petiole length (character 8) had the highest coefficient of variation for parental species by contrast with the hybrid where this character was the one of the lowest. The position of the broadest part (character 3) of leaf and number of veins on the half part of blade (character 6) had the lowest coefficient of variation for each of taxon.

Discriminant analysis indicated that character 6 (number of veins on the half part of blade) does not vary significantly between the species (Table 3). All 7 other characters discriminated between the species at the level of $p<0.01$. The number of teeth on the leaf edge of the half of blade (character 7), the apical angle of leaf (character 5), the blade width (character 2) had the highest F-ratios.

The distribution of particular populations of species in the space of the two first discriminant variables (U_1, U_2) confirmed the hybrid status of *V. ×intermedium* (Fig. 2). The discriminant function analysis indicated that all characters had an influence on differentiation of individuals. The Wilk's lambda components of par-

Table 2. Statistical description of the analysed 8 characters of *V. vitis-idaea*, *V. ×intermedium*, *V. myrtillus*

Calculated characteristics	Species	Characters							
		1	2	3	4	5	6	7	8
Mean	*V. vitis-idaea*	22.03	8.74	60.69	26.96	59.35	5.83	8.82	2.24
	V. ×intermedium	24.57	12.17	55.69	36.21	55.27	6.36	16.58	1.97
	V. myrtillus	23.05	14.17	39.32	66.89	44.73	6.82	30.46	1.86
Standard deviation	*V. vitis-idaea*	3.70	1.40	6.48	5.91	10.65	1.06	2.10	0.53
	V. ×intermedium	3.44	1.74	5.63	7.51	9.57	0.93	2.95	0.29
	V. myrtillus	4.63	2.77	5.56	12.78	9.65	0.98	4.52	0.57
Minimum	*V. vitis-idaea*	14	5	43	18	38	4	4	1
	V. ×intermedium	15	8	43	20	30	4	7	1
	V. myrtillus	13	7	21	35	15	4	20	1
Maximum	*V. vitis-idaea*	32	13	89	45	88	8	15	4
	V. ×intermedium	32	16	87	55	80	8	26	3
	V. myrtillus	36	21	52	90	75	10	41	3
Variability coefficient	*V. vitis-idaea*	16.80	15.99	10.67	21.91	17.94	18.18	23.82	23.59
	V. ×intermedium	14.01	14.32	10.11	20.74	17.31	14.59	17.80	14.78
	V. myrtillus	20.08	19.59	14.14	19.11	21.57	14.35	14.85	30.72

Table 3. Discriminant of power testing for leaves characters of *V. vitis-idaea*, *V.* ×*intermedium* and *V. myrtillus*

Character	F statistic	P value
1. Blade length	5.075	0.007
2. Blade width	33.768	0.000
3. Position of the broadest part of leaf	13.997	0.000
4. Basal angle of leaf	22.096	0.000
5. Apical angle of leaf	42.241	0.000
6. Number of veins on the part half of blade	0.092	0.913
7. Number of teeth on the leaf edge of the half of blade	105.813	0.000
8. Petiole length	12.434	0.000

ticular characters ranged from 0.5997 to 0.9994 with the lowest value for character 7. The first variable U_1 explained 97.6% of the whole variation of the samples. *V. myrtillus* showed the highest intrapopulation variation, whereas *V. vitis-idaea* and *V.* ×*intermedium* created more compact and close related groups with a few hybrid individuals occurring in *V. vitis-idaea* group.

