

Studies on variation and evolution in *Centaureum erythraea* Rafn and *C. littorale* (D. Turner) Gilmour in the British Isles, 3. Breeding systems, floral biology and general discussion

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ABSTRACT

Studies on the distribution of *Centaureum erythraea* Rafn subsp. *erythraea* and *C. littorale* (D. Turner) Gilmour subsp. *littorale* show them to meet only in a few places on the coasts of the British Isles and northern Europe, and where they do meet ecological isolation is reinforced by slight differences in flowering time and the tendency of *C. erythraea* to inbreed. However, there are no totally effective reproductive barriers to gene exchange and hybridization may occur where the habitats of these two inter-fertile species are adjacent. The low fertility of the F_1 hybrid acts as a further isolating mechanism and hybridization does not normally extend beyond the F_1 generation.

Extensive hybridization has, however, occurred in areas of the sand dune systems on the Lancashire coast. In these areas man's interference with the natural vegetation has led to the breakdown of the naturally effective ecological isolation, causing the two species to grow together in mixed populations.

The difference in the pattern of hybridization in the areas concerned is shown to be partly due to a difference in the habitats available to the backcross hybrids, and partly to a difference in the fertility of the backcrosses.

In two areas backcrossing to *C. erythraea* has resulted in the increased variability of *C. erythraea*, while in other areas hybridization has resulted in the production of a new, allohexaploid species closely resembling the tetraploid *C. littorale* parent.

The nomenclature of all plants of hybrid origin is discussed and the name *Centaureum intermedium* (Wheldon) Druce is applied to the new, allohexaploid species.

INTRODUCTION

Morphological analysis of mixed populations of *C. erythraea* Rafn subsp. *erythraea* and *C. littorale* (D. Turner) Gilmour subsp. *littorale* from the coasts of S. Lancashire, v.c. 59, W. Lancashire, v.c. 60, Anglesey, v.c. 52 and Germany has shown that hybridization has taken place between the two species. In nine populations from the Lancashire coast backcrossing to *C. littorale* has taken place, while in another two backcrossing to *C. erythraea* has occurred. This is in contrast to the situation in the population from Anglesey and in two from Germany in which only F_1 -like hybrids were found (Ubsdell 1976a).

Studies of cytology and pollen fertility showed both species to be tetraploid ($2n = 40$) and highly fertile, while the F_1 is tetraploid and almost sterile. Backcrossing to *C. erythraea* resulted in the formation of cytologically stable, fertile tetraploid plants, while backcrossing to *C. littorale* gave rise to cytologically stable, fertile hexaploid ($2n = 60$) plants (Ubsdell 1976b).

This paper is concerned with the isolating mechanisms that normally keep these two species distinct in the wild. A detailed study of the breeding systems, floral biology and ecology of these plants was carried out in order to find out why these mechanisms have broken down in certain areas, and why the outcome of hybridization differs in the areas studied.

BREEDING SYSTEMS

CROSS- AND SELF-FERTILITY

The amount of seed set in the wild under natural conditions by a representative number of plants of *C. erythraea* and *C. littorale* was calculated for individual plants as the mean number of seeds present

TABLE 1. SEED SET BY SELF-, OPEN- AND NATURAL-POLLINATION OF *C. ERYTHRAEA* AND *C. LITTORALE*, AND SEED VIABILITY

Cultivation ¹ code	No. of plants used in experiments	Method of pollination	No. of successful pollinations/ total attempts	\bar{x} seed ² set per capsule by each method	% ³ seed set	% ⁴ germ- ination
I. <i>C. erythraea</i>						
R6	2	Open ^a	5/5	241	{	96 ^d
		Self ^b	2/2	225		98 ^f
		Natural ^c	9/9	258		80 ^g
R9	2	Open	5/5	245	{	79
		Self	3/3	196		98
		Natural	8/8	255		94
R34	2	Open	4/4	262	{	99
		Self	5/5	260		100+
		Natural	12/12	253		94
R35	3	Open	6/6	237	{	100+
		Self	2/2	245		90
		Natural	5/5	269		95
R36	3	Open	9/9	233	{	90
		Self	10/10	207		93
		Natural	12/12	261		93
R46	2	Open	13/13	257	{	84
		Self	5/5	216		95
		Natural	8/8	241		93
II. <i>C. littorale</i>						
R4	2	Open	4/4	175	{	48
		Self	4/4	83		66
		Natural	9/9	160		52
R5	3	Open	4/4	158	{	57
		Self	2/2	90		62
		Natural	6/6	198		10
R7	5	Open	6/6	210	{	53
		Self	10/10	105		60
		Natural	12/12	223		16
R19	4	Open	7/7	217	{	44
		Self	4/4	94		60
		Natural	10/10	223		10

