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Parallel origins of apomixis in two diverged evolutionary lineages in tribe Potentilleae (Rosaceae)

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We synthesized the results from a flow cytometric seed screen and the literature to infer the phylogenetic origin and the geographical and taxonomic distribution of apomixis in tribe Potentilleae (Rosaceae). We distinguished between regular sexuality and apomixis, the zygotic and parthenogenetic origin of the embryo, and the pseudogamous (i.e. sexual) versus autonomous origin of the endosperm. The combined evidence provides information on reproductive modes for 11 genera and 120 species. For the first time records on reproductive mode are provided for the genus *Farinopsis*, 29 species (from five genera), and seven series of *Potentilla*. Regular sexuality was observed in *Aphanes*, *Argentina*, *Comarum*, *Dasiphora*, *Drymocallis*, *Farinopsis*, *Fragaria*, *Horkeliella*, *Potentilla*, and *Sibbaldia*. Reliable evidence for apomixis is restricted to two evolutionary lineages of Potentilleae: the *Potentilla* core group and *Alchemilla/Aphanes*. Early evolutionary divergence of these lineages (approximately 50 Mya), characterized by pseudogamous and autonomous apomictic seed formation, respectively, suggests parallel origins of apomixis. Apomixis is shown to be taxonomically widespread in the whole Northern Hemisphere distribution range of *Potentilla*, a pattern that is explained by hybrid transfer and repeated intercontinental dispersals. Taxonomic and geographical coverage is discussed with reference to species diversity centres of genera. *Botanical Journal of the Linnean Society*, 2015, **177**, 214–229.

ADDITIONAL KEYWORDS: evolution – FCSS – flow cytometry – phylogeny – *Potentilla* – reproduction – seed – sexuality.

INTRODUCTION

In the angiosperms, there are two principal modes of seed formation: sexuality and apomixis. Apomixis has two major variants, sporophytic apomixis (= adventitious embryony) and gametophytic apomixis (Asker & Jerling, 1992; Savidan, 2007). Gametophytic apomixis is an asexual reproductive mode prevailing in some major plant families, such as Asteraceae, Poaceae, and Rosaceae (Asker & Jerling, 1992; Carman, 1997). The formation of seeds via gametophytic apomixis involves a female gametophyte (embryo sac) formed by the modification or loss of meiosis (apomeiosis), embryo formation usually from an unfertilized egg cell (parthenogenesis), and the development of the endosperm with (pseudogamy) or without (autonomous) fertilization (Nogler, 1984). Gametophytic apomixis is, with few exceptions (Böcher, 1951; Siena *et al*., 2008), linked to polyploidy (Asker & Jerling, 1992; Carman, 1997). It evolved from sexual backgrounds, often in association with hybridization (Bayer, 1997; Dobeš, Mitchell-Olds & Koch, 2004), although autopolyploid gametophytic apomicts exist (Hojsgaard *et al*., 2008; Cosendai, Rodewald & Hörandl, 2011). Consequently, gametophytic apomixis stabilizes genotypes through generations and has great importance for evolutionary diversification and taxonomic differentiation.

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Reproductive modes in angiosperms have been inferred using embryology, progeny surveys (Leblanc & Mazzucato, 2001), specific screens developed for particular taxonomic groups (Matzk, 1991), and the flow cytometric seed screen (FCSS) (Matzk, Meister & Schubert, 2000). Based on estimates of the ploidy of the endosperm and the embryo, FCSS allows differentiation between meiotic and apomeiotic formation of the embryo sac, parthenogenetic and zygotic origin of the embryo, and autonomous and pseudogamous development of the endosperm. However, applicability of the method critically depends on knowledge of the cytology of seed formation. Notably, FCSS relies on the assumption of a binucleate female contribution to the endosperm (i.e. the involvement of two polar nuclei). Embryology has been the common choice to confirm the fulfilment of this condition (Rutishauser, 1969; Leblanc & Mazzucato, 2001), although it is limited by difficulties with respect to observing the moment of fusion of polar nuclei (Czapik, 1985). Alternatively, the binucleate female contribution to the endosperm can be indirectly established, as has been demonstrated for sexual and pseudogamous apomictic species of tribe Potentilleae, Rosaceae (Dobeš *et al*., 2013a). This has involved the use of an amplified fragment length polymorphism (AFLP)-based progeny survey for discriminating sexual and apomictic pathways with identical embryo : endosperm ploidy ratios but differing in the number of polar nuclei involved.

