Zeitschrift: Botanica Helvetica
Herausgeber: Schweizerische Botanische Gesellschaft
Band: 106 (1996)
Heft: 2

Artikel: Cytogeography of Medicago falcata L. and M. sativa L. in Switzerland
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DOI: https://doi.org/10.5169/seals-72199

Bot Helv 106: 197–207

0253-1453/96/020197-11 $1.50+0.20/0

Cytogeography of *Medicago falcata* L. and *M. sativa* L. in Switzerland

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Manuscript accepted May 22, 1996

Abstract

Savova D. 1996. Cytogeography of *Medicago falcata* L. and *M. sativa* L. in Switzerland. Bot. Helv. 106: 197–207.

The chromosome numbers of *Medicago sativa* L. and *M. falcata* L. from Switzerland were determined. *M. sativa* was always tetraploid with 2n = 32 chromosomes. *M. falcata* was tetraploid in Valais, Schaffhausen and the Rhine valley of Graubünden. In contrast, *M. falcata* was diploid (2n = 16) in Unterengadin providing an additional example of the affinity of floristic elements of that region with those of Eastern Europe, where this type is also present. The hybrid between *M. sativa* and *M. falcata, M.* × *varia* Martyn was found frequently in the areas where tetraploid *M. falcata* was present, but was very rare within the range of the diploid type. Our results are discussed in the context of conservation of genetic resources and risk of transgene escape.

Key words: Medicago, alfalfa, karyology, phytogeography, risk assessment, conservation biology, Switzerland.

Introduction

Cultivated alfalfa *Medicago sativa* L. (Fabaceae), one of the most important forage crops in the world, and its wild relative *M. falcata* L. are perennial species. Morphologically, they differ mainly in leaf shape, flower colour and pod shape. *M. sativa* has a deep tap root, erect stems, obovate leaves, cuneate at base, purple flowers and coiled pods, while *M. falcata* has a spread well-branched root system, prostrate stems, smaller and linear-obovate leaves, yellow flowers and falcate pods. The numerous intermediates between both species have been described as *M.* × *varia* Martyn.

M. sativa and *M. falcata* are either diploid or tetraploid. The original distribution of both diploid and tetraploid *M. sativa* covers the Mediterranean region, the Near and the Middle East, Caucasus, Middle, Central and South Asia (Quiros and Bauchan 1988). Tetraploid *M. sativa* was introduced in Middle, Central and South Asia (Quiros and Bauchan 1988). Tetraploid *M. sativa* was introduced in Europe, where it has been largely cultivated (Hegi and Gams 1924). According to Vollrath (1973) it is always present as an introgressed form with *M. falcata*. The recognition of the great economical value of

M. sativa was the reason for its introduction into South America, Mexico, Canada and the USA. It has been introduced also into Australia, New Zealand and South Africa (Freyer 1930). Diploid *M. falcata* is present mainly in Asia (from Siberia in the east to the Caucasus in the south) and in Eastern Europe, extending westwards to Southern Germany. It is a rare species for Denmark (Hojland and Poulsen 1994). According to Quiros and Bauchan (1988) the distribution area of tetraploid *M. falcata* coincides somehow with the one of the diploid form. However, it shows more western and northern distribution (from Portugal and France to Sweden) in Europe, in contrast to the diploid (Table 1).

In Switzerland *M. sativa* is widespread all over the country. It grows at colline level, mountain zone, and, rarely, in the subalpine zone. *M. falcata* is restricted to the north-eastern and eastern part of the country, to the canton Valais and the region of lake Geneva (Welten and Sutter 1982), from the colline level to the subalpine zone. According to these authors it has disappeared from most locations on the Swiss Plateau, where it was still mentioned at the beginning of the century. This decline can be confirmed by our analysis of historical herbarium sheets (unpublished data).

This paper reports the first chromosome numbers of *Medicago falcata* and *M. sativa* for Switzerland and describes the discovery of two ploidy levels for *M. falcata*. Moreover, these results are discussed in the perspective of potential gene flow between both species in the context of conservation genetics and risk of escape of transgenes.