1. Cultivation code and original locality:

C. erythraea

R6 Luccombe, Wight, v.c. 10

R9 Minsmere, E. Suffolk, v.c. 25

R34 Box Hill, Surrey, v.c. 17

R35 Sandown, Wight, v.c. 10

R36 Bude, E. Cornwall, v.c. 2

R46 Folkestone, E. Kent, v.c. 15

C. littorale

R4 Newborough, Anglesey, v.c. 52

R5 Holy Island, Cheviot, v.c. 68

R7 Ainsdale, S. Lancs., v.c. 59

R19 Ainsdale, S. Lancs., v.c. 59

2. Mean value for the number of seeds per capsule per plant set by each method—

(a) Open-pollination (b) Self-pollination (c) Natural-pollination.

3. (d) Mean seed set from self-pollination (b) expressed as a percentage of the mean seed set from open-pollination (a); and (e) Mean seed set from self-pollination (b) expressed as a percentage of the mean seed set from natural-pollination (c).

4. (f) Germination of self-pollinated seed (% seed sown/seed germinated) expressed as a percentage of the germination (% seed sown/seed germinated) of open-pollinated seed (a); (g) similar for self (b) and natural (c) seed.

per capsule. Using the same method the amount of seed set by open pollination in the greenhouse was determined.

For the study of self-fertility, individual flowers were enclosed in bags before the stigma and anthers were mature and left for some time for seed to set by self-pollination. Self-fertility for individual plants was assessed by expressing the average seed set by a number of selfed capsules per plant as a percentage of the maximum possible — as calculated from open and naturally-pollinated capsules.

Viability of the seed set by selfing was estimated by comparing the percentage germination of selfed seeds with the percentage germination of both open- and naturally-pollinated seeds.

RESULTS

Plants of *C. erythraea* were found to be highly self-fertile with 77-100% seed set (Table 1) while plants of *C. littorale* were much less self-fertile with 42-53% seed set. All seed set by *C. erythraea* showed a high percentage germination (Table 1) while seed set by *C. littorale* as a result of self-pollination showed a lower percentage germination than seed set by open- and natural-pollination of this species.

FLORAL BIOLOGY AND POLLINATION MECHANISMS

It was thought that a study of the relative positions of stamens and stigma and their different rates of maturation might give useful information on the pollination mechanisms involved.

C. ERYTHRAEA

In this species the anthers and stigma are always to be found well above the corolla-tube but enclosed within the corolla-lobes. In the young buds the anthers start at a level below that of the stigma but then the filaments grow faster than the stigma until the anthers are either on a level with or just above the receptive part of the stigma in the mature flower.

The stigma becomes mature a day or two before the anthers dehisce and in a few flowers the style and stigma were observed bending to one side of the newly-opened corolla-lobes while the unripe anthers were bending to the opposite side. After one or two days the anthers dehisce allowing pollen to fall down on to the stigma and self-pollination may occur if cross-pollination has not already taken place. In the flowers in which the stigma was observed bending to one side of the flower and the anthers to the opposite side, the style and filaments later moved back towards each other in the centre of the flower allowing pollen to fall on to the stigma. In many flowers the filaments were later seen to actually bend down so the anthers could touch against the stigma and ensure self-pollination.

In dull weather the pale pink flowers of this species readily close, so that although the pollination mechanisms of this species allows some cross-pollination to take place, self-pollination is ensured should the former not occur.

C. LITTORALE

In the early development of the buds of this species the immature stigma is often seen to protrude beyond the corolla-lobes, but after a few days it becomes enclosed within the corolla-lobes so that in the mature flower both stigma and stamens are to be found above the corolla-tube but within the corolla-lobes. In some of the flowers of this species that were examined the anthers were found to be just above the receptive part of the stigma, although the majority had the anthers either just below or well below it.