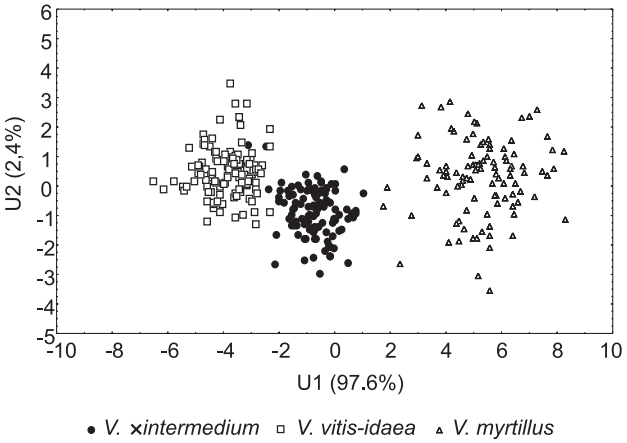

Fig. 2. Result of discriminant analysis based on 8 leaf characters for *V. intermedium*, *V. vitis-idaea* and *V. myrtillus* on the plane of the first two discriminant variables U_1, U_2

The morphological analysis is summed in Table 4. The differences in the morphology of these three taxa are shown in Fig. 3–5. All characters of *V.* ×*intermedium* revealed intermediate status or showed re-

Table 4. Comparison of morphological characters of *V. myrtillus*, *V.* ×*intermedium* and *V. vitis-idaea*

Characters	*V. myrtillus*	*V.* ×*intermedium*	*V. vitis-idaea*
Shoot:			
Form of branching	Sympodial	Sympodial	Monopodial
Stem pubescence	Glabrous	Puberulent	Pubescent
Hair shape	Absent	Hooked	Hooked
Stem shape	3-angled	Terete	Terete
Stem colour	Green	Green	Brownishred
Bud shape	Triangular, protruding	Triangular, protruding	Rounded, adhered
Leaf:			
Leaf colour	Light green, the same on top and below	Light green, with light gloss on top, more pale below	Dark green, with gloss on top, more light and matt-green below
Leaf duration	Annual	2–3 years	4–5 years
Blade edge	Flat	Weakly inrolled	Inrolled
Leaf underside	No gland	Few glands	Numerous glands
Flower:			
Blossom time	Earliest	Intermediate	Latest
Inflorescence	Axillary	Axillary and terminal	Terminal
Flower number	Single	2 (4)	6–10 (12)
Bracts	2	1	1
Bracteoles	Absent	2	2
Calyx teeth	Rounded, faint deeply cut	5 rounded, intermediate shape	4 acute, the biggest and the most deeply cut
Corolla shape	Globose, 5-not deeply cut	Urceolate, 5-not deeply cut	Campanulate, 4-deeply cut
Corolla colour	From white (greenish-white) to dark pink	Pale pink	White
Filaments	Golden-brown, glabrous	Golden-brown, puberulent	White, pubescent
Anthers	Awned, fused together edgeways	Awned half as long as V. myrtillus, not fused	Awned absent, not fused
Style	Included	Faintly exserted	Exserted
Ovary	5 chambers	5 or 6 chambers	4 chambers
Fruit:			
Berry colour	Blue-black	Reddish-purple	Red