Tribe Potentilleae (Rosoideae, Rosaceae) comprises approximately 19 genera and 1700 species (Soják, 2008; Soják, 2010a). Molecular phylogenetic studies (Eriksson *et al*., 2003; Potter *et al*., 2007; Dobeš & Paule, 2010) have suggested the division of the tribe into two major phylogenetic lineages corresponding to subtribes Fragariinae [*Alchemilla* L., *Aphanes* L., *Chamaerhodos* Bunge, *Comarum* L., *Dasiphora* Raf., *Drymocallis* Fourr. ex Rydb., *Farinopsis* Chrtek & Soják, *Fragaria* L., *Lachemilla* Rydb., *Potaninia* Maxim., *Sibbaldia* L. including *Sibbaldiopsis* Rydb., *Sibbaldianthe* Juz. = *Schistophyllidium* (Juz. ex Fed.) Ikonn.] and Potentillinae [*Argentina* Hill, *Horkelia* Cham & Schldl., *Ivesia* Torrey & A. Gray, *Potentilla* L., *Stellariopsis* (Baill.) Rydb.] (Soják, 2008). Based on the molecular evidence, *Argentina*, *Comarum*, *Dasiphora*, *Drymocallis*, *Farinopsis*, *Potaninia*, *Sibbaldianthe*, and *Sibbaldia p.p*. have been separated from *Potentilla s.s*. as segregate genera (Eriksson *et al*., 2003; Potter *et al*., 2007; Soják, 2008; Paule & Soják, 2009). *Potentilla* (in the circumscription used here; for alternative definitions, see Eriksson, Donoghue & Hibbs, 1998) and *Alchemilla* are the most taxonomically diverse genera of the tribe, comprising approximately 300–500 (Wolf, 1908; Airy Shaw, 1973; Soják, 2008) and 960–1100 species (Fröhner, 1995; Soják, 2008), respectively. *Potentilla* underwent a phylogenetically early divergence and consists of a derived core group ('core *Potentilla*') comprising the vast majority of the described species and several early diverged minor lineages representing the genera *Horkelia* Cham. & Schltdl., *Ivesia* Torr. & A.Gray and relatives (the so-called ivesioid clade), *Potentilla* series *Tormentillae*, and lineages corresponding to Wolf's (1908) *Trichocarpae Herbaceae* (Dobeš & Paule, 2010). Numbers of species in the other genera of Potentilleae range from one (the monotypic genera *Comarum*, *Farinopsis*, *Potaninia*) to approximately 30 (*Ivesia*) and 64 (*Argentina*) (Klackenberg, 1983; Ertter, 1995; Li, Ikeda & Ohba, 2003; Ertter, 2007; Soják, 2008; Paule & Soják, 2009; Soják, 2010a; Hummer, Bassil & Njuguna, 2011). Most genera of Potentilleae have a Northern Hemisphere distribution (or almost so) with species diversity centres in Asia (*Alchemilla*: Gehrke *et al*., 2008, *Argentina*: Ikeda & Ohba, 1999, *Chamaerhodos*: Li *et al*., 2003, *Dasiphora*: Klackenberg, 1983, *Fragaria*: Hummer *et al*., 2011, *Potentilla*: Dobeš & Paule, 2010) or North America (*Drymocallis*: Ertter, 2007, *Ivesia* and *Horkelia*: Ertter, 1995; Soják, 2008). By contrast, *Lachemilla* is a Central to South American genus and *Aphanes* occurs, apart from in the Northern Hemisphere, in Australia and South America (Gehrke *et al*., 2008). In addition, some genera extend with one to few species to the Southern Hemisphere: *Alchemilla* (Gehrke *et al*., 2008), *Argentina* (Ikeda & Ohba, 1999), *Fragaria* (Hummer *et al*., 2011), and *Potentilla* (Wolf, 1908).