The stigma becomes mature a day or two before the anthers dehisce and in the majority of flowers examined the ripe stigma appeared to bend towards one side of the open flower, while the unripe anthers were found bending towards the opposite side of the flower. After a few days the anthers dehisce and then the style and filaments move back towards the centre of the flower until the anthers and stigma come closer together. In those flowers in which the anthers and stigma can come into contact self-pollination can then take place, but in many flowers this is mechanically impossible because the stigma is well above the level of the anthers and so pollen cannot reach the stigma.

The flowers of this species are larger and brighter in colour (deep mauve) than those of *C. erythraea* and once open never close up again even in poor weather. It seems that cross-pollination is most likely to occur in this species, but if it fails self-pollination can take place in those flowers in which stigma and anthers can come into contact with each other.

HEXAPLOID PLANTS

The flowers of the hexaploid plants resemble those of *C. littorale* and the anthers were found to be either just above or just below the receptive part of the stigma. In nearly all flowers examined the filaments were seen to bend towards one side of the open flower while the stigma was held by the style towards the opposite side of the flower. Only after they had been apart for some time, presumably to allow cross-pollination to occur, did the stigma and anthers move back towards each other in the centre of the flower. In those flowers in which stigma and anthers can come into contact, self-pollination can take place.

POLLINATORS AND FLOWERING TIMES

FIELD OBSERVATIONS ON POLLINATING INSECTS

The flowers of *C. erythraea* are pink and fairly conspicuous while those of *C. littorale* are larger, deep mauve and even more conspicuous. Although the corolla forms a tube the stigma and anthers are exserted and thus open to pollination by many insects. Some field observations on insects active near *C. erythraea* and *C. littorale* were carried out to see which insects could be transferring pollen between these two species.

Butterflies (Lepidoptera), grasshoppers (Orthoptera), bees (Hymenoptera-Apidae), hoverflies (Diptera-Syrphidae) and other Diptera were observed but none was seen to visit either of the *Centaureum* species. Large patches of *Lotus corniculatus* and *Trifolium repens* were present in many of the habitats occupied by *Centaureum* and the bees were concentrating on those two species to the exclusion of all others. The only insects seen to visit flowers of *Centaureum* were small, black thrips (Thysanoptera), which were observed crawling all over the plants and appeared to move at random between plants of *Centaureum* and also over most of the other species in the area.

There is little recorded information about pollinators for *Centaureum* but Knuth (1909) gave a list of insects seen by Müller to visit flowers of *C. erythraea*. These included small flies of the families Empididae and Syrphidae (Diptera). Both families commonly visit open, unspecialized flowers, visiting nectarless ones for their pollen. Proctor & Yeo (1973), however, reported that flies of these two families often fail to effect pollination because they do not always touch against the stigma and anthers. Müller (1883) was of the opinion that the spiral twisting of the anthers of *Centaureum* is probably an adaptation to ensure their being touched by the thin proboscis of butterflies and moths (Lepidoptera). There is, however, some doubt as to whether they always effect pollination as Müller (1883) stated that *Lotus corniculatus* is also visited by Lepidoptera, which obtain nectar by boring at tissue at the base of the flowers and without effecting pollination.

A lot more observation is needed but it is possible that both cross- and self-pollination in these species of *Centaureum* could be carried out by casual visits from members of the Thysanoptera. Pollinators probably do not discriminate between *C. erythraea* and *C. littorale* on the basis of the attractiveness of the flowers and could visit flowers of both species at random. Thus, if the two species grow together in mixed populations there is no barrier to prevent cross-pollination between the two species from taking place.

FLOWERING TIMES

C. littorale starts to flower in the field about the middle of June and completes its main flowering period by the middle of July. *C. erythraea* starts to flower at the beginning of July and continues into August. There is, therefore, an overlap of two or more weeks in the flowering periods of the two species when cross-pollination could take place.

The stigmas of *C. littorale* become receptive in late June before the anthers of this species or those of *C. erythraea* have dehisced. The anthers of *C. littorale* soon dehisce but, at the time when most of the stigmas of this species are receptive, the only pollen available is its own.

