STUDIES IN *RANUNCULUS*

IV. ADDITIONAL EXPERIMENTS WITH *RANUNCULUS BULBOSUS* AND *R. ACRIS*

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We have previously described (Marsden-Jones & Turrill, 1929) experiments with British material of the two common species of buttercup. In a later paper (Marsden-Jones & Turrill, 1935) some further experiments regarding sex in *Ranunculus acris* were considered. Experiments were continued till the beginning of the last war and the results obtained are dealt with below.

Ranunculus bulbosus

Stock plant 13. Locality: Stevenage, Herts, 1920; communicated by W. E. T. Ingwersen, 1925.

A normal plant in respect of habit and leaves. Stems up to 30 cm. high. Flower colour pale, diameter 2·6 cm. Sex normal. Petals 1·3 cm. long, 1·0 cm. broad. Filaments 4·5 mm., anthers 3 mm. long.

Stock plant 17. Locality: Bradfield, Berks, 1930.

A low-growing plant with stems up to 20 cm. high but often much shorter. Basal leaves with blades slightly less divided than is normal in the species. Cauline leaves with blades simple to somewhat lobed, broad relative to their length, of rather thick texture. Flower colour full yellow, diameter from 8 mm. to 2·4 cm. Petals show a great range in shape and size, especially from flower to flower, from 3·5 mm. long and 1·5 mm. broad to 12 mm. long and 8 mm. broad, often asymmetrical. Sex minus normal. Filaments 3·5 mm., anthers 2·5 mm. long.

The following crosses are described below:

A.69 = S.P.17 × S.P.13.
A.82 = A.69 plant 1 × A.69 plant 3.
A.95 = A.69 plant 4 × A.69 plant 15.
A.96 = A.69 plant 15 × A.69 plant 4.
A.97 = A.69 plant 8 × A.69 plant 13.
A.98 = A.69 plant 16 × A.69 plant 4.
A.99 = A.69 plant 4 × S.P.17.
A.100 = A.69 plant 16 × S.P.17.

A69 = S.P.17 × S.P.13. 18 plants in the family.

Flower colour normal in all plants. Petals fully developed in all plants except in no. 1, which, in 1939, had many flowers with small petals. Three scorings were made for sex (11. v. 37, 10. vi. 37, 27. v. 39). No plant was fixed hermaphrodite or fixed female. Every plant had either intermediate flowers (minus normal or abnormal) or flowers that fluctuated seasonally or both.

A.82 = A.69 plant 1 × A.69 plant 3. 8 plants in the family.

Flower colour normal in all plants. Petals normal except in some flowers of no. 7. Two scorings were made for sex (10. v. 39 and 2. vi. 39): normal, 6, minus normal, 1, fluctuating, 1.

A. 95 = A. 69 plant 4 × A. 69 plant 15. 22 plants in the family.

Flower colour: normal, 21, pale, 1. Petals normal except in some flowers in nos. 7, 8, 9, 10, 17, 18, 22. Three scorings were made for sex (19. v. 39, 27. v. 39, 2. vi. 39): normal, 10, female, 1, intermediate or fluctuating, 11.

A. 96 = A. 69 plant 15 × A. 69 plant 4. 14 plants in the family.

Flower colour: normal, 12, pale, 1. Petals normal except in some flowers in nos. 4, 8, 11, 13. Three scorings were made for sex (19. v. 39, 26. v. 39, 2. vi. 39): normal, 6, intermediate or fluctuating, 8.

A. 97 = A. 69 plant 8 × A. 69 plant 13. 13 plants in the family.

Flower colour: normal, 11, pale, 1. Petals normal except in some flowers in no. 3. Three scorings were made for sex (19. v. 39, 27. v. 39, 2. vi. 39): normal, 8, female, 2, fluctuating or intermediate, 2.

A. 98 = A. 69 plant 16 × A. 69 plant 4. 17 plants in the family.

Flower colour: normal, 17. Petals normal except in some flowers in nos. 2, 15. Three scorings were made for sex (19. v. 39, 27. v. 39, 2. vi. 39): normal, 10, female, 4, fluctuating or intermediate, 3.

A. 99 = A. 69 plant 4 × S.P. 17. 9 plants in the family.

Flower colour: normal, 9. Petals normal except in some flowers in nos. 2, 7. Three scorings were made for sex (19. v. 39, 27. v. 39, 2. vi. 39): normal, 7, fluctuating, 2.