In Potentilleae, apomixis has been proven for the closely-related genera *Alchemilla* (Hjelmquist, 1956; Izmailow, 1986; Izmailow, 1994; Mandryk, 2009) and *Aphanes* (Böös, 1917, 1920, 1924; Hjelmqvist, 1959) and for *Potentilla* (see below). In both alliances, gametophytic apomixis is expressed, although reproductive pathways differ in some cytological aspects. Importantly, endosperm development is pseudogamous in *Potentilla* (Asker, 1970b) and autonomous in *Alchemilla* and *Aphanes* (Izmailow, 1986; Izmailow, 1994; Mandryk, 2009). Apomixis was claimed based on embryological studies for representatives of the *Potentilla* core group (Gentscheff, 1938; Gustafsson, 1947; Löve, 1954; Asker, 1970a; Nyléhn, Hamre & Nordal, 2003). Additional evidence for apomixis was obtained for this group from crossing and emasculation experiments, considering morphology, chromosome numbers (Müntzing, 1928; Asker, 1967; Asker, 1970c), and parental molecular markers as inherited traits (Holm, 1995; Holm & Ghatnekar, 1996a; Nyléhn *et al*., 2003), as well as using FCSS (Hörandl *et al*., 2011; Dobeš *et al*., 2013a, 2013b). In addition, apomeiotic embryo sac development was observed for *Potentilla* series *Tormentillae* (Czapik, 1975), which constitutes a separate phylogenetic lineage (Dobeš & Paule, 2010). However, evidence for apomictic seed

formation in *Potentilla indica* L. (series *Tormentillae*) is contradictory. FCSS indicated regular sexual reproduction because embryos and the endosperm were diploid and triploid, respectively. Seeds with diploid ($2n$) embryos and triploid ($3n$) endosperms are usually obtained from the double fertilization of a haploid egg cell and a diploid central nucleus (i.e. the fusion product of two haploid polar nuclei). This sexual pathway is also expected for Potentilleae, which usually have eight-nucleate embryo sacs containing two polar nuclei (Rutishauser, 1969; Asker & Jerling, 1992). However, an AFLP progeny survey suggested an apomictic origin of the offspring (Dobeš *et al*., 2013a). Under this scenario, triploidy of the endosperm was hypothetically explained by involvement of only one, unreduced ($2n$) polar nucleus. The result remained inconclusive because offspring were derived from selfing, and homozygosity of the selfed individual of *P. indica*, precluding a test for recombination, could not be excluded.

Most reproductive mode studies on Potentilleae have focused so far on selected species from the *Potentilla* core group [e.g. *Potentilla argentea* L.: Asker, 1970c; Holm & Ghatnekar, 1996a; Holm & Ghatnekar, 1996b; Paule, Sharbel & Dobeš, 2011; *Potentilla crantzii* (Crantz) Beck ex Fritsch: Czapik, 1961; Smith, 1963b; *Potentilla verna* L. group: Czapik, 1962; Smith, 1963a; Dobeš *et al*., 2013b] and *Alchemilla* (Murbeck, 1901; Mandryk, 1976; Glazunkova & Myatlev, 1983; Izmailow, 1986; Izmailow, 1994; Mandryk, 2009), as well as accessions of European provenance. In particular, the taxonomically diverse *Potentilla* core group is understudied in both Asia, where the centre of species diversity is (Shah *et al*., 1992) and the origin of the tribe presumed to be (Dobeš & Paule, 2010), and North America.

Providing a comprehensive literature survey and performing a FCSS, the present study (1) inferred the geographical, taxonomic and phylogenetic distribution of reproductive modes in Potentilleae; (2) documented the three basic cytological elements of seed formation, the origin of the embryo sac (meiotic versus apomeiotic), the embryo (zygotic versus parthenogenetic), and the endosperm (pseudogamous versus autonomous); and (3) determined whether seeds with $2n$ embryos and $3n$ endosperms were derived from regular sexuality or apomixis in *Potentilla indica* (*Tormentillae*). To answer this question, the progeny of a controlled cross was genotyped using AFLPs and tested for recombination.

MATERIAL AND METHODS

Plant material

The plant material was obtained either via the Index Seminum seed exchange during the years 2004–2009 (64 accessions; 30 of known geographical origin) or fruitlets were collected directly in the field (14 accessions; see Supporting information, Doc. S1). Herbarium vouchers were prepared from field-collected and from cultivated mature plants. Vouchers are deposited in the herbaria HEID, HBU, W, WUP, and the private collection of Elvira Hörandl (University of Göttingen, Germany). Fruitlets were bagged in airtight aluminium bags and stored at 4 °C prior to flow cytometric analysis. *Argentina*, *Dasiphora*, *Drymocallis*, *Farinopsis*, *Potentilla*, and *Sibbaldia sensu* Soják (2008) were included in the FCSS covering 54 species and 18 out of the 31 series distinguished in the last worldwide monograph of *Potentilla* (Wolf, 1908) (Table 1).