Materials and methods

Living plants from 13 populations, including where possible all three species *M. sativa*, *M. falcata* and *M.* × *varia*, were collected in the summer of 1993 and the autumn of 1995 in Valais, Schaffhausen, and Graubünden (Rhine valley and Unterengadin) (Appendix 1, Fig. 1). The shoots of each plant were dried for herbarium collection and for biometrical studies (Pia Rufener Al Mazyad, in preparation), while the base and the roots were kept and cultivated in the Botanical Garden of Neuchâtel. Herbarium specimens have been deposited at the Botanical Institute of the University of Bern (BERN). In addition, the historical herbarium samples of the three species in Graubünden were examined.

Chromosomes of at least five individuals per population were counted from root tips of potted plants. The root tips were pretreated with 0.002 M 8-hydroxyquinoline for two hours at room temperature and then fixed in Carnoy's solution (3 : 1 ethanol : acetic acid). After at least one day, they were stained in 1% aceto-orcein for two hours at room temperature, followed by heating for two minutes. The somatic chromosome number was determined on metaphases.

Results and discussion

M. sativa was always found to be tetraploid ($2n = 4x = 32$) (Appendix 1, Fig. 2a).

M. falcata was found to be tetraploid ($2n = 4x = 32$) in Valais, Schaffhausen and the Rhine valley of Graubünden. Contrasting with that, all populations from Unterengadin were diploid ($2n = 2x = 16$) (Appendix 1, Fig. 2b).

M. × *varia* was always found to be tetraploid ($2n = 4x = 32$) and was present in all populations, except in those from Unterengadin. The examination of historical herbarium revealed its presence on the whole territory of Graubünden. In Unterengadin it was mentioned only in two locations – Ramosch and Schuls (Appendix 2).

Table 1. Chromosome counts of *Medicago sativa, M. falcata*, and *M.* × *varia* from the literature

Country	References
Diploid *Medicago sativa* (2n = 16)	
Armenia	Lesins 1952, Clement 1962
Canada	Mulligan 1984
Caucasus	Magulaev 1980
Iran	Lesins 1952
Transkaukasia	Lesins 1952
Turkey	Lesins 1952, Gillies 1972, Small and Bauchan 1984
Tetraploid *M. sativa* (2n = 32)	
Algeria	Clement 1962
Azerbaidzan	Kliphuis and Wiefferings 1979
Caucasus	Magulaev 1980
France	Ghimpu 1930
Greece	Strid and Franzen 1981
India	Bhaumik 1976
Iraq	Al Mayah and Al-Shebaz 1977
Italy	Gadella and Kliphuis 1970, Falistocco 1987
Poland	Bijok et al. 1971
Turkey	Small and Bauchan 1984
Slovakia	Majovsky et al. 1978
USA	Clement 1962
Diploid *M. falcata* (2n = 16)	
Bulgaria	Kozugharov and Kuzmanov 1965, Kozugharov et al. 1973, Lesins and Lesins 1964
Canada	Clement 1962
Caucasus	Lesins 1952, Lesins and Lesins 1964, Clement 1962
Denmark	Hojland and Poulsen 1994
Georgia	Lesins and Lesins 1964
Germany	Lesins and Lesins 1964
Greece	Strid and Franzen 1981
Kazakhstan	Lesins and Lesins 1964
Moldova	Lesins and Lesins 1964
Romania	Lesins and Lesins 1964
Russia	Lesins and Lesins 1964
Siberia	Lesins and Lesins 1964
Slovakia	Majovsky et al. 1974
Turkey	Small and Bauchan 1984
Ukraine	Kliphuis 1977
USA	Clement
Tetraploid *M. falcata* (2n = 32)	
Azerbaidzan	Kliphuis and Wieffering 1979
Caucasus	Magulaev 1980
France	Ghimpu 1930
Poland	Skalińska et al. 1966
Portugal	Fernandes and Queiros 1978
Siberia	Belaeva and Siplivinsky 1981
Slovakia	Majovsky et al. 1970
Sweden	Clement 1962, Lesins and Lesins 1964
Turkey	Small and Bauchan 1984
Yugoslavia	van Loon and Kieft 1980
***Tetraploid M.* × *varia* (2n = 32)**	
Slovakia	Majovsky et al. 1978
Turkey	Small and Bauchan 1984