A. 100 = A. 69 plant 16 × S.P. 17. 6 plants in the family.

Flower colour: normal, 6. Petals normal except in some flowers in no. 1. Three scorings were made for sex (19. v. 39, 27. v. 39, 2. vi. 39): normal, 4, fluctuating, 2.

Ranunculus acris

Leaf blotch

In some plants of *R. acris* the leaves are entirely green throughout the growing season. In others there is a well-marked dark purplish red blotch on the upper surface of the blade towards its base. An intermediate condition with much paler coloration also occurs. The dark leaf blotch is due to suffusion with purplish red anthocyanin of a single definite layer of cells immediately below the upper colourless epidermis. This layer is a layer of the palisade, and that with the most clearly marked palisade structure, though at least the next layer below should, on structural appearance, be included as palisade tissue. The anthocyanin-containing cells, like the cells of the rest of the mesophyll, have chloroplastids, but in these hypodermal cells the green colour is masked by the purplish red anthocyanin.

We have used the following scoring symbols: G = green, with no anthocyanin; I = intermediate, with pale blotch; F = full development of anthocyanin blotch. Scoring has been done in spring, since there is more or less fading out of the colour with increasing age of the leaves.

The following tabulation is of nine quadrats, every one of one square metre on Nettens pasture field, Potterne, Wilts, 30. iv. 1937:

(1) G 7 : F 1.	(4) G 13 : F 3.	(7) G 2 : F 1.
(2) G 1 : F 1.	(5) G 13 : F 0.	(8) G 5 : F 2.
(3) G 27 : F 11.	(6) G 6 : F 2.	(9) G 4 : F 1.

Totals: G 78; F 22.

Leaf blotch in stock plants

S.P. 2, 7, 8, 30 were G.

S.P. 23, 24 were F.

Leaf blotch in selfings

A. 87 = S.P. 23 selfed, gave I 10, F 12.

A. 88 = S.P. 24 selfed, gave I 10, F 10.

Leaf blotch in F_1 and F_2 families from crossings

A. 70 = S.P. 23 × S.P. 24 gave I 17, F 33.

A. 71 = S.P. 23 × S.P. 2, gave G 1, I 22, F 24.

A. 72 = S.P. 24 × S.P. 2 gave I 24, F 26.

A. 73 = S.P. 23 × S.P. 30 gave I 24, F 25.

A. 74 = S.P. 24 × S.P. 30 gave I 22, F 15.

A. 84 = S.P. 7 × S.P. 24 gave I 22, F 19.

A. 85 = S.P. 7 × S.P. 8 gave G 2, I 29, F 2. (The two full-blotched plants were fixed females.)

A. 89 = A. 71 plant 26 (F) selfed gave I 13, F 12.

A. 90 = A. 71 plant 42 (I) selfed gave G 2, I 5, F 3.

A. 91 = A. 71 plant 3 (I) × A. 71 plant 26 (F) gave I 17, F 13.

A. 92 = A. 71 plant 3 (I) × A. 71 plant 42 (I) gave G 2, I 38.

A. 93 = A. 71 plant 12 (F) × A. 71 plant 26 (F) gave G 1, I 12, F 17.

A. 94 = A. 71 plant 12 (F) × A. 71 plant 42 (I) gave G 1, I 21, F 11.

A. 101 = A. 74 plant 5 (I) selfed gave G 6, I 12, F 9.

A. 102 = A. 74 plant 10 (F) selfed gave G 4, I 11, F 42.

A. 103 = A. 74 plant 12 (I) selfed gave G 4, I 26, F 3.

A. 104 = A. 74 plant 24 (F) selfed gave I 1, F 3.

A. 105 = A. 74 plant 5 (I) × A. 74 plant 10 (F) gave G 6, I 21, F 14.

A. 106 = A. 74 plant 5 (I) × A. 74 plant 12 (I) gave G 7, I 77.

A. 107 = A. 74 plant 24 (F) × A. 74 plant 10 (F) gave G 8, I 14, F 25.

A. 108 = A. 74 plant 24 (F) × A. 74 plant 12 (I) gave G 3, I 34, F 34.

A. 109 = A. 74 plant 10 (F) × A. 74 plant 12 (I) gave G 1, I 3, F 8.

A. 110 = A. 74 plant 5 (I) × A. 74 plant 24 (F) gave G 3, I 8, F 19.

It is possible to recognize male plants, from the correlated leaf characters, in the young non-flowering condition. The original male had green leaves. It is of interest to record that in families A. 101 to A. 110 inclusive, there were 82 male plants that are not included in the scorings for leaf blotch given above, and of these 20 had a blotch and 62 had no blotch. The leaf-blotch genes had been transferred to the gene combination for maleness in 20 plants.