Flow cytometric seed screen

The fluorescence intensities of embryo and endosperm nuclei were determined by FCSS. The results from two experiments using different methodological designs were merged. In the first experiment (A), measurements were performed without the use of an internal standard and separately for each fruitlet. One to 18 (but mostly two to five fruitlets) were analyzed per accession (241 in total). The samples were measured in the linear mode using two flow cytometric systems: (1) a Partec Ploidy Analyzer (Partec GmbH) equipped with a mercury arc lamp and a Partec CyFlow Ploidy Analyzer equipped with a 532-nm green laser. 4′-6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI) served as DNA-selective stains, respectively. Alternatively, (ii) the FACSCalibur cytometer (Becton Dickinson) equipped with a 532-nm laser was employed. PI was used as DNA-stain and both forward and side-scatter were recorded as fluorescence parameters. In all cases, samples were prepared *sensu* Matzk *et al*. (2001) using a slightly modified seed buffer (5 mM $MgCl_2 \times 6H_2O$, 85 mM NaCl, 100 mM Tris, 0.09% Triton-X-100, 6.1 mM sodium citrate dihydrate, pH 7). The extraction buffer contained 4 μg mL^{-1} DAPI or 50 μg mL^{-1} PI, respectively. After removal of the pericarp of the fruitlets and the testa, seeds were chopped in 0.5 mL of extraction buffer using a razor blade. After a lag of approximately 15 min, the homogenate was filtered through a 20-μm nylon mesh filter (Partec CellTrics). DAPI-stained samples were immediately measured without further manipulation. PI stained samples were RNA-digested for 40 min at 30 °C using 20 μL of RNase A solution (3 mg mL^{-1}) per sample. In the second experiment (B), *Pisum sativum* L. ' Kleine Rheinländerin' (Greilhuber & Ebert, 1994) and *Vicia faba* L. 'Inovec' (Doležel, Sgorbati & Lucretti, 1992) co-chopped with the sample served as internal standards. Fourteen to 42 seeds were

Table 1. Summary statistics on reproductive modes observed in tribe Potentilleae inferred using FCSS

