Apomictic biotypes in *Potentilla intermedia* and *P. norvegica*

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Material from localities in N. Scania, Sweden, was used in the present investigation. The chromosome number was $2n=56$ in *P. intermedia* (as previously counted in Polish material) and $2n=70$ in *P. norvegica* (as in many previous counts). Crossing experiments indicated pseudogamy in both species. Apomictic reproduction has been reported previously in *P. intermedia*, while *P. norvegica* has been supposed to be sexual. Embryologic studies gave evidence of obligate apospory in *P. intermedia*, while in *P. norvegica* some of the primary EMC:s undergo meiosis. In the latter species, partial sexuality is not quite excluded, although it reproduces mainly by apomixis. Meiosis on the male side is fairly irregular in *P. intermedia* while on the other hand *P. norvegica* has a quite regular bivalent formation, in spite of its high degree of polyploidy. *P. intermedia* is supposed to be of hybrid origin, being the result of crosses between *P. argentea* and *P. norvegica*. This remains to be proved experimentally, however, since crossing experiments between *P. argentea* and *P. norvegica* have so far failed to give any offspring.

*Potentilla intermedia* L. and *P. norvegica* L. are supposed to be two fairly closely related species. In the monograph by WOLF (1908) both are assigned to the grex *Rivales* of the subsection *Conostylae*, series *Orthotrichae*. JANICH (1957) places them within the series *Acephalae* (= *Rivales*), giving to *Orthotrichae* the standing of subsection and to *Conostylae* that of section. In any case, in its morphological characteristics, *P. intermedia* shows affinities to *P. norvegica*, but also to *P. argentea*. According to WOLF, the latter species belongs to another series, *Eriotrichae*, of the same subsection. By some taxonomists, *P. intermedia* is regarded as a species of hybrid origin, representing a stable hybrid between *P. norvegica* and *P. argentea* (GUSTAFSSON 1947 a; JANICH 1957; SKALINSKA and CZAPIK 1958).

*P. norvegica* has a wide distribution in Europe and Asia. *P. intermedia* has its main area of distribution in European Russia, from where it has been spreading westwards. Both species are anthropochorous at least in part of their ranges, for instance in Scandinavia. As introduced, they are also found in North America.

The chromosome number usually reported for *P. norvegica* is $2n=70$ (for instance, by GENTCHEFF 1938; A. LÖVE 1954 a, b; A. and D. LÖVE 1956; MULLIGAN 1959). However, POPOFF (1939) also stated the numbers $2n=42$ and $2n=56$ for material from Royal Bot. Garden, Kew, and Hort. Bot. Bergianum, Stockholm, respectively. In Northern America, where *P. norvegica* is only occasionally introduced, instead the “substitution species” *P. monspeliensis* is indigenous. This species has $2n=56$ (LÖVE 1954 a, b).

Concerning *P. intermedia* three chromosome numbers have been published. $2n=28$ was given by SHIMOTOMAI (1930 a, b; 1935); unfortunately, the origin of his material is unknown. POPOFF (1939) found $2n=42$ in collections obtained from the Botanical Gardens of Copenhagen and the Belorussian scientific academy. The number $2n=42$ was also found in introduced Canadian material, belonging to var. *canescens*, by MULLIGAN (1959); while $2n=56$ was counted in Polish plants by SKALINSKA and CZAPIK (1958). Some of the previous counts cited in MULLIGAN’s paper are erroneously attributed by him to *P.
intermedia var canescens, and refer instead to the species P. canescens Besser.

In the surveys on apomixis in the angiosperms by Gustafsson (1947 b) and Nygren (1954) P. intermedia, but not P. norvegica, is included among species with known or suspected apomixis. As for P. intermedia, this refers to the only mode of reproduction of this species known to me, by Shimotomai (1935). The cross P. intermedia \( (2n=28) \times P. \) pimpinelloides gave rise to maternal intermedia plants only, while castrated flowers did not set any seeds without pollination. This indicated pseudogamy here, but no embryological investigation on P. intermedia seems to have been carried out.

For P. norvegica, a sexual mode of reproduction was indicated, according to Gentcheff (1938). A cross P. norvegica \( (2n=70) \times P. \) argyrophylla \( (2n=63) \) gave rise to hybrids only, as judged by the morphology of the offspring. However, the chromosome numbers of the F\(_1\) plants were not determined. Meiosis in P. norvegica was spoken of as "normal". On the contrary, A. Löve (1954 a) stated that both P. norvegica and P. monspeliensis "seem to be obligate agamosperms". Neither in these species has the embryology been studied.

