

STUDIES IN *RANUNCULUS* SUBGENUS *BATRACHIUM* (DC.)
A. GRAY. III. *RANUNCULUS HEDERACEUS* L. AND
R. OMIOPHYLLUS TEN.

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ABSTRACT

An account is given of *Ranunculus hederaceus* and *R. omiophyllus* including information on distribution, ecology, floral biology, hybridization, variation and karyotype analysis. Formal descriptions with full synonymies and typifications are also included. Both species are morphologically and ecologically very similar and can exist as diploids or tetraploids. They are largely sympatric throughout their geographical ranges and, occasionally, grow intermingled. They show complete hybrid inviability at the diploid level and hybrid sterility at the tetraploid level but at the same time can be regarded as belonging to a single potential common gene-pool (hologamodeme) as they form fertile hybrids with *R. tripartitus* and *R. baudotii*. In spite of a high degree of inbreeding and numerous isolated populations no genotypic differentiation has been detected in either *R. hederaceus* or *R. omiophyllus*.

INTRODUCTION

R. hederaceus and *R. omiophyllus* are the only species of *Ranunculus* subgenus *Batrachium* that lack throughout their life the ability to produce divided leaves with capillary segments (Cook 1963) and are essentially terrestrial plants of wet places. Salisbury (1934) reviewed these two species and concluded, mainly on the basis of their geography, ecology and meristic variation, that they both originated from a common stock and that because *R. omiophyllus* occupied more specialized habitats than *R. hederaceus* and showed less marked evidence of trimery it was of more recent origin. Salisbury's studies were carried out exclusively on British material collected in the field. During the investigation reported here I have endeavoured to study these species throughout their geographical ranges and have cultivated plants under different conditions, carried out breeding experiments and incorporated cytological observations with the result that some of Salisbury's conclusions need reconsideration.

DISTRIBUTION*

R. hederaceus occurs in Europe and N. America. In Europe it shows an atlantic distribution but is widespread in inland districts of Britain (Fig. 1a). In North America it is confined to Newfoundland and the Chesapeake Bay region (Fig. 1b). It has been recorded from Dalmatia, Italy, Sicily, Iceland, Greenland and north-west America (Salisbury 1934) but I have not been able to trace herbarium material to support these records. It is likely that the Italian and Sicilian records are due to misidentifications of *R. omiophyllus*.

The status of *R. hederaceus* in North America is problematical. It was first recorded from Carolina by Bosc (Bosc & Baudrillard 1821). Gray (1886), Fernald (1929), Drew (1936) and Benson (1948) considered it to be native while Macoun (1891) and Morris (1900) regarded it as introduced. Morris wrote 'patches . . . (Patuxent River) badly cut up by the passage of teams over a temporary farm road,' while Fernald wrote : 'apparently indigenous in Newfoundland especially since it shares natural and undisturbed habitats with or near other European types'. In Europe it is usually found in disturbed habitats. There seems little doubt that it was introduced in Carolina. Walker did not record it in 1788 in *Flora Caroliniana* : it was recorded by Bosc in 1821 and reported extinct by Elliot

*Material from the following herbaria has been examined : CGE, COI, C, E, K, LE, LIVU, LU, BM, M, NAP, W.

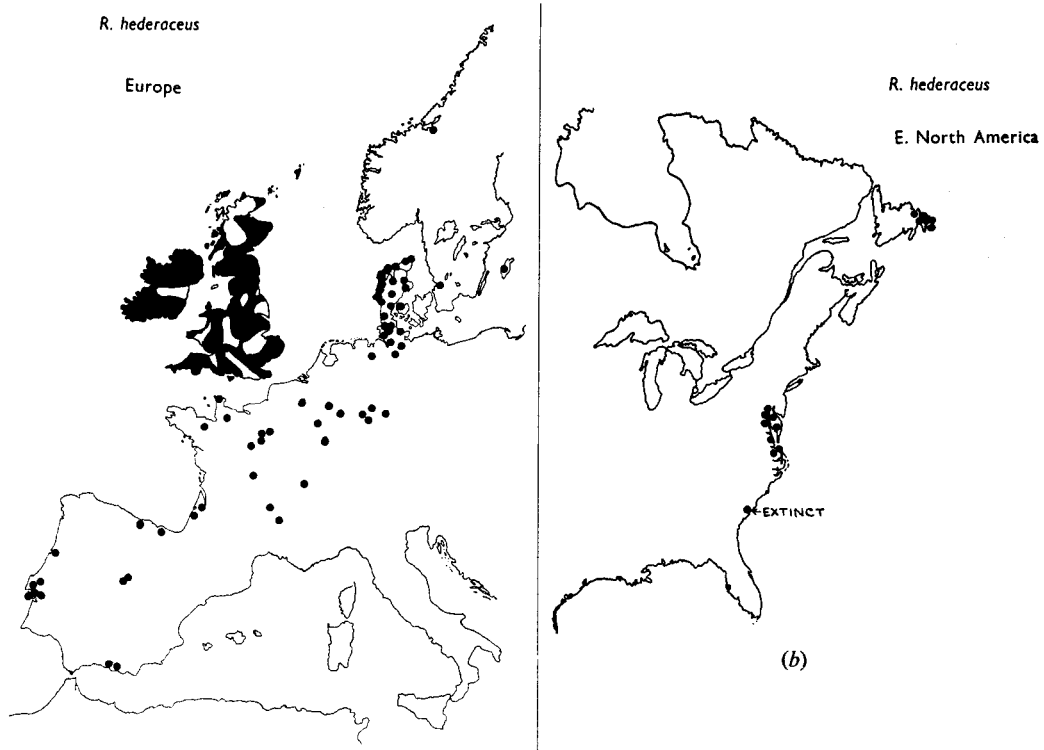


Fig. 1. Distribution of *R. hederaceus* (a) in Europe, and (b) in North America.