Petal colour and sex in stock plants (S.P.)

S.P. 2 pale, hermaphrodite (H).

S.P. 7 type, female (F).

S.P. 8 type, male (M).

S.P. 23 type, hermaphrodite (H).

S.P. 24 type, hermaphrodite (H).

S.P. 30 type, male (M).

Petal colour and sex in selfings

A.87 = S.P.23 selfed, gave type 12, H 8, intermediate 1.

A.88 = S.P.24 selfed, gave type 11, H 8, F 1, fluctuating 1.

Petal colour and sex in F_1 and F_2 families from crossings

A.70 = S.P.23 × S.P.24, gave type 50, H 50.

A.71 = S.P.23 × S.P.2, gave type 47, H 47.

A.72 = S.P.24 × S.P.2, gave type 50, H 50.

A.73 = S.P.23 × S.P.30, gave type 49, functionally more or less hermaphrodite 49.

A.74 = S.P.24 × S.P.30, gave type 37, functionally more or less hermaphrodite 37.

A.84 = S.P.7 × S.P.24, gave type 41, F 41.

A.85 = S.P.7 × S.P.8, gave type 33; scorings for sex were made on 17. v. 39, 26. v. 39, 3. vi. 39, with the results H 7, F 10, fluctuating 16.

A.89 = A.71 plant 26 selfed, gave type 24, pale 1; scorings for sex were made on 19. v. 39, 26. v. 39, 4. vi. 39, with the results H 22, fluctuating or intermediate 3.

A.90 = A.71 plant 42 selfed, gave type 10; scorings for sex were made on 19. v. 39, 26. v. 39, 3. vi. 39, with the results H 9, intermediate 1.

A.91 = A.71 plant 3 × A.71 plant 26, gave type 19, pale 9; scorings for sex were made on 19. v. 39, 26. v. 39, 4. vi. 39, with the results H 28.

A.92 = A.71 plant 3 × A.71 plant 42, gave type 31, pale 9; scorings for sex were made on 20. v. 39, 26. v. 39, 4. vi. 39, with the results H 39, fluctuating 1.

A.93 = A.71 plant 12 × A.71 plant 26, gave type 26, pale 1; scorings for sex were made on 20. v. 39, 26. v. 39, 4. vi. 39, with the results H 21, intermediate or fluctuating 6.

A.94 = A.71 plant 12 × A.71 plant 42, gave type 27, pale 6; scorings for sex were made on 20. v. 39, 26. v. 39, 4. vi. 39, with the results H 31, intermediate 2.

Families A.101 to A.110 inclusive had all type colour flowers.

A.101 = A.74 plant 5 selfed, gave H 39, M 10 (2 H showed some fluctuation).

A.102 = A.74 plant 10 selfed, gave H 184, M 12 (5 H showed some fluctuation).

A.103 = A.74 plant 12 selfed, gave H 136, M 28 (5 H showed some fluctuation).

A.104 = A.74 plant 24 selfed, gave H 21, M 5 (6 H showed some fluctuation).

A.105 = A.74 plant 5 × A.74 plant 10, gave H 111, M 24 (7 H showed some fluctuation).

A.106 = A.74 plant 5 × A.74 plant 12, gave H 200, M 51 (26 H showed some fluctuation).

A.107 = A.74 plant 24 × A.74 plant 10, gave H 172, M 49 (10 H showed some fluctuation).

A.108 = A.74 plant 24 × A.74 plant 12, gave H 147, M 45 (36 H showed some fluctuation).

A.109 = A.74 plant 10 × A.74 plant 12, gave H 228, M 48 (22 H showed some fluctuation).

A.110 = A.74 plant 5 × A.74 plant 24, gave H 115, M 38 (17 H showed some fluctuation).