Material and methods

P. norvegica was collected on the railway bank in the parish of Farstorp, N. Scania. P. intermedia was collected from some localities along highroads in the same parish and in the adjacent one, Vankiva. Although the latter species must be considered as rare in Sweden, it is rather frequent in this area.

Besides, in the crosses listed in Table 1, biotypes of the species P. argentea, crantzii, tabernaemontani and erecta were used as staminate parents. The material belonging to the three first-mentioned species was studied by A. Müntzing (1928, 1931, 1958) and A. and G. Müntzing (1941).

Chromosome numbers were determined on preparates from root tips fixed in the Svalöv modification of Navashin-Karpechenko (Müntzing 1933) after refrigeration for at least 12 hours in order to get chromosome contraction. The root tips were embedded in paraffine, cut into sections of 14 \( \mu \) and stained with crystal violet, after bleaching of the cytoplasm in hydrogen peroxide and mordanting in 1% chromic acid.

For meiotic studies, buds were fixed in a 3: 2: 1 mixture of ethyl alcohol, chloroform and acetic acid for 1 hour and then in a 3: 1 alcohol and acetic acid for 12–24 hours. For staining,

### Table 1. Species crosses with P. intermedia and P. norvegica as pistillate parents

| Pistillate parent | Localities | Chrom. numb. | Staminate parent | Localities | Chrom. numb. | Year | Flowers crossed | Seeds Obtained |
|------------------|------------|--------------|------------------|------------|--------------|------|----------------|----------------|
| P. intermedia    | Farstorp   | 2\( n = 56 \) | P. argentea      | Dalby      | 2\( n = 14 \) | 1964 | 7              | 33             |
|                  |            |              | P. crantzii      | Lomma      | 2\( n = 42 \) | 1968 | 10             | 100            |
|                  |            |              | P. tabernaemontani | Bondkyrka | 2\( n = 42 \) | 1964 | c. 15           | c. 500         |
|                  |            |              | P. norvegica     | Västergötland | 2\( n = 42 \) | 1964 | c. 10           | c. 100         |
|                  | Vankiva    | 2\( n = 56 \) | P. crantzii      | Farstorp   | 2\( n = 70 \) | 1968 | 10             | 2              |
|                  |            |              | P. erecta        | Farstorp   | 2\( n = 28 \) | 1964 | 6              | 3              |
|                  |            |              | P. tabernaemontani | Bondkyrka | 2\( n = 42 \) | 1964 | ?              | ?              |
| P. norvegica     | Farstorp   | 2\( n = 70 \) | P. argentea      | Blekinge   | 2\( n = 28 \) | 1965 | 5              | c. 200         |
|                  |            |              |                  | Lomma      | 2\( n = 42 \) | 1964 | 8              | 13             |
|                  |            |              |                  |            |              | 1965 | 6              | 75             |
|                  |            |              |                  |            |              | 1968 | 6              | 100            |
|                  |            |              |                  |            |              | 1968 | 106            | 0              |
|                  |            |              |                  |            |              | 1968 | 0              | 0              |

*Hereditas* 66, 1970
alcoholic hydrochloric-acid carmine was used (Snow 1963). The anthers were squashed in Hoyer's medium (Beeks 1955).

The percentages of morphologically good pollen were estimated on preparates stained with lactophenolic fuchsine.

Embryological studies were performed on buds fixed in Navashin-Karpechenko after a short prefixation in 1:3 acetic alcohol. After embedding in paraffine and sectioning, the material was stained with Heidenhain's haematoxylin.

Before controlled crossings, buds were emasculated about one day before opening. The pollination was performed by twirling flowers of the staminate parent around the gynaecia of the pistillate parent.

The distribution of phenolic compounds in leaves was studied by means of thin-layer chromatography according to the Balsgard system (Nybom 1968; Askér and Fröst 1970).

Results

1. *P. intermedia*

The chromosome numbers of two collections from Farstorp and one from Vankiva were found to be \(2n=56\), the same as in the Polish material studied by Skalinska and Czapik (1958). No morphological differences could be observed between plants from different localities after cultivation in uniform environment. As, furthermore, the offspring of individual plants was very uniform, the suspicion arose that the plant material belonged to one single apomictic biotype.

To check the mode of reproduction, some crossing experiments were performed in 1964, and were in part repeated in 1965 and 1968 (Table 1). Only interspecific crosses were made, since no other *intermedia*-types were available.