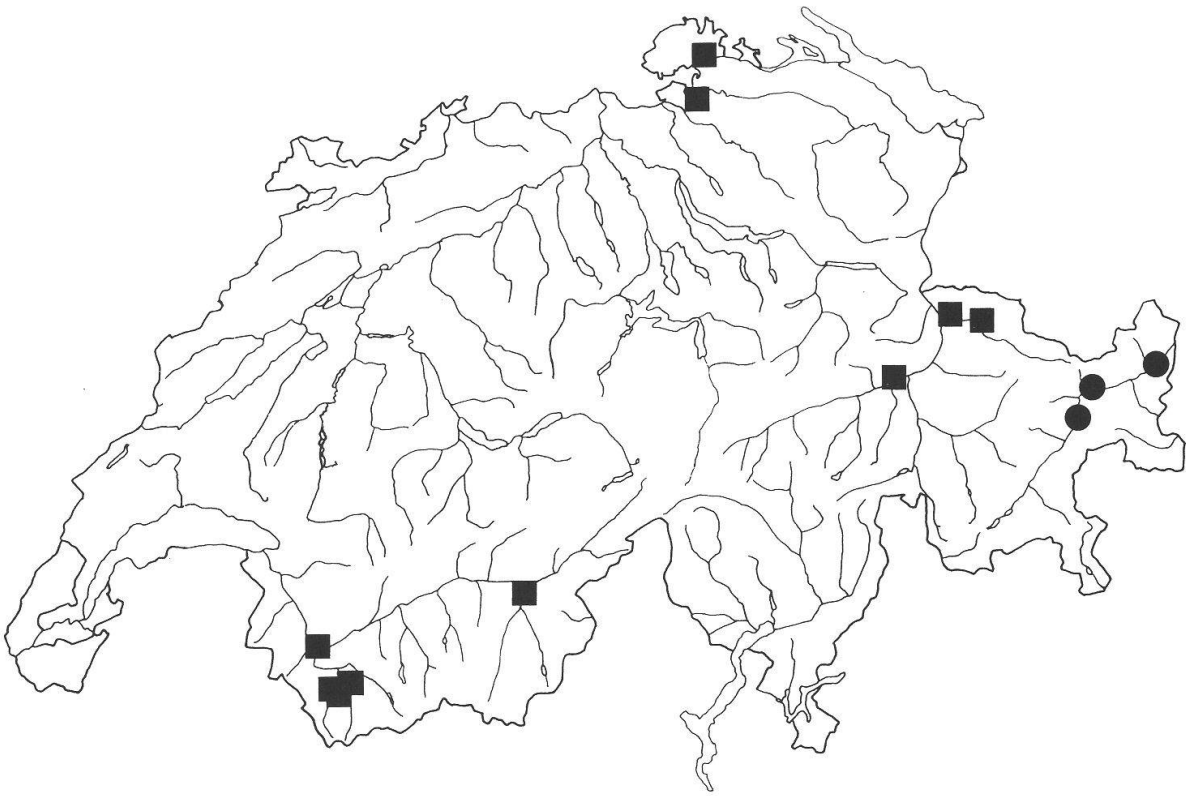

Fig. 1. Sampling sites of diploid (●) and tetraploid (■) *Medicago falcata*.