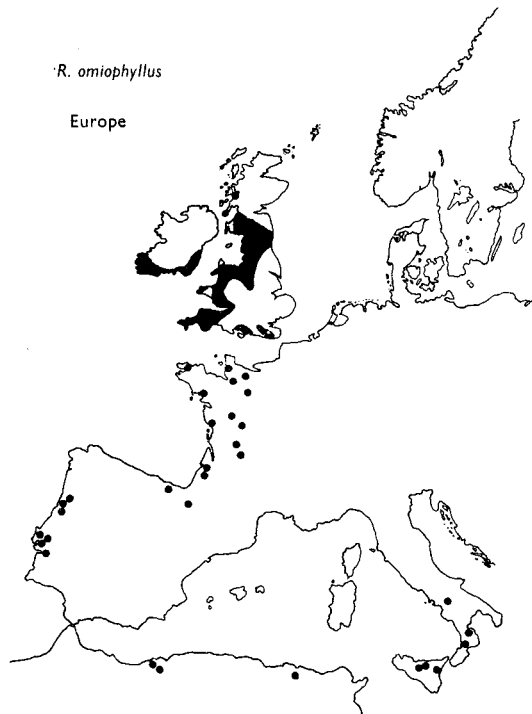


Fig. 2. Distribution of *R. omiophyllus*.

in 1824. The general distribution in North America would suggest that it was introduced, but there are two fern species, *Schizaea pusilla* Pursh and *Woodwardia areolata* (L.) Moore which share almost the same distribution in North America and which are certainly not European introductions.

Faegri (1960) considered *R. hederaceus* to have been introduced in Norway where it is confined to a small coastal area near Trondheim.

R. omiophyllus is confined to Europe and North Africa (Fig. 2). In Europe it shows a distinct Atlantic distribution from North France to Portugal but is also present in mountainous districts in South Italy and Sicily. In Britain the limits of distribution follow extremely closely the August 3in isohyet. In North Africa it is confined to the coastal mountains of Algeria. According to Moggi (1963) most species common to Atlantic Europe and South Italy migrated along the Apennine Mountains and are not found in North Africa. *Genista anglica* L. and *R. omiophyllus* are, perhaps, the only two Atlantic south Italian species that are absent from the Apennines but present in North Africa.

Morris (1837) described a plant called *R. aquatilis* L. var. *homophyllus* from Sardinia. The description is inadequate and as I have seen no herbarium specimens some doubt about this record must remain.

FLORAL BIOLOGY

The flowering times of *R. hederaceus* and *R. omiophyllus* show a considerable range of variation. When cultivated in cool but frost-free greenhouses at Cambridge, München and Liverpool Botanical Gardens, occasional flowers were formed throughout the year but most flowers were produced between February and November. Both species are fairly sensitive to constant high temperature and if cultivated in a greenhouse with a continuous temperature of 21° C or more they soon die. In nature *R. hederaceus* has been seen in flower and fruit in March 1958 in South Cornwall while in East Scotland in the same year flowering was just starting in May. In Norway and in the higher parts of the Sierra Nevada in Spain flowering begins as late as August. *R. omiophyllus* is equally variable and plants have been seen in flower in North Wales in November and February, in the Madonie Mountains of Sicily flowering commences during April. In cultivation flowering does not cease abruptly at any particular time of the year. In nature, however, flowering is usually stopped by the plants being smothered by later-growing species such as *R. sceleratus* L., *Peplis portula* L. and *Montia fontana* L. or by the habitat drying out or freezing.

Pollination and fertilization have been described elsewhere (Cook 1963)—see also p. 252. After fertilization the pedicel bends downwards forcing the developing fruits into the substratum. The achenes are probably dispersed in mud, carried by animals or machines. Occasionally they are liberated in water and then they may be dispersed by water currents or by surface contact with larger animals.

Salisbury (1934) reported both species to be winter annuals but this appears to be an over-simplification. If cultivated in waterlogged soil and not subjected to inter-specific competition they behave as perennials and survive for at least six years. In winter, both species form small tight cushions which are very resistant to desiccation, freezing and shade, but in summer they develop a spreading habit and are very susceptible to desiccation and shade. When cultivated in mud with *Peplis portula* both species behave as winter annuals. Seed germination is very irregular when the achenes are kept wet, but if the achenes are dried and then rewetted nearly complete germination takes place. Both species in nature may behave as spring or winter annuals or as perennials depending on the local water regime and competing species.

POLYPLOIDY AND CHROMOSOME MORPHOLOGY

I have already reported (Cook 1962) a chromosome count of $2n = 16$ for a plant of *R. omiophyllus* from Sicily and $2n = 32$ for a plant from Dartmoor, Devon, also *R. hederaceus* with $2n = 16$ from plants collected from six populations. During the summer of

1963 and 1964 the same number has been obtained from four more populations of *R. hederaceus*,* viz.:

- Jackson's Pond, Childwall, Liverpool, Lancashire.
- Bwlch-y-Rhiw, 4 m east of Aberdaron, Caernarvonshire.
- Ditch 1 m south-east of Penrhos Lligwy, Anglesey.
- Wet flush near Porthmynawyd, Pembroke.

An artificial autotetraploid of *R. hederaceus* has been induced using aqueous colchicine solution. The method used consisted of maintaining a droplet of 0.04 per cent. colchicine solution between the cotyledon leaves of a young seedling and allowing the young apex to grow through the droplet. To maintain humidity the plants were kept in a sealed tank partly filled with water. The treatment lasted four or five days depending on the growth of the apex. The 0.04 per cent. colchicine had a profound effect on all seedlings—the rate of growth was slowed down and any new leaves produced were markedly deformed and succulent. Only one polyploid was obtained from 150 treated seedlings.