The crossing result strongly supported the opinion that apomictic reproduction was predominating here. The offspring was almost exclusively of maternal plants. Only in the crosses to *P. crantzii* and *P. tabernaemontani*, one hybrid plant was obtained in each combination. The hybrids were weak, non-flowering, and in appearance most similar to their pistillate parent.

As their chromosome numbers were found to be \(2n=c.\ 80\) and \(2n=77\), respectively, they were undoubtedly B III hybrids in the sense of Rutishauser (1948), arisen through occasional fertilizations of unreduced egg cells. Castrated flowers gave no seed-set after isolation, indicating that the *intermedia* biotype was pseudogamous like other *Potentilla* apomicts.

Pollen fertility, measured as the percentage of morphologically good pollen, was 64.4% when counted in the summer of 1970 (somewhat lower in earlier counts). On the same occasion, pollen fertility was 82.0% in *P. norvegica* and 83.5% in a hexaploid *argentea* biotype from Lomma.

The rather low pollen fertility in *P. intermedia* indicated meiotic disturbances, which was also verified by a study of meiosis in the anthers. The course of meiosis here was in its broad outline the same as that described by Rousi (1965) for the *P. anserina* complex (Fig. 4–6). Univalents occurred in most cells in first metaphase, as many as 6–7 being sometimes observed. Laggards were frequent in first anaphase. Probably trivalents and quadrivalents occurred in a few cells in first metaphase.

Embryological studies indicated that *P. intermedia* is obligately aposporous (Fig. 1–3). The development of the archespore proceeds in the way described by Hunziker (1954) and Rutishauser (1967). The primary EMC:s arrive at a pachytene-like stage; no later meiotic stages were

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**Table 1**

| Sown | Germ. | Mature plants | Hybrids |
|------|-------|---------------|---------|
| 33   | 3     | 2             | None    |
| 50   | 33    | 23            | None    |
| 30   | 26    | 25            | 1 BHH \((2n=\text{c.}\ 80)\) |
| 30   | 28    | 28            | 1 BHH \((2n=77)\)          |
| 2    | 1     | 1             | None    |
| 3    | 3     | 3             | None    |
| 30   | 29    | 28            | None    |
| 15   | 14    | 14            | None    |
| 60   | 55    | 55            | None    |
| 13   | 13    | 13            | None    |
| 60   | 49    | 49            | None    |
| 50   | 15    | 4             | None    |
| 50   | 14    | 8             | None    |

*Hereditas* 66, 1970
observed. Somatic cells in the chalazal region enlarge, become vacuolized and finally give rise to unreduced aposporous embryosacs of the usual Polygonum-type. Often several embryosacs in various stages of development are seen in the same ovule.

2. P. norvegica

The biotype from Farstorp had the chromosome number $2n=70$, which has also been obtained in most previous counts from this species. Also in this species offspring from seeding appeared to be uniform.

In the crosses with P. argentea and P. intermedia, only maternal plants were obtained. If hybrid seeds had arisen, either they did not germinate, or the seedlings died at an early stage. Thus, even here, an apomictic mode of reproduction was indicated. No B III hybrids resulted from the crosses, as in P. intermedia. However, the species P. crantzii and P. tabernaemontani, which gave hybrids when crossed to P. intermedia, were not used as pollinators for P. norvegica (cf. Table 1).

The high pollen fertility, as compared to that of P. intermedia, indicated a fairly regular meiosis, in spite of the high degree of polyplody. Meiotic studies showed that this was the case, and many cells with 35 bivalents were observed (Fig. 7–9). Univalents were much more scarce than in P. intermedia. If tri- or quadrivalents occurred, they must have been very rare.

Unfortunately, the embryologic observations in P. norvegica are still inconclusive. The primary EMS:s sometimes seem to undergo meiosis, since tetrads were observed in some cases. It is uncertain if reduced embryosacs are formed.

Thus, after pollination by other species, only a parthenogenetic development of unreduced egg cells takes place. A partially sexual mode of reproduction can, however, not be quite excluded, since reduced egg cells might function in other cross combinations. Further crosses, especially with other norvegica biotypes, as well as continued embryological studies, are needed to ascertain the degree and type of apomictic reproduction in P. norvegica. Pseudogamy is indicated

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Fig. 1–3. Embryosac development in Potentilla intermedia.—Fig. 1a and b. Section of a young nucellus in two different foci. Top: Primary EMC in early prophase I of meiosis. Bottom: Enlarging and vacuolized somatic cell becoming an embryosac initial. Fig. 2. Some young aposporous embryosacs in an ovule. Fig. 3. Mature embryosac. Scale equals 10 μ, Fig. 1 and 2 have the same magnification.