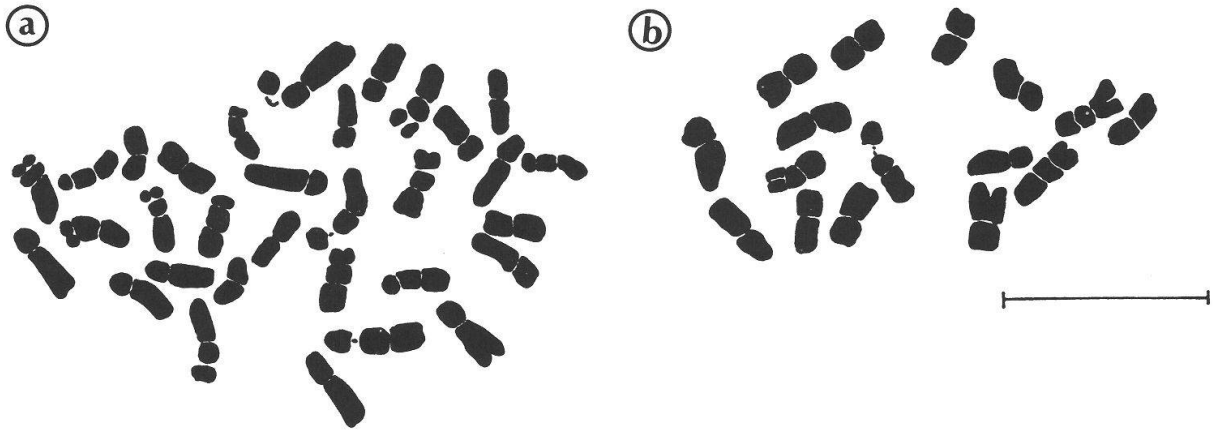

Fig. 2. Somatic metaphases from root tips; a) tetraploid *Medicago sativa*, 2n = 32 (13a 2/0); b) diploid *M. falcata*, 2n = 16 (13a 14.9/1.34). Scale bar = 10 μm.

Our chromosome counts of *M. sativa* are in accordance with the previous records from Europe (Table 1) where it is not indigenous (Hegi and Gams 1924). The absence of diploids was expected as there is not report for their presence in Europe.

Few chromosome counts of *M. falcata* have been reported for Europe. Nevertheless, the presence of tetraploids in most parts of Switzerland is consistent with previous reports from Portugal and the Balkans (Table 1).

The western distribution boundary of diploid *M. falcata* runs through the Balkans and Eastern Europe to Southern Germany (Table 1). The populations in Unterengadin lie in the margin of its range. Diploid *M. falcata* is an additional example of the affinity of floristic elements of the Unterengadin with Eastern Europe, where this cytotype is also present. At the beginning of the postglacial period (ca. 10 000 years BP) the climate favoured the expansion of the steppes. Unterengadin was probably invaded through the Inn valley, where other relicts from that period have subsisted, e.g. *Dorycnium germanicum* (Gremli) Rikli (Landolt 1986). Contrasting with that, Swiss tetraploid *M. falcata* might have migrated either from the Balkans or from Western and Northern Europe, according to its present distribution. It is moreover not ruled out that the *Medicago* colonization of Switzerland originates from different regions.

Both cytotypes present in Switzerland are probably geographically separated. A detailed study of the distribution of diploid and tetraploid *M. falcata* would determinate whether they really coexist as Quiros and Bauchan (1988) claim or whether they are geographically or ecologically separated.

In the local literature for Graubünden, no evidence of morphological differentiation mentioning both ploidy levels of *M. falcata* has been found. Even Hegi and Gams (1924), who describes a whole range of variations of *M. falcata* does not mention such a differentiation in Switzerland. A comparison of the morphology of both types of *M. falcata* by biometrics is in progress.

At the tetraploid level, *M. sativa* hybridizes freely with *M. falcata* (Ledingham 1940). Our observations of natural populations (unpublished data) support the presence of extensive gene exchange between both tetraploid species. Some of the populations we collected in the Valais, Schaffhausen and the Rhine valley of Graubünden were in apparently pure zones of *M. sativa* and pure zones of *M. falcata*, joined by a hybrid zone. Others consisted exclusively of intermediate individuals between both species (*M.* × *varia*). Thus, there is a strong evidence for intensive genetic erosion of the native tetraploid *M. falcata*. This phenomenon is not recent as Schinz and Keller mentioned already in 1923 that the bybrid *M.* × *varia* could outcompete *M. falcata*.