The stigmas of *C. erythraea* become receptive at the beginning of July before the anthers have dehisced. So for a short period only pollen of *C. littorale* is available, and it is quite feasible for insects to transfer pollen from *C. littorale* to the stigmas of *C. erythraea* before most of its own pollen is ready. However, if *C. erythraea* is not fertilized by pollen from *C. littorale*, self-pollination will take place when its own pollen is ready by the flowers closing and the anthers and stigma coming into contact. Transfer of pollen from *C. erythraea* to the stigmas of *C. littorale* is unlikely to occur because of the different

times of maturation. As already stated, insects probably do not discriminate between flowers of the two species and probably visit flowers of both at random. Transfer of pollen in early July could result in the pollination of *C. erythraea* by *C. littorale*, but transfer of pollen in early June can only result in the pollination of *C. littorale* by its own pollen.

Hybrid plants come into flower after *C. littorale* and at about the same time as *C. erythraea*. The hybrid stigmas are, therefore, receptive at the same time as are the stigmas of *C. erythraea*, when for a short time the only available pollen is that of *C. littorale*. Thus, pollen could easily be transferred by non-discriminating insects from *C. littorale* to the stigmas of the hybrid plants while their own pollen is immature. By the time the hybrid pollen is shed the stigmas of *C. littorale* will have been fertilized by their own pollen, and many of the flowers of *C. erythraea* will have closed for self-pollination. This may explain why backcrossing to *C. littorale* seems to have taken place most commonly, although some backcrossing to *C. erythraea* has also occurred. This could have happened by transfer of pollen in early or mid July before the flowers of *C. erythraea* close for self-pollination. However, the amount of backcrossing also depends upon the abundance of the backcross parent and upon the habitats available in the different areas; the importance of the latter in determining the presence of the various backcross types is stressed later.

GENERAL DISCUSSION

The relative importance of the various isolating mechanisms which normally keep *C. erythraea* and *C. littorale* distinct in the wild can now be considered. Artificial crosses between the two species have shown that there are no internal, reproductive barriers to gene exchange (Ubsdell 1976b). Reciprocal crosses gave a high seed set and high percentage germination, producing vigorous but almost sterile F_1 hybrids.

Distribution maps of *C. erythraea* and *C. littorale* indicate that the two species grow apart over much of their range but do meet in a few places on the coasts of the British Isles, France, Belgium, Holland, Denmark, Germany and Sweden. However, recent reports and field studies show that they actually meet in fewer places than this overlap suggests, and this is partly due to the destruction of many of their coastal habitats by man. They are known to occur together in the British Isles at Harlech and Morfa Dyffryn, Merioneth, v.c. 48, at Newborough Warren, Anglesey, v.c. 52, at Hightown, Freshfield, Ainsdale and Hesketh, S. Lancs., v.c. 59, at St Annes, W. Lancs., v.c. 60 and on parts of the coasts of Belgium, Denmark, Schleswig-Holstein in northern Germany, southern Sweden and the Swedish islands of Gotland and Öland.

Where they meet they are generally effectively isolated by different habitat preferences, *C. littorale* being found in more halophytic habitats such as young, wet dune-slacks and the more saline communities of salt-marsh sea meadow, while *C. erythraea* is found in old, dry dune-slacks and occasionally in the least saline of the salt-marsh sea meadow communities.

The results obtained from experiments on seed set by self- and open-pollination, and from observations on the floral biology and pollination mechanisms, indicate that *C. erythraea* is highly self-fertile and more likely to be inbreeding than *C. littorale*, which is less self-fertile and largely outbreeding. It is possible that the high degree of self-pollination shown by *C. erythraea* may act as a weak, external reproductive barrier reducing the amount of gene exchange between the two species if they come into contact.

There is also a difference in flowering periods, with *C. littorale* coming into flower at least two weeks before *C. erythraea*, and this would also help to reduce the amount of gene exchange, but there is an overlap of at least two weeks in which transfer of pollen, chiefly from *C. littorale* to the stigmas of *C. erythraea* by non-discriminating insects, could take place.

Thus, there are no totally effective reproductive barriers to gene exchange and so hybridization may take place where these two inter-fertile species come into contact. This has occurred at all the British localities listed above and at St Peter and Falshoft on the coast of Schleswig-Holstein.