It has been found when diploid and tetraploid plants of *R. hederaceus* and *R. omiophyllus* of the same age are cultivated side by side there are a few morphological differences between them, see Table 1. This table was compiled from measurements made on single plants of each species at both diploid and tetraploid levels. The plants in this experiment were cultivated side by side in a cool greenhouse at the Botanical Gardens at München, Germany and gathered in June 1961. Five flowering shoots from each plant were taken. Stomatal length was measured on 25 stomata from one mature leaf from each shoot and the mean and standard deviation (*s.d.*) calculated. Three anthers from one flower from each shoot were heated in 45 per cent. propionic orcein solution. Pollen diameter was measured on 50 well formed grains from each flower. Apparent male fertility was calculated as an absolute percentage of well formed grains. Female fertility was calculated as a percentage of well-formed achenes in mature heads. Stamen number and carpel number is given for each flower examined. From Table 1 it can be seen that there are considerable differences between the diploid and the tetraploid.

TABLE 1

	<i>R. hederaceus</i>		<i>R. omiophyllus</i>	
	<i>diploid</i>	<i>autotetraploid</i>	<i>diploid</i>	<i>tetraploid</i>
Stomatal length (mm)	0.0275	0.0358	0.0267	0.0361
s.d.	0.0017	0.0028	0.0015	0.0021
Pollen diameter (μ)	27.3	33.3	26.9	30.9
s.d.	1.6	2.5	0.5	1.8
Apparent male fertility (%)	99	85	99	96
Female fertility (%)	80	69	74	75
Stamen number	7 8 9 10 10	6 9 9 10 11	8 8 8 10 10	8 9 9 10 10
Carpel number	20 24 24 26 32	24 27 28 28 36	28 30 31 41 42	30 34 34 37 43

If, however, these plants are cultivated in different conditions or sampled at different times, the above characters are modified and no clear separation can be made between diploid and tetraploid. For example, Fig. 3 shows pollen grain diameters of diploid and autotetraploid *R. hederaceus*. I, II and III represents ramets of a clone of diploid *R. hederaceus* (collected from Ponsongath, The Lizard, Cornwall by C. D. K. Cook, 30th March 1958) while IV and V represent the autotetraploid derived from seeds from this clone. All were cultivated at Liverpool University Botanic Garden and gathered on the same day

*Herbarium specimens have been deposited in the herbarium at the Hartley Botanical Laboratories, Liverpool (LIVU).

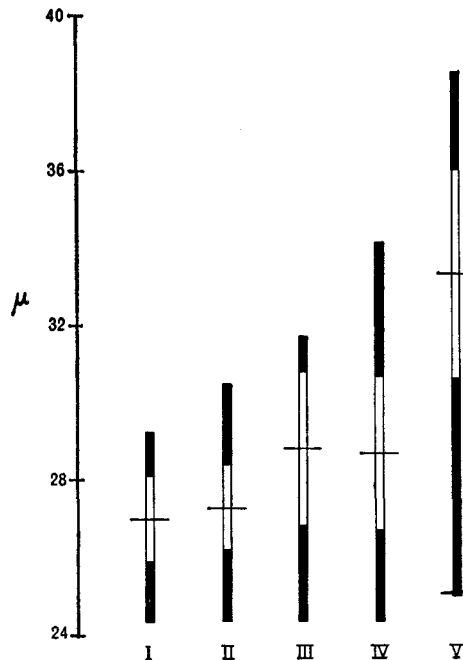


Fig. 3. Pollen diameters of *R. hederaceus* (central line represents the mean, unshaded area the standard deviation and the black area the total range). I, II and III represent ramets of a single clone of 16 *R. hederaceus* (Lizard) and IV and V represent the autotetraploid derived from this clone. I and IV were cultivated terrestrially, side by side outdoors, II and V were cultivated terrestrially, side by side in an unheated greenhouse and III was cultivated in 5 cm. of water outdoors. All plants were grown at Liverpool University Botanic Gardens and gathered on 16th May 1962.

(16th May 1962). I and IV were cultivated terrestrially, side by side outdoors ; III was cultivated in 5 cm of water outdoors, unfortunately the autotetraploid did not survive in 5 cm of water in this experiment.

It can be seen that plants II and V show a marked difference, I and IV show less difference while III and IV are nearly the same. In this experiment the mean pollen diameter of the aquatically cultivated diploid was slightly greater than that of the terrestrially cultivated tetraploid. A similar situation is found in *R. omiophyllus*. In both species stomatal length is extremely variable and when plants are placed under water the stomatal length in successive leaves may increase or decrease but the environmental factors that induce these changes are not understood. The mere fact that pollen grain diameter and stomatal length are plastic and under environmental control precludes the recognition of the level of polyploidy from herbarium material. Sokolovskaya (1962) has found that pollen diameter is not a good indicator of polyploidy in *Saxifraga*, and Schwanitz (1952) demonstrated that both stomatal size and pollen diameter can be altered by changes in the environment and that this variation may obscure differences due to polyploidy.

The other differential characters such as male and female fertility and numbers of stamens and carpels vary considerably as a result of small environmental changes. Nevertheless, living plants of the autotetraploid of *R. hederaceus* can usually be distinguished from the diploids as they are more shiny, somewhat succulent, slower growing with a more compact habit and show a marked tendency to become fasciated. Flowering normally starts about two weeks later than the diploid and continues about two months after the diploid. There is no evidence that the autotetraploid occurs wild in nature. Diploid and tetraploid plants of *R. omiophyllus* are extremely similar and no separation can be made on gross morphological grounds. A similar situation has been described by Solbrig (1964) ; he was unable to distinguish diploids from tetraploids of *Gutierrezia sarothrae* after a

detailed study of their ecology, geography and 11 morphological characters from 53 wild populations.

The karyotypes of diploid *R. hederaceus* and *R. omiophyllus* are similar (Fig. 4) and no convincing difference has been found. The autotetraploid of *R. hederaceus*, as expected, simply has the diploid set represented twice (Fig. 4) with the exception that there is only one pair of satellited chromosomes. The chromosomes that carry the satellites are the smallest in the karyotype and have a sub-terminal centromere. These chromosomes have been seen in all species of *Batrachium* so far investigated (Cook 1962) but except for autotetraploid *R. hederaceus* and its hybrids never more than two satellited chromosomes have been seen. It is possible that the satellites act as nucleolar organizers (they are much larger at prophase than at metaphase) and with time the extra satellites may be lost; this loss of satellites has been described by Navashin (1934).