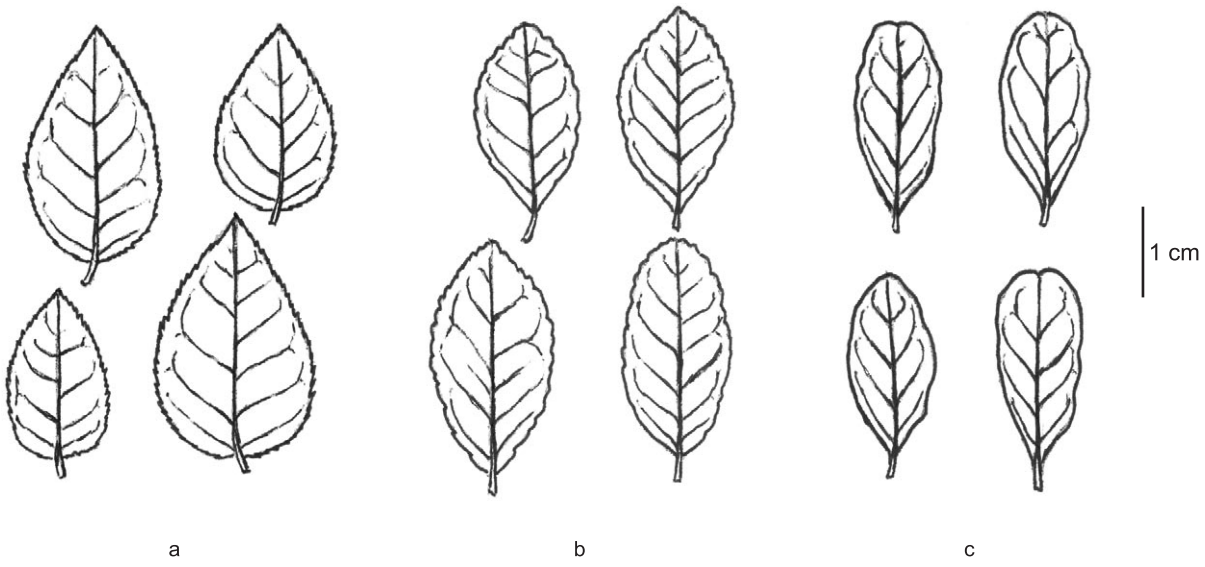

Fig. 3. Leaves shapes: a – *Vaccinium myrtillus*, b – *V.* ×*intermedium*, c – *V. vitis-idaea*

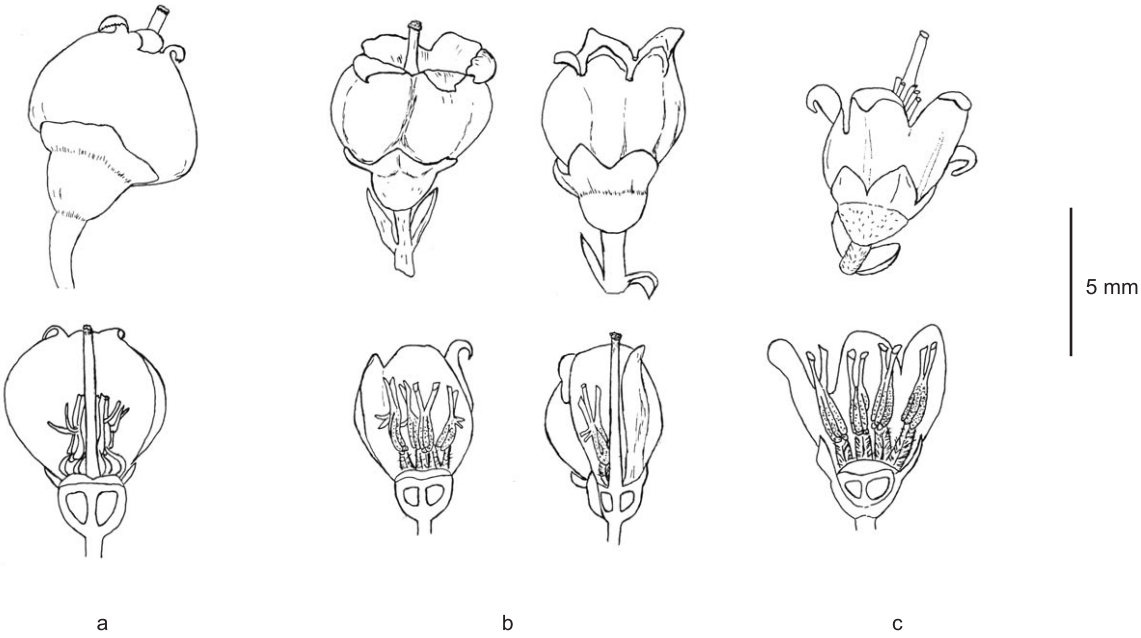

Fig. 4. Floral morphology: a – *Vaccinium myrtillus*, b – *V.* ×*intermedium*, c – *V. vitis-idaea*