Evidence of apomixis. Flowers of A.74 plant 12 were deanthered and not pollinated in the insect proof breeding house. 26 apparently 'good seeds' (achenes) were produced and on sowing 17 germinated (one seedling had 3 cotyledons) to produce H 13, M 4.

Colchicine treatment. The soaking of 240 achenes with 0·2, 0·4, 0·8, 1·0% colchicine solution for 10 days produced no mutations from the seeds that germinated.

Discussion

(1) Ranunculus bulbosus

Petal colour. In F_2 families from the cross type colour × pale colour there were obtained type 69, pale 3 (=23:1). In two families resulting from back-crossing with the type-coloured parent, the total was type 15, pale 0.

Sex. In F_1 from the cross intermediate (minus normal) × hermaphrodite all plants were intermediates or fluctuating. The F_2 families summated as H 40, F 7, intermediate or fluctuating 26.

Petal development. Poor petal × normal petal gave normal 17, poor petal 1 (in A. 69) and normal 7, poor petal 1 (in A. 82). The summation for F_2 families from the crossing of F_1 plants with normal petals was normal 52, poor petal 14 and for backcrosses of F_1 plants with normal petals by the poor petal parent it was normal 12, poor petal 3. It is possible that there is here a monohybrid scheme modified by apomixis.

It is of importance to note that the 'poor petal' effects are not all seen or all seen equally in every flower or every petal. Morphologically the conditions appear to be comparable with what occurs in *R. auricomus* (see Weiss, 1927).

(2) Ranunculus acris

Leaf blotch. The class termed 'intermediate' (I) is not a uniform class, since the anthocyanin is developed in various degrees, varying in intensity and the area covered, from plant to plant, but it is always much less than in the full blotch (F) class. The difficulty is in demarcating the I (intermediate) and G (green) classes. It has not been possible to explain the figures satisfactorily. Attention may be called to the following: (1) No plants with full blotch have bred true for this character. (2) Relatively very few plants with completely green leaves appeared in either F_1 or F_2 families. (3) The ratios 1:1 and 2:1 were frequent, particularly if classes G and I are united and contrasted with F. (4) I plants bred together (A. 92 and A. 106) gave no F plants. (5) Offspring from S.P. 7 (A. 84), presumably produced apomictically, were female as the parent, but segregated for leaf blotch. In a previous experiment with S.P. 7 there was also segregation for leaf blotch but also some for sex (see Marsden-Jones & Turrill, 1935, p. 368). (6) It is possible that three or more independent genes are concerned and that certain combinations of these result in lethality.

Petal colour. S.P. 2 had pale petals. Used as pollen parent on two plants with type-coloured petals and homozygous for this character only plants with type-coloured petals appeared in F_1 (A. 71, A. 72). In F_2 families from the former the summated figures were: type colour 137, pale colour 26 = 5·27: 1. Deleting the one true breeding F_2 family (A. 90) the corresponding figures are: type colour 127, pale colour 26 = 4·88: 1. S.P. 2 on selfing gave type colour 8: pale colour 27 (Marsden-Jones & Turrill, 1929, p. 174). Pale colour of petals is constantly correlated with reduced size, growth, and vigour of the plant as a whole.

Sex. A female (S.P. 7) pollinated by a hermaphrodite (S.P. 24) gave only females but pollinated by a male (S.P. 8) there was segregation for hermaphrodite, female, and fluctuating conditions in F_1. We assume that apomixis accounts for the former since we know from previously published work that S.P. 7 was prone to apomixis. The 10 female plants in the second of the above crosses were also probably the result of apomixis.

In A. 74 a hermaphrodite × male gave in F_1 only functionally more or less hermaphrodite plants. Summating F_2 families the figures were: functionally more or less hermaphrodite

1353, male 310, a ratio of 4·36:1. It is known from previous work that the number of male plants appearing in F_2 families is often below expectation. There is thus a probability that the figures represent a basic 3:1 ratio and the **FFMM** scheme holds (see Marsden-Jones & Turrill, 1935).

SUMMARY

This paper is a continuation of two previous papers on the genetics of *Ranunculus bulbosus* and *R. acris*. Characters studied are flower colour and sex in both species, poor development of petals in the former and leaf blotch in the latter species. Evidence is given that these have all a genetic basis, but the ratios are often disturbed by the occurrence of apomixis and, particularly in *R. acris*, semi-lethality and perhaps of lethality with certain gene combinations.

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