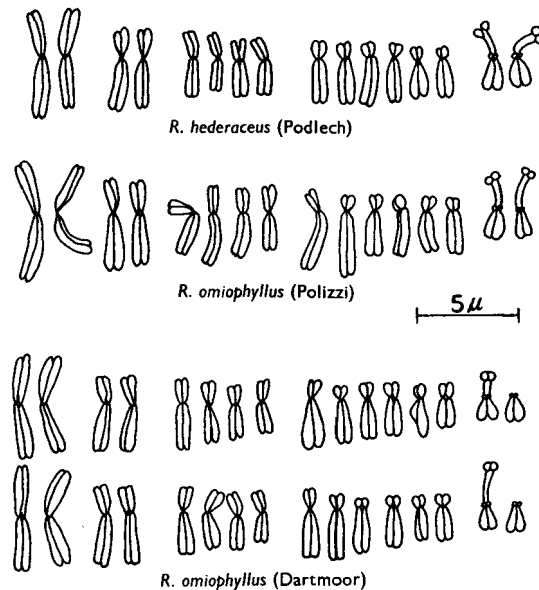


Fig. 4. Karyotypes of diploid *R. hederaceus* and *R. omiophyllus* and tetraploid *R. omiophyllus*.

VARIATION

Plants from the following populations have been maintained in cultivation.

R. hederaceus

1. Near Port Logan, Galloway, Wigtownshire. O. R. Staples, 9 August 1956.
2. Sellindge, near Hythe, Kent. O. R. Staples, 28 August 1956.
3. Ponsongath, The Lizard, Cornwall. C. D. K. Cook, 30 March 1958.
4. Near Mora, prov. Alto Alentejo, Portugal. B. V. Rainha, 12 June 1958, sent by A. R. Pinto da Silva.
5. Eifel, Wirftal, Stadtkyll, Prüm, Germany. D. Podlech, 25 May 1961.
6. Bwlch-y-Rhiw, near Aberdaron, Caernarvonshire. C. D. K. Cook, 14 May 1962.
7. Wet flush near Porthmynawyd, Pembroke. J. J. B. Gill, April 1964.

R. omiophyllus

8. Wet flush just below Wistman's Wood, Two Bridges, Dartmoor, Devon. C. D. K. Cook, 4 May 1958.
9. Small spring about 6 km north of Polizzi Generosa, Madonie Mountains, Sicily. C. D. K. Cook, 21 April 1961.

Plants from collection numbers 1, 2, 3, 4, and 8 were cultivated in the Cambridge Botanic Garden, Cambridge between 1956 and 1960 ; 2, 3, 4, 5, 8, and 9 were cultivated in the Botanic Gardens, München, Germany between 1960 and 1961 ; 3, 4, 5, 6, 7, 8, and 9 in the Liverpool University Botanic Gardens, Ness, Cheshire from 1961 to 1965. Crosses between 3, 4 and 5 have been made (see page 253) and batches of 25 seedlings from selfed 3, 4 and 5 have been raised. All these cultivated plants have been compared and no genotypic variation has been detected. No suggestion of possible genotypic variation has been seen from herbarium material examined (see page 246).

Glück (1924, 1936) recognized one variety, *R. hederaceus* var. *coenosus* (Gussone) Cosson (*R. coenosus* Gussone) which is referable to *R. omiophyllus* Tenore. Under *R. hederaceus* sensu stricto Glück described six formae (*natans* Glück, *amphibius* Glück, *latifolius* Glück, *terrestris* Glück, *pumilus* Glück, and *reniformis* Glück). Under *R. omiophyllus* Glück described three formae (*natans* Glück, *amphibius* Glück and *terrestris* Glück) and plants matching the descriptions of each of these formae have been induced from ramets of a single clone. The plastic variation pattern of this species closely matches that found in *R. hederaceus* and may be due to homologous variation.

To a great extent Glück based his formae on more or less arbitrarily chosen size categories, and his descriptions, tables and illustrations (Glück 1924, p. 580–591, plaet) V give a very good indication of the plasticity of these species. Cultivation experiments suggest that these formae are no more than phenodemes (Gilmour & Gregor 1939). Plants matching the descriptions of each of Glück's formae have been induced from ramets of a single clone. However the behaviour of both species is not always predictable and merely cultivating plants terrestrially does not necessarily cause them to resemble forma *terrestris*. Each phenodeme results from the interaction of many environmental factors. The water regime is very important but so are light intensity, photo-period, competition, substrate conditions, age of plant etc.

Leaf shape and size are particularly plastic. In *R. hederaceus*, for example, a terrestrially cultivated plant in summer will usually be compact with leaves 4–8 mm long and 3–10 mm wide, while a plant of the same age at the same time cultivated in 5 cm of water will be spreading and normally have leaves 17–25 mm long and 25–35 mm wide. When a plant is growing quickly in favourable conditions the leaves are usually three-lobed with the lobes widest at the base. If the plant is grown in less suitable conditions, such as in deep water, shade or dry soil, the leaves become distinctly five-lobed and the sinus may become deeper making the base of the lobe narrower, or the sinus may disappear producing a typically reniform leaf. It is interesting to note that the synthesized autotetraploid (see page 249) nearly always has five lobes with a very much reduced sinus.

In spite of the extreme phenotypic plasticity in size and leaf-shape the recognition of these species is not difficult since highly modified leaf-shapes are only manifest on mature or over-mature leaves while the immature leaves always show the characteristic form of each species.

HYBRIDIZATION

R. hederaceus and *R. omiophyllus* are protogynous but pollination usually takes place before the flower buds open. The carpels are usually ready to receive pollen from three to six days before the flower opens, but the timing is difficult to judge as opening depends largely on the weather. The lower (outer) anthers dehisce about 24 hrs before the flower opens but at this stage pollen rarely gets beyond the outer carpels. Later when the flower has opened the filaments elongate, shedding pollen on the inner carpels. In spite of there being an opportunity for outbreeding there are no reports of any naturally occurring hybrids between these species.