Taxon	Clade	Series	Geographical origin ($N_{accessions}$)	apo	pa	po	sex	zy	Total
Dasiphora fruticosa (L.) Rydb.	A		As (1)					4	4
Drymocallis fissa (Nutt.) Rydb.	A		NAm* (2)					8	8
Drymocallis geoides (M.Bieb.) Soják	A		As, E* (1)					2	2
Drymocallis glandulosa (Lindl.) Rydb.	A		NAm (1)				25		25
Drymocallis lactea (Greene) Rydb.	A		NAm* (1)					13	13
Drymocallis rupestris (L.) Soják	A		E (2)					2	2
Drymocallis sp.	A		NAm (1)					4	4
Farinopsis salesoviana (Steph.) Chrtek & Soják	A		As (1)					2	2
Sibbaldia cuneifolia (Bertol.) Paule & Soják	A		As (2)					15	15
Sibbaldia procumbens L.	A		N* (1)					3	3
Sibbaldia tridentata (Aiton) Paule & Soják	A		E (1)					2	2
Argentina leuconota (D.Don) Soják	B		As (1)					4	4
Potentilla megalantha Takeda	C	Not assigned	As* (2)				20	1	21
Potentilla argentea L.	C	*Argenteae*	E (1)		2				2
Potentilla calabra Ten.	C	*Argenteae*	E* (1)					1	1
Potentilla aurea L.	C	*Aureae*	E (2)					8	8
Potentilla crantzii (Crantz) Beck ex Fritsch	C	*Aureae*	E (1)	20					20
Potentilla frigida Vill.	C	*Aureae*	E (1)				20		20
Potentilla velutina Lehm.	C	*Aureae*	E (1)					1	1
Potentilla adscharica Sommier & Levier	C	*Chrysanthae*	As* (1)		3			2	5
Potentilla grandiflora L.	C	*Grandiflorae*	E (2)		1			5	6
Potentilla pyrenaica Ram. ex DC.	C	*Grandiflorae*	E (1)				20		20
Potentilla umbrosa Stev.	C	*Grandiflorae*	E (1)				20		20
Potentilla atrosanguinea Lodd. ex. D. Don.	C	*Haematochroae*	As* (2)	15			1	6	22
Potentilla venusta Soják	C	*Haematochroae*[1]	As* (1)		2				2
Potentilla chinensis Ser.	C	*Multifidae*	As* (1)				23		23
Potentilla hippiana Lehm.	C	*Multifidae*	E (2)	22	1				23
Potentilla multifida L.	C	*Multifidae*	As, E* (2)	18	7				25
Potentilla ornithopoda Tausch	C	*Multifidae*	As (2)	21	4				25
Potentilla pensylvanica L.	C	*Multifidae*	NAm (1)				23		23
Potentilla pulcherrima Lehm.	C	*Multifidae*	NAm* (1)	19		1			20
Potentilla nevadensis Boiss.	C	*Persicae*	E* (1)				21		21
Potentilla detommasii Ten.	C	*Rectae*	E (1)		3				3
Potentilla recta L.	C	*Rectae*	E (4)	36	4				40
Potentilla semilaciniosa Borbás	C	*Rectae*	E (1)		3				3
Potentilla norvegica L.	C/E	*Rivales*	E (1)					2	2
Potentilla supina L.	C/E	*Rivales*	E (2)					6	6
Potentilla fulgens Wall. ex Hook.	C	*Rupestres*	As* (1)	12			7		19
Potentilla longifolia Willd. ex Schlecht.	C	*Tanacetifoliae*	As, E* (1)				23		23
Potentilla erecta (L.) Räuschel	F	*Tormentillae*	E (2)				41	4	45
Potentilla reptans L.	F	*Tormentillae*	E (1)				26		26
Potentilla caulescens L.	G/H	*Caulescentes*	E (1)					4	4
Potentilla clusiana Jacq.	G/H	*Caulescentes*	E (1)					3	3
Potentilla valderia L.	G/H	*Crassinerviae*	E* (1)					2	2
Potentilla alba L.	G/H	*Fragariastra*	E (1)				20		20
Potentilla sterilis (L.) Garcke	G/H	*Fragariastra*	E (1)					2	2
Potentilla alchimilloides Lapeyr.	G/H	*Nitidae*	A, E* (2)				23	5	28
Potentilla nitida L.	G/H	*Nitidae*	E* (1)					3	3
Potentilla speciosa Willd.	G/H	*Speciosae*	As, E* (1)					3	3
Potentilla biflora Willd. ex. Schlecht.	I	*Biflorae*	As (1)				8		8
Total				163	30	1	321	117	632

apo, apomictic; pa, parthenogenetic; po, polyploidization; sex, regular sexual; zy, zygotic. Taxa were assigned to the major phylogenetic lineages ('clades') of Potentilleae (Dobeš & Paule, 2010). Series refer to Wolf (1908). The geographical origin of the studied material is provided at the level of continents (A, Africa; NAm, North America; As, Asia; E, Europe; N, northern hemisphere). Where collection sites are unknown, the distribution range of the species (*sensu* Wolf, 1908; Kurtto *et al*., 2004; Soják, 2012; B. Ertter, pers. comm.) is given (indicated by an asterisk). The number of successfully analyzed accessions is given in parentheses. The number of analyzed seeds is provided for each reproductive mode-taxon combination. For a definition of reproductive modes, see text: regular sexuality, apomixis, zygotic and parthenogenetic origin of the embryo, and polyploidization. Underlined taxa were studied for the first time in the present study.

analyzed per accession (542 in total). At least five seeds per accession were prepared and measured separately. The remaining seeds were pooled: two and five seeds per sample. Samples were measured in the logarithmic mode (log3) using (1) a CyFlow Ploidy Analyzer equipped with a 532-nm green laser, PI staining and in accordance with the protocol described above, and (2) a CyFlow Ploidy Analyzer equipped with a 365-nm LED. DAPI served as DNA-selective stain. Sample preparation was conducted *sensu* Dobeš *et al*. (2013a). Fluorescence peaks were manually gated using the Partec operating and analysis software FLOWMAX, version 2.4d, and CELLQUEST PRO, version 5.2.1 (Becton Dickinson).