Hereditas 66, 1970
Fig. 4-9. Meiosis in *P. intermedia* (Fig. 4-6) and *P. norvegica* (Fig. 7-9): - Fig. 4 Pachytene. - Fig. 5 Metaphase I with univalents outside the plate. - Fig. 6 Anaphase I with lagging dividing univalents. - Fig. 7 Diplotene. - Fig. 8 Diakinesis with 35 bivalents. - Fig. 9 Anaphase II. - Scale indicates 10 μ.

*Hereditas* 66, 1970
even here, since no seed set occurs after emasculation and isolation.

The chromatographic pattern of phenolic compounds was studied and compared to that of other Potentilla species by ASKER and FRÖST (1970). Rather few compounds showed their presence in the chromatograms of P. intermedia and P. norvegica. However, these species showed rather great similarities inter se. Their patterns were also somewhat related to those of P. argentea, but not to those of P. collina, tabernaemontani and crantzii.

Discussion

As stated in the beginning of this paper, P. intermedia has been suspected to represent stabilized hybrids between P. argentea and P. norvegica, in the same way as the series Collinae is thought to have arisen through hybridization between members of Argenteae and Aureae, and P. canescens (by some authors at least) from crosses between Argenteae and Rectae. My primary intention, when beginning this study, was to hybridize P. norvegica (which was then thought to be sexual) with P. argentea in the hope of getting hybrids of intermedia phenotype. Also the chromosome number 2n=56 of the intermedia biotype studied here supported the view that it could have been formed by crosses between P. norvegica (2n=70) and P. argentea (in which 2n=42 is the most frequent number, at any rate outside Scandinavia). The origin of intermedia-biotypes with 2n=28 and 2n=42 could not, however, be explained in this way.

In this connection the report on sexuality in P. norvegica by GENTCHEFF (1938) should be considered. The Potentilla type assigned by GENTCHEFF to P. norvegica had 2n=70 and, obviously, a sexual reproduction. However, CHRISTOFF and PAPASOVA (1943) had to revise many classifications of Potentilla species in the Botanical Garden of the Agricultural Faculty of Sofia. This material had earlier been studied by POPOFF (1939) who still used erroneous names of several species. Some of the chromosome numbers given by POPOFF are thus open to some doubt, for instance the statement of the numbers 2n=42 and 2n=56 in P. norvegica, which have not been met with elsewhere. Furthermore, CHRISTOFF and PAPASOVA found that the "P. norvegica" from the Botanical Garden of Frankfurt with 2n=70, mentioned by POPOFF, in reality belonged to the species P. rivalis NUTT. GENTCHEFF (1938) did not mention the origin of his material, but since at that time he was also working in Sofia, it seems very probable that he used the same plant material as the preceding workers and that his sexual type with 2n=70 was in reality P. rivalis instead of P. norvegica.

So far, the attempts to synthesize P. intermedia from crosses between P. argentea and P. argentea have not been successful. Owing to apomixis in P. norvegica, only maternal plants were obtained from the crosses P. norvegica × argentea. The reciprocal cross (argentea × norvegica), using as pistillate parent a sexual autotetraploid derived from a mainly apomictic diploid (ASKER 1967), failed completely. No seeds were obtained, although the cross was repeated three different times. On the contrary P. intermedia, crossed to the same argentea type, gave rise to some viable and slightly fertile hybrids (ASKER, unpubl.).

The Potentilla norvegica biotype used in this study gave no hybrids following pollination from other species, not even B III hybrids which were obtained from P. intermedia in two cases (Table 1). Since the former species has a very extensive distribution area, it is likely that forms will be found with other chromosome numbers than 2n=70, the only one so far ascertained; possibly also sexual types exist somewhere within its range.

Summing up, the origin of P. intermedia remains to be tested experimentally. This species probably consists of apomictic biotypes, which have resulted from crosses between P. argentea and P. norvegica, or perhaps even other members of the series Argenteae and Acephalae (Rivales). To make this clear, material of P. intermedia and its proposed parental species has to be studied from a greater number of localities in different parts of Europe and Asia.

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Hereditas 66, 1970
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