The dune system at Newborough Warren has been little disturbed by man and although both species grow in the area they are mostly effectively isolated by the natural, isolating mechanisms described above. However, a few F_1 -like plants were found where the habitats of the two were adjacent and where they were growing in close proximity. Hybrids have also been reported (Benoit & Richards 1963)

from Merioneth but the area concerned has not been visited and so no information is available on the ecological situation.

In direct contrast, the dune systems on the Lancashire coast have been subject to much disturbance by man. At Hightown and Ainsdale urban developments have destroyed the habitats favoured by *C. erythraea* and it is now found in areas more suited to *C. littorale*, while at St Annes the situation has been reversed and habitats favoured by *C. littorale* have been destroyed and it is now found in those more suited to *C. erythraea*. In all these areas and at Freshfield, which was subject to disturbance prior to being made into a Nature Reserve in 1965, the two species grow intermingled in the same dune-slacks and extensive hybridization has taken place.

Hybrid plants have also been found in disturbed places on the coast of Schleswig-Holstein, but no ecological information is available on the situation in France, Belgium, Holland, Denmark and Sweden.

The availability of suitable habitats is one of the main factors governing the establishment of hybrids. The areas of sea meadow in Schleswig-Holstein are frequently denuded of vegetation when the turf is cut to provide material for the banks protecting the cultivated land from the sea, and such open habitats are ideal for the establishment of F_1 hybrids; as are the extensive areas of open ground found in the dune systems at Newborough Warren, Anglesey and on the Lancashire coast. These areas of open ground are particularly extensive around Hightown and Ainsdale because of constant trampling by weekend-trippers and holiday-makers.

Dune-slack habitats are also highly variable and Ranwell (1959, 1960) has demonstrated the gradient environments in dune systems. Such areas will contain many habitats intermediate between the young, wet dune-slacks favoured by *C. littorale* and the old, dry dune-slacks preferred by *C. erythraea*, in which F_1 hybrids can become established, while habitats most closely resembling those favoured by one or other of the two species will be suitable for the respective backcross hybrids. Stebbins & Anderson (1954) have pointed out the significance of habitat gradients for the survival of hybrids, and Bradshaw (1958) has shown that gradient environments in the dune system at Newborough Warren favour the development of hybrids between *Agrostis stolonifera* (a plant of wet slacks) and *A. tenuis* (a plant of drier slacks).

The relationship between the types of hybrids present in an area and the types of habitats available is clearly shown on the Lancashire coast, with backcrosses to *C. littorale* predominating in those areas (Hightown and Ainsdale) in which only *C. littorale*-like habitats are present, backcrosses to *C. erythraea* predominating at St Annes where only *C. erythraea*-like habitats are present, and both backcrosses occurring at Freshfield where both habitats still exist. This demonstrates that one of the major reasons for the difference in the outcome of hybridization between the different areas is the types of habitats available.

However, the outcome of hybridization also depends on the vigour and fertility of the F_1 hybrid, and on the degree of isolation from the parents. Previous work (Ubsdell 1976b) has shown that the F_1 hybrids are almost sterile, and so in order to survive they must overcome this sterility since they are unable to persist by vegetative reproduction. Selfing of the F_1 and crossing with other F_1 plants does not produce any increase in fertility, and the F_2 is equally sterile. At Newborough Warren the few F_1 plants do not grow near enough to the parents to backcross, and so hybridization does not extend beyond the F_1 generation, although there are plenty of suitable habitats for the hybrid derivatives. It is difficult to understand the situation in Schleswig-Holstein as little work has been done on the Continental plants, but it does seem that stabilization of hybrid segregates at the tetraploid level and in the absence of parents may have taken place.

On the Lancashire coast the F_1 hybrids grow intermingled in the same slacks as the parents and backcrossing to both has taken place. In both cases this has resulted in an increased fertility and a diminution of the cytological abnormalities exhibited by the almost sterile, tetraploid F_1 hybrid, although it seems to have been achieved by two separate methods.

The amount of backcrossing in any area will depend upon the frequency of the parents, the breeding systems of the parents, and the flowering periods of hybrids and parents. Despite the high degree of inbreeding shown by *C. erythraea*, some backcrossing to this parent has occurred at St Annes and in one population from Freshfield. This is because this parent is the most frequent in these areas where *C. erythraea*-like habitats occur, and the highly fertile, tetraploid backcross plants will be well adapted to survive in such habitats. In these areas introgression has taken place, and genes of *C. littorale* have been transferred to *C. erythraea* by backcrossing of the F_1 hybrid to *C. erythraea*, followed by natural

selection of favourable recombinant types resembling the latter. This has resulted in increased variability of *C. erythraea* and some of the new gene combinations may be at a selective advantage in habitats other than those favoured by this species.