Occasionally, flowers are produced underwater. When this happens a gas bubble is formed within the closed bud allowing pollination to take place. If a newly opened flower is repeatedly wetted there is a low degree of pollination.

The flowers produce visible quantities of nectar and have a sweet-smelling scent somewhat like *Crataegus monogyna* Jacq. In spite of this no nectar or pollen-taking insects have been seen visiting the flowers. Some chalcids, psychodids, chironomids, *Corixa* sp. and females of *Scopeuma stercorarium* L. have been collected from the flowers. In order to cross these plants it is necessary to emasculate the flowers while they are in bud. This is normally done under a microscope as it is very easy to burst an anther or damage the carpels. Polythene tubing sealed with cotton wool is used to prevent foreign pollen reaching the stigmas. Initially, while the flower is erect the polythene tubing is held in position by wire but later after pollination, when the pedicel starts bending the supporting wire is removed because if prevented from bending the pedicel breaks.

Pollen of *R. bulbosus* L., *R. sceleratus* L., *R. ophioglossifolius* Vill. and *Papaver somniferum* L. was placed on the stigmas of *R. hederaceus* to test for pseudogamy in the restricted sense of Gustafsson (1946). The pollen of these species germinated but soon died and no pseudogamy was detected. Embryological investigations were carried out on emasculated flowers but failed to reveal any agamosperous process. It is probable that faulty technique and not apomixis was to blame for the few maternal plants obtained in F₁ inter-specific hybrid progenies. *R. hederaceus* and *R. omiophyllus* are self-compatible. From hybridization experiments it was found that diploid *R. hederaceus* could be used only as a maternal parent when crossed with other species. To study this crosses between male *R. hederaceus* and female *R. tripartitus* were examined embryologically. The growth of the pollen tube appeared normal and apparently some fertilization took place as the endosperm started normal development but the embryo did not divide and remained dormant.

The following is a list of successful crosses involving *R. hederaceus* and *R. omiophyllus*. The origin of the material used is shown in a separate list after the hybrids. Herbarium material of these hybrids is deposited in the herbarium of the Hartley Botanical Laboratories, Liverpool (LIVU). The female parent is cited first. The somatic chromosome number of the parent is given before the name (for details of chromosome numbers see Cook, 1962). In most cases the hybrid was obtained after the first attempt but in all cases after not more than three attempts.

1. 16, *R. hederaceus* (Podlech) × 16, *hederaceus* (Lizard).
2. 16, *R. hederaceus* (Lizard) × 16, *hederaceus* (Podlech).
3. 16, *R. hederaceus* (Lizard) × 16, *hederaceus* (Portugal).
4. 16, *R. hederaceus* (Lizard) × 32, *omiophyllus* (Dartmoor).
5. 16, *R. hederaceus* (Lizard) × 48, *tripartitus* (Lizard).
6. 16, *R. hederaceus* (Lizard) × *peltatus* (Coimbra).
7. 32, *R. hederaceus* (Aut. Lizard) × 16, *hederaceus* (Lizard).
8. 32, *R. hederaceus* (Aut. Lizard) × 32, *omiophyllus* (Dartmoor).
9. 32, *R. hederaceus* (Aut. Lizard) × 48, *tripartitus* (Lizard).
10. 32, *R. hederaceus* (Aut. Lizard) × 32, *trichophyllus* (Wicken).
11. 32, *R. omiophyllus* (Dartmoor) × 32, *hederaceus* (Aut. Lizard).
12. 32, *R. omiophyllus* (Dartmoor) × 48, *tripartitus* (Lizard).
13. 48, *R. tripartitus* (Lizard) × 32, *omiophyllus* (Dartmoor).
14. 32, *R. baudotii* (Brading) × 32, *hederaceus* (Aut. Lizard).
15. 32, *R. baudotii* (Brading) × 32, *omiophyllus* (Dartmoor).

The following is a list of unsuccessful crosses. The number of attempts made is shown after each cross.

- 16, *R. hederaceus* (Lizard) × 32, *hederaceus* (Aut. Lizard) (2).
- 16, *R. hederaceus* (Lizard) × 16, *omiophyllus* (Polizzi) (10).
- 16, *R. hederaceus* (Lizard) × 16, *ololeucos* (Maarn) (4).
- 16, *R. hederaceus* (Lizard) × 32, *baudotii* (Brading) (2).
- 16, *R. hederaceus* (Lizard) × 16, *circinatus* (Wicken) (7).
- 16, *R. hederaceus* (Lizard) × 16, *fluitans* (Quorndon) (3).
- 16, *R. hederaceus* (Lizard) × 32, *fluitans* (Suffolk) (2).

- 32, *R. hederaceus* (Aut. Lizard) × 16, *omiophyllus* (Polizzi) (5).
 32, *R. hederaceus* (Aut. Lizard) × 16, *ololeucos* (Maarn) (2).
 32, *R. hederaceus* (Aut. Lizard) × 16, *circinatus* (Wicken) (2).
 32, *R. hederaceus* (Aut. Lizard) × 32, *fluitans* (Suffolk) (2).
 16, *R. omiophyllus* (Polizzi) × 16, *hederaceus* (Lizard) (10).
 16, *R. omiophyllus* (Polizzi) × 32, *hederaceus* (Aut. Lizard) (2).
 16, *R. omiophyllus* (Polizzi) × 32, *omiophyllus* (Dartmoor) (3).
 16, *R. omiophyllus* (Polizzi) × 16, *ololeucos* (Maarn) (2).
 32, *R. omiophyllus* (Dartmoor) × 16, *hederaceus* (Lizard) (8).
 32, *R. omiophyllus* (Dartmoor) × 16, *omiophyllus* (Polizzi) (2).
 32, *R. omiophyllus* (Dartmoor) × *peltatus* (Coimbra) (2).
 32, *R. omiophyllus* (Dartmoor) × 48, *aquatilis* (Stretham) (1).
 32, *R. omiophyllus* (Dartmoor) × 32, *trichophyllus* (Wicken) (2).
 32, *R. omiophyllus* (Dartmoor) × 32, *fluitans* (Suffolk) (1).
 16, *R. ololeucos* (Maarn) × 16, *hederaceus* (Lizard) (3).
 16, *R. ololeucos* (Maarn) × 32, *hederaceus* (Aut. Lizard) (1).
 16, *R. ololeucos* (Maarn) × 16, *omiophyllus* (Polizzi) (4).
 48, *R. tripartitus* (Lizard) × 16, *hederaceus* (Lizard) (12).
 48, *R. tripartitus* (Lizard) × 32, *hederaceus* (Aut. Lizard) (2).