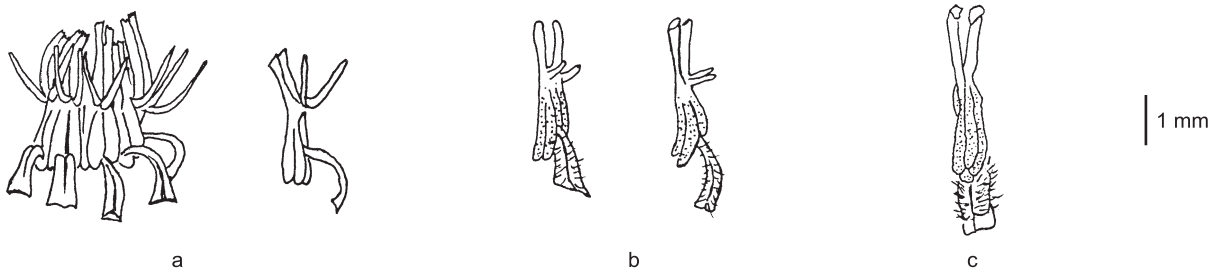

Fig. 5. Stamen morphology: a – *Vaccinium myrtillus*, b – *V. intermedium*, c – *V. vitis-idaea*

semblance to one of the parental species. However, much of vegetative characteristics of the hybrid were similar to those of *V. vitis-idaea*, which is corroborated by the results of discriminant analysis (Fig. 2).

Discussion

The results presented here confirm the suggestions of earlier research that *V. ×intermedium* displays intermediate characteristics of leaf and floral morphology concordant with hybrid status.

It seems necessary to continue research into both the morphological differences, especially within- and between-population variability, and the hybrid ecology. None of the previously published investigations provide an explanation of either the origin of the hybrid or the factors controlling its restricted distribution compared to that of the parents. Questions about the factors favouring hybridisations were first raised by Gourlay and Vevers (1919) while examining the plants of Cannock Chase in Staffordshire. A more detailed examination of the possible isolating mechanisms controlling hybridisation between these species, as well as consideration of the possible role of habitat disturbance was reported by Ritchie (1955a, b).

In the case of *Vaccinium*, with the exception of groups of polyploid taxa, mechanisms preventing hybridisation are not well understood. In closely related species, the isolating mechanisms are either prezygotic (ecological or fenological factors) or postzygotic (failure of survival of F_2 generation). Future research should focus on genetic aspects of hybridisation in European species of *Vaccinium*, as an extension of the work reported by Rousi (1967) who concludes (p. 355) from his cytological investigations that: "…although the chromosomes of *V. myrtillus* and *V. vitis-idaea* are capable in some cases, there are impediments to regular pairing" and he adds "…irregularities in pairing and even in complete pairing lead to the formation of pollen grains and embryo sacs with duplications and deficiencies". Irregularities in production of gametes considerably lower the probability of origin of F_2 generation of *V. ×intermedium*. Nevertheless fruits including full-developed seeds are produced, so it is possible to come to the conclusion, that the hybrid's sterility is not total. But it is not known, as was suggested by Ritchie (2003, personal communication), what percentage of fruits appear as a result of the self-fertilization, and what percentage as a result of the back-crossing with the parent species.

For the first time since the eighties of the 20th century, on September 2003 one fruit was found in the population located on the edge of a pine forest in Trzcianka District. The population bloomed very poorly that year, during repeated blooming on August there were observed only several shoots with single flowers, in contrast to the population in the young pine forest, which had most shoots with racemes consisted of up to four corollae. On September fruits of *V. myrtillus* fell in most cases or very sporadically stayed withered on shoots, however *V. vitis-idaea* had fruits in various stages of development and even flowers. In the authors opinion, in the light of observations of above mentioned the fruit, back-crossing seems very probable, considering that fruit occurred on the edge of poorly bloomed population, very close *V. vitis-idaea* shoots. But for the sake of a range of hybridisation, manner of reproduction, as well as longevity of *V. ×intermedium* and parent species, introgression in natural habitat is difficult to observe and was not described so far.

This investigation has provided both enhanced understanding of the taxonomic characteristics of the hybrid in relation to the parental species, and has opened avenues for future research into the evolutionary aspects of this example of plant hybridization.

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