Inference and phylogenetic distribution of reproductive modes

We distinguished the zygotic (i.e. sexual *s.s*.) and the parthenogenetic origin of the embryo for accessions measured without an internal standard (Experiment A). Under the condition of a binucleate female contribution to the endosperm, peak indices (the endosperm: embryo fluorescence ratio) of < 2 indicate an zygotic origin, and values > 2 indicate parthenogenesis (Dobeš *et al*., 2013a). The embryo : standard fluorescence ratio as a measure of relative genome size was established for accessions measured with the internal standard (Experiment B). The embryo : standard fluorescence ratio and the peak index was used to calculate the female genomic contribution to the embryo (haploid versus diploid) for zygotically derived embryos *sensu* Dobeš *et al*. (2013a). Embryos receiving half of their genome via the female genomic contribution were interpreted to be derived via regular sexuality (i.e. involving female meiosis and fertilization of the egg cell), if the contribution is fairly constant within an accession (see Discussion). The apomictic origin was assumed for parthenogenetically derived embryos recovering the genome of the maternal individual. Because the maternal individuals were not available for most accessions, we used the median of the embryo : standard fluorescence ratio observed for the parthenogenetically derived embryos of an accession as a proxy for the sample: standard fluorescence ratio of the maternal individual. According to Greilhuber (2005), we use n (i.e. the haplophasic chromosome number) to indicate the number of holoploid genomes (i.e. the whole chromosome complement with chromosome number n).

Reproductive modes inferred using FCSS were compared with the record in the literature. Evidence for apomixis was based on (1) the combined occurrence of apomeiotic development of embryo sacs and the parthenogenetic development of embryos in cyto-embryological studies and (2) maternal inheritance of morphological, karyological or molecular characters in controlled crossing experiments. Sexuality was inferred based on (1) the observation of female meiosis and zygotic embryo development and (2) the biparental inheritance of markers. Data were summarized at the level of species, except for *Alchemilla*/*Aphanes* and *Fragaria*, for which data are presented at the genus level. Studies on natural and synthetic hybrids were not considered.

The phylogenetic distribution of reproductive modes is interpreted on the basis of the plastid DNA-based phylogenetic tree for Potentillae provided by Dobeš & Paule (2010).

Crossing experiments, AFLP genotyping, and progeny tests

Seeds with diploid ($2n$) embryos and triploid ($3n$) endosperms derived from eight-nucleate embryo sacs can be formed via two cytological pathways: regular sexuality involving a binucleate female contribution to the endosperm (i.e. the embryo receives an n female + n male contribution; the endosperm an $n + n$ female + n male contribution) and pseudogamous apomixis involving a mononucleate female and haploid male contribution to the endosperm (embryo $2n + 0$; endosperm $2n + n$) (Matzk *et al*., 2000; Dobeš *et al*., 2013a). We exploited this relationship and tested for the inheritance of maternal AFLP-markers in the progeny obtained in a controlled cross of genetically divergent individuals. We expected recombination in the case of regular sexuality and matroclinal inheritance in the case of apomixis, thereby also indirectly solving the question of whether one or two polar nuclei are involved in endosperm formation. A crossing experiment with *P. indica* was performed using pollen recipient Ptl8202 from Dobeš *et al*. (2013a) and a pollen donor Ptl8581 originating from a geographically distant population (Austria, Vienna, Lainzer Tiergarten; collected by Christoph Dobeš 27.05.2011, voucher 2012-02870 in W). Flowers were emasculated and bagged a few days before anthesis. At stigma maturity, flowers were outcrossed by rubbing mature anthers against the recipient stigmas. Mature seeds were sown in sterilized Neuhaus N3 substrate (Klasmann-Deilmann, Geeste) in a temperate green house of the Department of Pharmacognosy. Seedlings were collected after the development of two or three leaves. The sample : standard fluorescence ratio of the parental individuals and the seedlings was established by flow cytometry on leaf petioles using DAPI staining and *P. sativum* 'Kleine Rheinländerin' as the internal standard. The flow cytometric protocol was conducted *sensu* Paule *et al*. (2011). Total DNA was isolated from silica gel-dried leaves and seedlings using the NucleoSpin Plant 96 II extraction kit

(Macherey Nagel). Parental individuals, 12 seedlings, and two repeats were genotyped using AFLPs. AFLP were analyzed using the protocol established by Vos *et al*. (1995) with a few modifications as described by Paule *et al*. (2011) using EcoRI-AGG [NED]/MseI-CTC, EcoRI-AAC [6-FAM]/MseI-CTT, and EcoRI-AGC [VIC]/MseI-CTG as three selective primer pairs. Differentially fluorescence-labelled polymerase chain reaction products and the GS600 LIZ size standard (Applied Biosystems) were multiplexed and the fragments were separated on a 3730 DNA Analyzer (Applied Biosystems). Raw data were visualized and scored using GENEMARKER, version 1.95 (SoftGenetics) and exported as a presence/absence matrix. Recombination within the F_1 was estimated using GENODIVE, version 2.0b25 (Meirmans & Tienderen, 2004) based on pairwise differences between pairs of genotypes. A threshold value was applied based on the estimated genotyping error and the number of observed AFLP fragments.