The following is a list of the origins of the material used in the crossing experiments.

- 48, *R. aquatilis* (Stretham). Roadside ditch about 2 km south of Stretham, Cambridgeshire. C. D. K. Cook, 20 September 1956.
 16, *R. baudotii* (Brading). On the saltmarsh at Brading, Isle of Wight. C. D. K. Cook, 20 May 1958.
 16, *R. circinatus* (Wicken). In the Lode at Wicken Fen, Cambridgeshire. C. D. K. Cook, 2 June 1959.
 16, *R. fluitans* (Quorndon). In the Markeaton Brook, Quorndon, Derbyshire. C. D. K. Cook, 11 June 1957.
 32, *R. fluitans* (Suffolk). In the River Lark at Temple Bridge, Tuddenham, Suffolk. C. D. K. Cook, 19 June 1958.
 16, *R. hederaceus* (Lizard). By a wet farm-track at Ponsongath, The Lizard, Cornwall. C. D. K. Cook, 30 March 1958.
 16, *R. hederaceus* (Portugal). Collected wild near Mora. prov. Alto Alentejo, Portugal by B. V. Rainha, 12 June 1958, sent by A. R. Pinto da Silva.
 16, *R. hederaceus* (Podlech). In a ditch at Eifel, Wirftal about 1 km south of Stadtykll, Prüm, Germany. D. Podlech, 25 May 1961.
 32, *R. hederaceus* (Aut. Lizard). An autotetraploid induced from seeds of 16, *hederaceus* (Lizard) in September 1958, at Cambridge Botanic Garden.
 16, *R. ololeucos* (Maarn). In a ditch at Maarn, 15 km east of Utrecht, Holland. C. D. K. Cook and T. W. J. Gadella, 1 April 1960.
 16, *R. omiophyllus* (Polizzi). Small spring about 6 km north of Polizzi Generosa, Madonie Mountains, Sicily. C. D. K. Cook, 21 April 1961.
 32, *R. omiophyllus* (Dartmoor). Wet flush just below Wistman's Wood, Two Bridges, Dartmoor, Devon. C. D. K. Cook, 4 May 1958.
R. peltatus (Coimbra). Seed sent from the Botanic Gardens of the University of Coimbra, Portugal. Received March 1957 but wild origin unknown.
 32, *R. trichophyllus* (Wicken). In the ditch around the bird sanctuary in Adventurers' Fen, Wicken, Cambridgeshire. C. D. K. Cook, 20 September 1956.
 48, *R. tripartitus* (Lizard). In a pool by the air-raid shelter, Kynance, The Lizard, Cornwall. C. D. K. Cook, 21 March 1957.

Hybrids 1, 2 and 3 are between plants of *R. hederaceus* collected from Britain, Germany and Portugal. All hybrid plants were fully fertile and indistinguishable from the parents.

Hybrid 7 is the result of a cross between maternal tetraploid and paternal diploid *R. hederaceus*; the reciprocal cross has been attempted twice without success. Stebbins (1958) pointed out that in crosses involving diploids and autotetraploids the diploid usually is the poorer maternal parent. This hybrid is triploid ($2n = 24$) and highly sterile, and has produced no fertile achenes. In summer it closely resembles the diploid parent and in winter the tetraploid parent. All attempts at inducing a hexaploid from this triploid have, so far, failed.

Hybrid 4 (diploid *R. hederaceus* × tetraploid *omiophyllus*) is a highly sterile triploid ($2n = 24$). Apart from a little more vigour this hybrid is indistinguishable from *R. omiophyllus* on all gross morphological characters. Using the technique described on p. 249 it has an apparent pollen-fertility of 4 per cent. but no fertile achenes have been produced. The reciprocal cross has been attempted eight times but no offspring have been obtained.

Hybrid 7 (autotetraploid *R. hederaceus* × tetraploid *omiophyllus*) and hybrid 11, the reciprocal cross, are highly sterile tetraploids ($2n = 32$) which are morphologically indistinguishable from the triploid hybrid described above. Both these tetraploid hybrids have an apparent pollen fertility of about 15 per cent. but neither have produced mature achenes. This apparently fertile pollen stains well and looks perfectly formed but when used for backcrossing to either parent it will not germinate. Reciprocal backcrosses have been made and parent pollen will germinate on the hybrid stigmas but no fertile achenes have been formed.

The nature of this hybrid sterility appears to be genic rather than chromosomal. From studies of mitosis there are no visible differences between the karyotypes of *R. hederaceus* and *R. omiophyllus* (Fig. 4). In the tetraploid hybrids there is some univalent formation (Fig. 5a) which is not found in diploid *R. hederaceus* (Fig. 5b). Although the