Due to the ploidy barrier, diploid *M. falcata* does not readily cross with tetraploid *M. sativa*. Crosses between diploid and tetraploid froms result in low fertility (Ledingham 1940, Lesins 1952). If *M. falcata* ($2n=16$) is the female, the ovules abort while the ovary may develop a well-formed pod. When *M. sativa* is the female, fertilization delaies and the development of the endosperm and of the embryo usually stops. The offspring are either sterile triploids or, rarely, tetraploids. These tetraploids are highly fertile (Ledingham 1940). In the populations from the Unterengadin neither triploid plants nor *M.* × *varia* were observed, which confirms the existence of the ploidy barrier.

From the historical floristic literature such as Killias (1888) and according to N. Bischoff, Ramosch, Unterengadin (personal communication) *M. sativa* has been a commonly cultivated crop in the Unterengadin. Feral populations are present in many places. *M. falcata* is a frequent plant in the whole valley up to 1500 m.

The presence of the hybrid *M.* × *varia* in Unterengadin is described in the literature as a rarity, which has been confirmed by local plant specialists. The origin of *M.* × *varia* in Unterengadin is unclear. It could be the result of successful hybridization between diploid *M. falcata* and tetraploid *M. sativa*. Another, more probable possibility is the introduction of seeds as a contamination within those of *M. sativa*.

Apart from genetic pressure from the more competitive *M. sativa*, the present decline of habitats as a result of intensive land use is an additional danger for the conservation of tetraploid *M. falcata*. Landolt (1991) describes *M. falcata* as *endangered* on the

Plateau and *extremely endangered* for the western part of the Jura and the Western part of the Northern Alps. He considers that this species is not threatened in the other regions of Switzerland. We believe that except from Unterengadin, where the diploid type grows, there is a danger of genetic erosion suppressing *M. falcata* in the whole country. Therefore, protection of this indigenous species as a part of our flora and as a potential genetic ressource for the improvement of cultivated *M. sativa* is urgent (see Vollrath 1973). Protection measures could consist in the prescription of a minimal distance between cultures of *M. sativa* and present populations of *M. falcata*.

Our results are also interesting for risk assessment of the escape of transgenes to the wild flora, in which we are involved through the Swiss Priority Program Biotechnology. *M. sativa* has genetically been modified (D'Halluin et al. 1990, Hill et al. 1991, Gold et al. 1991, Larkin et al. 1990, Schroeder et al. 1991, Sarul et al. 1995). The risk of transfer of modified genes to tetraploid *M. falcata* is obviously very high in any field release scenario of the Swiss Plateau and most alpine valleys and creates an additional problem for the genetical conservation of all related rare species also outside of Switzerland. Contrasting to that the risk is low for the diploid *M. falcata*. Bibliographical studies on *Medicago* indicate a high risk of gene flow between *M. sativa* and *M. falcata* (Jacot 1994, Jacot and Jacot 1994). Thus, the genetics of a species may vary geographically and may influence the ability of hybridization with other species. Our results stress the importance of empirically regionalized data for risk assessment in addition to a careful examination of the existing literature.

We wish to thank Nicolin Bischoff, Ramosch for the exciting discussions about the situation of *Medicago* in Unterengadin. We are also grateful to Konrad Lauber, Bern and Klara Röthlisberger, Langnau, both members of the Botanical society of Bern and Romedi Reinalter, Brail, who helped us to find suitable populations for our study of *Medicago* and who indicated us many local plant specialists. Thanks to Dr. Klaus Ammann, Geobotanical Institute of the University of Bern, and Prof. Phillippe Küpfer, Botanical Institute of the University of Neuchâtel, for the critical remarks and helpful comments in preparing the manuscript. Our gratitude is for Julia Keller Senften, who helped with the field work and contributed to the discovery of the diploid *M. falcata* in Unterengadin.

This work was funded by Swiss National Science Foundation (grant n° 5002-035207).