Backcrossing to *C. littorale* has occurred in all other areas on the Lancashire coast where this species is the most frequent parent. Backcrossing to this parent is also favoured by its tendency to outbreed, and pollen from this parent can be transferred to the stigmas of the hybrids during the short period before their own pollen is ripe. However, it has also been shown (Ubsdell 1976b) that backcrossing to this parent does not increase the fertility of the hybrids at the tetraploid level, since all tetraploid backcrosses to this species were found to be almost as sterile as the F_1 hybrids, unlike the backcrosses to *C. erythraea* which became more fertile. There may, therefore, be a strong selective advantage for plants that can overcome this sterility, and polyploidy is one way of achieving this. Backcrosses to *C. littorale* collected from Hightown, Ainsdale and Freshfield have been shown (Ubsdell 1976b) to be hexaploid, highly fertile and cytologically stable. They have also been shown (Ubsdell 1976b) to breed true and to be genetically isolated from the tetraploid parents and all tetraploid hybrids by the difference in chromosome number. They are able to compete successfully with the parents at Hightown, Freshfield and Ainsdale, and in some areas, notably at Hightown and Ainsdale, they are more abundant than the parents, and they may be at a selective advantage in these disturbed, somewhat intermediate habitats.

This study of variation and evolution has consequently shown that, in addition to the more expected process of introgression, a new species resulting from both hybridization and polyploidy has been produced by abrupt speciation. The origin of this species seems to be closely related to man's disruptive effect on the natural environment and, since there is little reason to assume any direct connections between the areas in which it was formed (Hightown, Freshfield and Ainsdale), it must be considered an example of the polytopic origin of an allopolyploid species.

It is possible to establish an approximate date for the formation of this new species, since the major disturbance of the dune systems of the Lancashire coast appears to have taken place during the building of the Liverpool-Southport railway in 1884 and the first record of hybrid plants was made by Wheldon (1897). His plants (**BM**) collected in 1894 from Hightown are identical to those of this new species, the nomenclature of which will now be discussed.

NOMENCLATURE OF PLANTS OF HYBRID ORIGIN

The nomenclature of all plants of hybrid origin has been confused in the literature by the lumping together of the tetraploid F_1 hybrids, the tetraploid backcrosses to *C. erythraea* and the hexaploid plants under the general name of *Centaureum intermedium* (Wheldon) Druce, although the original description of *Erythraea littoralis* var. *intermedia* Wheldon, upon which the name *C. intermedium* was based, refers only to the hexaploid plants. This present study has shown that the hexaploid plants should be recognized as a new allopolyploid species to which the name *C. intermedium* (Wheldon) Druce should be given, while the tetraploid F_1 and backcross hybrids to *C. erythraea* should be included under the general hybrid formula *C. erythraea* \times *C. littorale* (Melderis 1972), as follows:

Tetraploid hybrids between *C. erythraea* and *C. littorale*

C. erythraea \times *C. littorale*

C. \times *intermedium* sensu Gilmour (1937) *p.p.* et O'Connor (1955) *p.p.*, non Druce (1905) nec *Erythraea littoralis* var. *intermedia* Wheldon, *Sci. Gossip*, n.s., 4: 111 (1897).

Hexaploid plants

Centaureum intermedium (Wheldon) Druce, *Ann. Scot. Nat. Hist.*, 53: 48–49 (1905)

Erythraea littoralis var. *intermedia* Wheldon (1897)

Lectotype (selected here): South Lancashire (v.c. 59), Hightown, "sand dunes", 21 July 1894, Wheldon (**BM**)

Wheldon described *Erythraea littoralis* var. *intermedia* from plants collected at Hightown, S. Lancashire. These specimens are at **BM** and are identical with the hexaploid plants. Druce's *C. intermedium* therefore also refers to these hexaploid plants. Gilmour and O'Connor lumped tetraploids and hexaploids together, while O'Connor and Melderis considered the hexaploid to be a polyploid form of *C. littorale*.

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