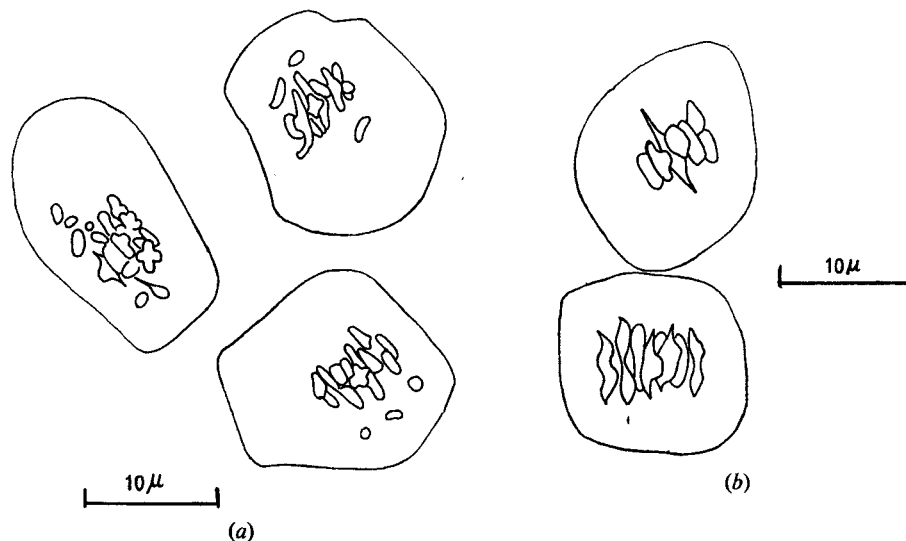


Fig. 5. Preparations of pollen-mother cells to show meiotic metaphase in (a) 32 *R. hederaceus* (Aut. Lizard) × 32 *omiophyllus* (Dartmoor) and, (b) 16 *R. hederaceus*.

meiotic preparations are not satisfactory there is no great chromosomal unbalance and hybrids between tetraploid *R. hederaceus* and *R. baudotii* show similar meiotic figures and apparent pollen-fertility but produce fertile achenes which germinate and give rise to F_2 progeny which show segregation. Similar situations are found in *Nicotiana* (Lammerts 1931) and *Geum* (Gajewski 1957). In spite of repeated attempts no allopolyploids have been induced from these hybrids, so it is not possible to rule out chromosomal sterility; but as both diploid *R. hederaceus* and tetraploid *R. omiophyllus* will form fertile hybrids with *R. tripartitus* it looks as if the sterility may be genic, especially as the F_2 progeny from these

hybrids show segregation in the degree of fertility. It may appear that an important stage in this sterility is in the formation of the pollen tube, but backcrosses using parental pollen have also been inviable, so that the sterility between *R. hederaceus* and *R. omiophyllus* is present on both the maternal and paternal sides.

DISCUSSION

R. hederaceus and *R. omiophyllus* are largely sympatric, morphologically very alike, and share a similar ecological niche, occasionally being found growing together. Salisbury (1934) reported that *R. omiophyllus* was invariably associated with peaty waters while *R. hederaceus* was more tolerant of waters well supplied with mineral salts, but he had, however, seen both species growing intermingled. In Britain, his generalization may be correct, but in the Madonie Mountains of Sicily *R. omiophyllus* was found growing in eutrophic conditions around cattle troughs on calcareous substrata, the kind of conditions, in fact, where one expects to find *R. hederaceus* in Britain. It may be that where these two species are not competing they exhibit wider ecological amplitudes.

In cultivation, British material of each species grows well on calcareous and non-calcareous soils and no interspecific differences in vigour have been detected. Similarly Sicilian and British material of *R. omiophyllus* do not show any differences in vigour when cultivated side by side under different conditions. In spite of a high degree of inbreeding and the presence of many isolated populations of these species it is remarkable that no local genotypic differentiation has been detected. This is especially noteworthy as they are related species that may compete with each other in one area or may compete with different species in different areas.

R. hederaceus and *R. omiophyllus* show complete hybrid inviability at the diploid level and hybrid sterility at the tetraploid level, but at the same time can be regarded as belonging to a single potential gene pool (hologamodeme) as diploid *R. hederaceus* and tetraploid *R. omiophyllus* will form fertile hybrids with *R. tripartitus*, while tetraploid *R. hederaceus* and tetraploid *R. omiophyllus* will form fertile hybrids with *R. baudotii*. The genetic isolating mechanism is apparently genic and operates between *R. hederaceus* and *R. omiophyllus* alone; one can visualize it as being the result of a comparatively simple mutation. If this is the case then it is possible that sympatric speciation, not dependent on polyploidy, has taken place. Putative records of *R. omiophyllus* and *R. hederaceus* from inter- and post-glacial times are given by Godwin (1956), but after re-examination of some fossil material I find it impossible to distinguish between these species from their quaternary remains so, unfortunately, no acceptable information can be reported about their geographical ranges in the past.

It is possible that *R. hederaceus* and *R. omiophyllus* differentiated allopatrically but if this were the case it seems unlikely that a simple genic sterility mechanism would be present in areas where the species are allopatric and that this mechanism would affect these two species alone within the whole subgenus.

Perhaps of more immediate interest is the phenomenon of two closely related species living in the same area. Harper *et al* (1961) in a review on this subject point out that much interest has been concentrated on the mechanisms whereby productive isolation is achieved and fixed but that this is only part of the process leading to systematic divergence.

R. hederaceus and *R. omiophyllus* are morphologically very similar, only differing in quantitative characters such as the degree of fusion of the stipules to the petiole, depth of sinus between leaf-lobes, ratio of petal length to sepal length and relative position of style. These two species have more attributes in common than they do with any other species of the subgenus *Batrachium*; they also show homologous phenotypic variation patterns which are seen in other aquatic species of the subgenus. I can find no evidence to suggest that these two species have not differentiated fairly recently (in terms of *Batrachium* evolution) from some common stock. It is hoped to discuss the position of these two species in the evolutionary pattern of *Ranunculus* subgenus *Batrachium* in a later paper but the leaf morphogenesis with extreme proleptic growth of stipules (Cook 1963) and presence of

stomata on the upper leaf-surface suggests that these species have had an aquatic ancestor.

Aquatic plants that are partially emergent are subjected to an extreme disruption in space as the lower parts may be submerged in water while the upper are in air. Bradshaw (1965) points out that any disruption that approaches the size of an individual plant will form a selection pressure favouring phenotypic plasticity. It is a safe generalization to say that emergent aquatics do show a considerable amount of phenotypic plasticity in many characteristics; Glück (1923, 1924 and 1936), for example, gives many examples. An aquatic ancestry may well explain how, or perhaps where, *R. hederaceus* and *R. omiophyllus* acquired their considerable degree of plasticity, but once acquired, phenotypic responses will require special selection pressures to restrict them before selection favouring different genotypes can take place.