Appendix 1. Description of localities and results of the chromosome counts

Graubünden, Bonaduz, Tadi, 560 m, 749.490/186.050; tetraploid *M. falcata*, 2n = 32 (4a 26/1, 4a 0.2/14, 4a 22/1.5, 4a 13.1/1, 4b 5/2.7); tetraploid *M. sativa*, 2n = 32 (4a 17/0, 4a 6.90/0.10, 4a 19.60/0); tetraploid *M.* × *varia*, 2n = 32 (4a 21.2/3, 4a 11.0/1, 4b 3.9/7.1, 4b 10/7.1).

Graubünden, Malans, Bau, 570 m, 763.300/205.250; tetraploid *M. falcata*, 2n = 32 (5a 3.2/7.4); tetraploid *M. sativa*, 2n = 32 (5a 0.2/6.8, 5a 3/11, 5a 1.7/1, 5a 3.65/0.20, 5a 6.20/1.60, 5a 0.9/4, 5a 0.1/2.9).

Graubünden, Seewis, station Seewis-Valzeina, 595 m, 766.775/205.175; tetraploid *M. falcata*, 2n = 32 (6a 1.6/16); tetraploid *M. sativa*, 2n = 32 (6a 1.5/24.4, 6a 2.4/3.6).

Graubünden, Zernez, Sosa., 1460 m, 802.700/175.700; diploid *M. falcata*, 2n = 16 (11a 8.95/3.3, 11a 19.6/4.70, 11a 16.7/3.25, 11a 10/5, 11a 3.9/2.3, 11a 4.65/2.37, 11a 4.68-3.27, 11a 18.6/4.7).

Graubünden, Ramosch, Chants, 1400 m, 825.550/191.750; diploid *M. falcata*, 2n = 16 (12a 2, 12a 4, 12a 8).

Graubünden, Ramosch, Chants, 1350 m, 825.225/191.725; diploid *M. falcata*, 2n = 16 (12b 2, 12b 3, 12b 6, 12b 7, 12b 8, 12b 9, 12b 10).

Graubünden, Guarda, Suot la Sassa, 1450 m, 807.225/183.650, diploid *M. falcata*, 2n = 32 (13a 13.2/2.05, 13a 4.3/4, 13a 15.2/3.55, 13a 15,6/2.4, 13a 16.05/1.2, 13a 8/2.7, 13a 2.8/2.54, 13a 4.3/4.25, 13a 18.55/2.3, 13a 19.3/0.8, 13a 13.4/1.2, 13a 17.6/2.85, 13a 12/26, 13a 12.8/2.2, 13a 13.8/2.2, 13a 14.9/1.34, 13a 18/1.15, 13a 4.6/1.4, 13a 15.5/2.4, 13a 0.7/1.3, 13a 13.8/2.2), tetraploid *M. sativa*, 2n = 32 (13a 0/0, 13a 2.0/0, 13a 2.8/0, 13a 5/0, 13a 6/0, 13a 9/0, 13a 11/0, 13a 12/0, 13a 13/0, 13a 16/0, 13a 17/0, 13a 19/0, 13a 20/0, 13a 28/0).

Valais, Martigny, channel of the Drance, 465 m, 571.660/106.300, tetraploid *M. falcata*, 2n = 32 (10a 1.0/14.0, 10a 4.3/4.4); tetraploid *M. sativa*, 2n = 32 (10a 1.0/0, 10b 1/1); tetraploid *M.* × *varia*, 2n = 32 (10a 1.1/9.2, 10a 3.1/4.1, 10a 1.1/1).

Valais, Orsières, Cenaire, 1000 m, 577.700/97.000; tetraploid *M. falcata*, 2n = 32 (1a 3/10.9, 1a 1.7/12, 1a 3/8, 1a 2.2/8).

Valais, Orsières, Cenaire, 1000 m, 577.700/96.700; tetraploid *M. sativa*, 2n = 32 (1c 4/1.6, 1c 9/3, 1c 4.7/6.3).