Peplis portula and *Montia fontana* frequently occupy the same habitat and have essentially the same life-form and ecology as *R. hederaceus* and *R. omiophyllus* but show very different phenotypic response patterns (Glück 1923). The genera *Peplis* and *Montia* contain no submerged aquatic species nor have they any living aquatic relatives so it is unlikely that they have evolved from aquatic ancestors. This difference in phenotypic response may provide further evidence that *R. hederaceus* and *R. omiophyllus* have retained their phenotypic plasticity from aquatic ancestors.

In conclusion, *R. hederaceus* and *R. omiophyllus* are a pair of related species that appear to occupy the same ecological niche. They are genetically isolated and differ in quantitative morphological features. Because they grow together it is impossible to suggest any adaptive advantage for any of the differential morphological characteristics. It is unlikely that any of these morphological differences are due to genetic drift as both species are found in numerous spacially isolated populations and they show a very high degree of inbreeding. My own view is that these two species represent two distinct homozygous strains that have survived from a polymorphic species-group that evolved from some aquatic species of *Ranunculus* subgenus *Batrachium* by loss of capillary leaves, and that these two species owe their success today to plastic responses carried over from their aquatic ancestors.

FORMAL DESCRIPTIONS

RANUNCULUS HEDERACEUS L., Sp. Pl. 556 (1753).

R. hederaceus Salisb., Prod. Stirp. 373 (1796);

Batrachium hederaceum (L.) S. F. Gray, Nat. Arr. Brit. Pl. 2, 721 (1821);

R. hydrocharis B, *homioiophyllus* var. *hederaceus* (L.) Spenner, Fl. Frib. 3, 1008 (1829);

R. hydrocharis Spenner, 'form'* *hederaceus* (Salisb.) Hiern, J. Bot. Lond. 9, 67 (1871);

R. asarifolius Diard, exsicc. fide Rouy & Fouc., Fl. Fr. 60 (1893).

Typification

Benson (1954) designated the specimen on sheet number 74(Savage 1945) in the Linnaean Herbarium, Linnaean Society, London (LINN) the lectotype of *R. hederaceus*. I have examined this specimen and can find no reason to doubt Benson's designation.

Description

Prostrate annual or perennial. Leaves opposite or alternate; stipules adnate to petiole for more than half their length, apex acute; petiole 2-4 times as long as the lamina; lamina 4-25 mm long, 3-35 mm wide, reniform to subcordate with 3 or 5 lobes; lobes broadest at the base; margin entire or crenulate. Sepals (1.0-) 2-2.5 (-3.0) mm long, spreading. Petals (1.25-) 2.5-3.5 (-4.25) mm long, † ovate, not continuous; nectary pits lunate. Stamens (4-) 7-10 (-11). Carpels (9-) 18-24 (-42), glabrous; style lateral. Receptacle glabrous.

*Hiern's 'forms' or 'ultimate forms' as he calls them on p. 44 *loc. cit.* have no definite taxonomic rank.

†Petal length and sepal length are positively correlated.

- RANUNCULUS OMIOPHYLLUS* Ten., Fl. Nap. 4, 338 (1830).
R. coenosus Guss., Suppl. Fl. Sic. Prod., 187 (1832);
 ?*R. aquatilis* L. var. *homophyllus* *Sensu* Moris, Fl. Sard. 1, 26 (1837).
R. lenormandii F. Schultz, Bot. Zeit. 20, 726 (1837);
R. reniforme Desportes, Fl. Sarthe and Mayenne 3 (1838);
R. hederaceus L. var. *grandiflorus* Bab., Man. Brit. Bot. 5 (1843);
Batrachium lenormandii (F. Schultz) F. Schultz, Arch. Fl. Fr. & Allem. 70 (1844);
B. coenosum (Guss.) Nym., Bot. Notis. 100 (1852);
R. hydrocharis Spenner, 'form' *homoeophyllus* (Ten.), 'form' *lenormandii* (F. Schultz) Hiern, J. Bot. Lond. 9, 66 (1871);
R. mauritanicus Pommel, Nouv. Mat. Fl. Atl. 249 (1874);
R. hederaceus L. subsp. *coenosus* (Guss.) Arcangeli, Comp. Fl. Ital. 8 (1882);
R. hederaceus L. proles *homoeophyllus* (Ten.) Rouy & Fouc. Fl. Fr. 60 (1893);
R. hederaceus L. var. *homoeophyllus* (Ten.) Fiori & Paoletti, Fl. Anal. Ital. 1, 500 (1898);
B. omiophyllum (Ten.) C. D. K. Cook, Mitt. Bot. Staatss. München, 3, 600 (1960).

Typification

Written below Tenore's original description (*loc. cit.* p. 339) appears 'Nasce ne' fossi fangosi della Basilicata e della Calabria : a Balvano, Cassano, Corigliano.' In Tenore's own herbarium at Naples (NAP) the most complete specimen is one collected from Balvano; it is affixed to a label bearing *R. omiophyllus* in Tenore's own handwriting. I designate this specimen the lectotype of *R. omiophyllus*.

Description

Prostrate annual or perennial. Leaves opposite or alternate; stipules adnate to petiole for half or less their length, apex obtuse; petiole 3-6 times as long as lamina; lamina 2-26 mm. long, 3-32 mm. wide; reniform to sub-orbicular with 3 or 5 lobes; lobes narrowest at base; margin crenate. Sepals (1.75-) 2.5-3 (-3.75) mm. long, reflexed. Petals (3.75-) 5-6 (-7.00) mm. long, ovate not contiguous; nectary pits lunate. Stamens (5-) 7-10 (-13). Carpels (15-) 20-50 (-67), glabrous; style terminal. Receptacle glabrous.

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