Valais, Orsières, Cenaire, 1000 m, 577.775/96.675; tetraploid *M. falcata*, 2n = 32 (2a 0.5/10.6, 2a 17.8/5.86, 2a 11.7/7.2, 2a 16.35/5.5); tetraploid *M. sativa*, 2n = 32 (2a 4.1/6.9); tetraploid *M.* × *varia*, 2n = 32 (2a 15.7/6.8, 2a 17.8, 2a 17.8/5.6).

Valais, Orsières, Fin, 1020 m, 576.650/97.025, tetraploid *M. falcata*, 2n = 32 (3a 0/3.88, 3a 1.6/3.8); tetraploid *M. sativa*, 2n = 32 (3a 1.2/0.5, 3a 4/7, 3a 31.80/20.0, 3a 1.2/0.5, 3a 7.7/9.3); Valais, Orsières, Fin, 1020 m, 576.650/97.025, tetraploid *M.* × *varia*, 2n = 32 (3a 7.9/8.3).

Valais, Stalden, Ribe, 780 m, 634.500/125.600, tetraploid *M. falcata*, 2n = 32 (9a 0.75/2.2, 9a 4.3/20.2); tetraploid *M. sativa*, 2n = 32 (9a 6.3/9).

Schaffhausen, Stetten, Rotacker, 580 m, 691.875/288.650, tetraploid *M. falcata*, 2n = 32 (8b 3.0/8.3, 8b 2.7/5.0); tetraploid *M. sativa*, 2n = 32 (8a 0.4/11.3, 8a 1.2/1, 8a 1/8); tetraploid *M.* × *varia*, 2n = 32 (8a 1.9/11.0).

Zürich, Flaach, Thurhus, 350 m, 687.800/271.575, tetraploid *M. falcata*, 2n = 32 (7a 1/8, 7a 3.7/1.3, 7a 3.65/8.2); tetraploid *M. sativa*, 2n = 32 (7b 32.30/2); tetraploid *M.* × *varia*, 2n = 32 (7a 39/50).

Appendix 2. Historical herbarium samples, literature data and oral communications of *Medicago* × *varia* in Graubünden

Historical herbarium samples

Prättigau and Herrschaft:

Küblis, 07.1914. leg. F. Sprecher, comm. E. Baumann. Z.
Maienfeld, 1929. H. Dübi. LUGANO.

Churer Rheintal and Schanfigg:

Castiel, wayside, 1200 m, 19.09.1913. H. Berger. Z.
Chur, 31.07.1904. A Mermod. LAU.
Chur, Schanfiggerstr., 22.06.1905. Candrian, Samaden. Z.
Meadows above Chur, 1912. R. Haller. BAS. [Bot. Ges]
Near Ems, dry hills: Turruna Casti, 620 m, 25.06.1944. H. Reese, BAS. [Bot. Ges.]
Lüen, meadow edges towards Castiel, 1070 m, 30.06.1914. H. Berger. Z.
Reichenau, along the road to Bonaduz, 600 m, 24.07.1905. E. Steiger, Basel. BAS. [Bot. Anst.]
Pathway Zizers-Trimmis, 26.07.1990. A. Amsler, Zürich. Z.

Albula:

Conters, 28.06.1919. W. Knecht, Flums. Z.
Davos-Platz, near the Hotel Belvédère, dry meadow balks, frequent, 1500 m. Very close to *M. falcata*, but nevertheless showing some bluish colour. Summer 1942. P. Lauser, Davos. Z.
Prada, between Mistail and Tiefencastel, grassy waysides, dry meadow, 900 m, 02.09.1912. W. Schibler, Davos. Z.
Between Savognin and Conters, 13.08.1918. Hans Schinz. Z.
Savognin, 1966. A. Becherer. LUGANO.
Northeast above Savognin, wayside, a group, 10.08.1966. A Becherer. BE.
Tiefencastel, towards Prada, meadow edge, 890 m, 1912. W. Schibler, Davos. Z.
Wiesen, 1200 m, 12.08.1921. W. Schibler, Davos. Z.

Unterengadin:

Remüs [= Ramosch], roadside towards Pazza, inter parentes, 1916. A. Thellung, Zürich. BAS. [Bot. Anst.]
Remüs, roadside towards Pazza, 09.08.1916. A Thellung, Zürich. Z.
Schuls [= Scuol], 12.07.1913. Candrian, Samaden. Z.
Schuls, south of the church, 02.08.1966. Anklin, Biel. BE. [det. P. Rufener Al Mazyad rect. *M. falcata*]

Val Müstair (Münstertal):

Müstair, together with *M. falcata*, 1968. A. Becherer. LUGANO.

Puschlav:

Campocologno, wasteland at the railway below the station. 09.07.1949. A. Becherer. BE.
Campocologno, 1940. A. Becherer. G.

Literature data and oral communications

Prättigau and Herrschaft:

Braun-Blanquet and Rübel 1934: Wide-spread in the Herrschaft (B.-B.) [Braun-Blanquet]; Schiers (Bgg. in S.) [Brügger Ch. in Seiler].
Seiler 1909: Maienfeld (G.) [Marie v. Gugelberg in Maienfeld] [Brügger], Schiers [Brügger].

Churer Rheintal and Schanfigg:

Braun-Blanquet and Rübel 1934: Chur, near St. Luzi, Rosenhügel, (Bgg. in S.) [Brügger Ch. in Seiler]; roadside between Reichenau and Bonaduz 600 m (Stg.) [Steiger E.] and elsewhere common in the Churer Rheintal (B.-B.) [Braun-Blanquet]; Lüen 1070 m; Castiel 1220 m (Beg. mss.) [Beger H., Manuskript der Seiler 1909: Chur 700 m 5.7.72, Fl. Cur. [Flora Curiensis] 102, St. Luzi [Brügger].

Vorderrhein:

Braun-Blanquet and Rübel 1934: Ilanz (Bgg. in S.) [Brügger Ch. in Seiler].
Seiler 1909: Flims, Trins, Ilanz [Brügger].

Hinterrhein:

Braun-Blanquet and Rübel 1934: Thusis; Sils (Bgg. in S.) [Brügger Ch. in Seiler] and elsewhere (B.-B.) [Braun-Blanquet].
Seiler 1909: Thusis, Domleschg (Sils) [Brügger].
Röthlisberger, Klara, Langnau i. E.: Paspels (Domleschg), together with *M. falcata.*

Albula:

Braun-Blanquet and Rübel 1934: Between Lain and Muldein in Obervaz 1300 m; Filisur, Lenz; Bergün (Bgg. in S.) [Brügger Ch. in Seiler].
Seiler 1909: Alvaschein (Lain) 1300 m, Lenz, Filisur, Bergün, between Lein and Muldein in Obervaz [Brügger].

Unterengadin:

Braun-Blanquet and Rübel 1934: Found once in the area of Schuls. Also observed in the Unterengadin (near Fetan) by Brügger (Kill.) [Killias E.].
Killias 1888: Once found around Schuls. Also observed in the region by Prof. Brügger.
Seiler 1909: Schuls [= Scuol] (Kill. Fl.) [Killias, Flora des Unterengadin] [Brügger], Fetan [Brügger].
Bischoff, Nicolin, Ramosch: He confirms to have seen greenish flowering *M.* × *varia* in the Unterengadin but can not remember the site.
Moser, Daniel-Martin, Bern: On an excursion in the Unterengadin, he found a greenish flowering *M.* × *varia* between Guarda and Ramosch.
Reinalter, Romedi, Brail: He does not know localities of *M.* × *varia* from the Unterengadin, in particular also from the upper part around Zernez. The climate there is too rough for cultivation, the chance for hybridization is therefore scanty.

Puschlav:

Braun-Blanquet and Rübel 1934: Brusio (Bgg. in S.) [Brügger Ch. in Seiler].
Seiler 1909: Brusio (Leonh.) [Leonhardi, das Poschiavinotal] [Brügger].

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