RESULTS

Performance of FCSS and inferred reproductive modes

At least one clearly interpretable fluorescence peak was observed for 675 samples, 538 of which showed one additional distinct peak. The variation coefficient of the embryo peaks ranged between 1.80 and 9.96 (mean 4.63) and that of the endosperm peaks ranged between 1.45 and 9.41 (mean 3.46). Based on the endosperm : embryo ratios of 1–2 and > 2 expected for zygotically and parthenogenetically derived embryos under the condition of fertilization of two polar nuclei, 531 samples were interpreted to exhibit distinct signals for these tissues (Fig. 1). The corresponding peak indices were in the ranges 1.29–1.85 and 2.52–4.30 (Fig. 2, see also Supporting information, Doc. S2). Peaks of approximately double the fluorescence intensity of the embryo peak (1.93–2.10) were observed in 39 samples. In these cases, the peak of higher fluorescence intensity was considered to represent embryo nuclei in the G2-phase (or endopolyploidy). This interpretation is in agreement with the co-occurrence of an additional third peak of a fluorescence intensity expected for the endosperm in 33 of these samples and indicates an origin of the endosperm from fertilization. Parthenogenetically derived embryos measured with an internal standard invariably recovered the female genome (1.92*n*–2.14*n*), indicating apomictic origin. The contribution of half of the female genome (42.6–63.6%) to zygotically derived embryos in 225 out of 226 measurements performed with an internal standard indicated a high functionality of female meiosis. The individual female genomic contributions deviated from the accession mean by 3.3–16.1% and 1.0–7.0% for zygotically and parthenogenetically derived embryos, respectively, indicating the contribution of the same number of holoploid genomes. Accounting for the number of seeds analyzed per measurement, we inferred a regular sexual and apomictic origin for 321 and 163 embryos (Experiment B) and a zygotic and parthenogenetic origin for 117 and 30 additional embryos (Experiment A), respectively (Table 1). Reproductive modes were established for 66 out of the 78 accessions and for 50 out of the 54 studied taxa (the screening failed for *Potentilla delphinensis* Gren. & Godr., *Potentilla gracilis* Dougl. ex Hook, *Potentilla thurberi* A.Gray ex Lehm., and *Potentilla thuringiaca* Bernh.).

Progeny test

The sample: standard fluorescence ratios of *P. indica* parental individuals used in the controlled crossing experiment and of their progeny differed by 0.1–3.4%, indicating the same ploidy. Three AFLP primer combinations resulted in 80 clearly scorable fragments sized from 92–539 bp and the parental genotypes differed by 19 fragments. The repeatability of the data was 96.25%. Thus, a threshold value of three AFLP band mismatches was applied for the identification of genotypes (Fig. 3). Differences above the threshold value were considered as evidence for recombination in the F_1 generation. Frequency distributions of pairwise genotypic distances among mother and F_1 individuals, defined as the total number of different marker alleles between two individuals, suggested genetic recombination for all tested samples (Fig. 3).

Taxonomic, phylogenetic, and geographical distribution of reproductive modes

Forty-six out of 66 accessions successfully analyzed using FCSS produced embryos exclusively from fertilization (i.e. zygosis) and 16 exclusively via parthenogenesis. In four accessions, both modes co-occurred. Regular sexuality, apomixis, and the co-occurrence of these reproductive modes could be established for 14, five, and three of these accessions. In terms of taxa, zygotic, parthenogenetic, and both types of embryo formation was observed in 36, nine, and five species (Table 1). Parthenogenesis and apomixis was exclusively found in series assigned to clade C, core *Potentilla* (series *Argenteae* Th.Wolf, *Aureae* Th.Wolf, *Chrysanthae* Th.Wolf, *Grandiflorae* Th.Wolf, *Haematochroae* Th.Wolf, *Multifidae* Th.Wolf, *Rectae* Th.Wolf, *Rupestres* Th.Wolf) (Fig. 4). Except for series *Rectae*, regular sexuality (and zygosis) was observed in all the series of clade C, demonstrating the co-occurrence of

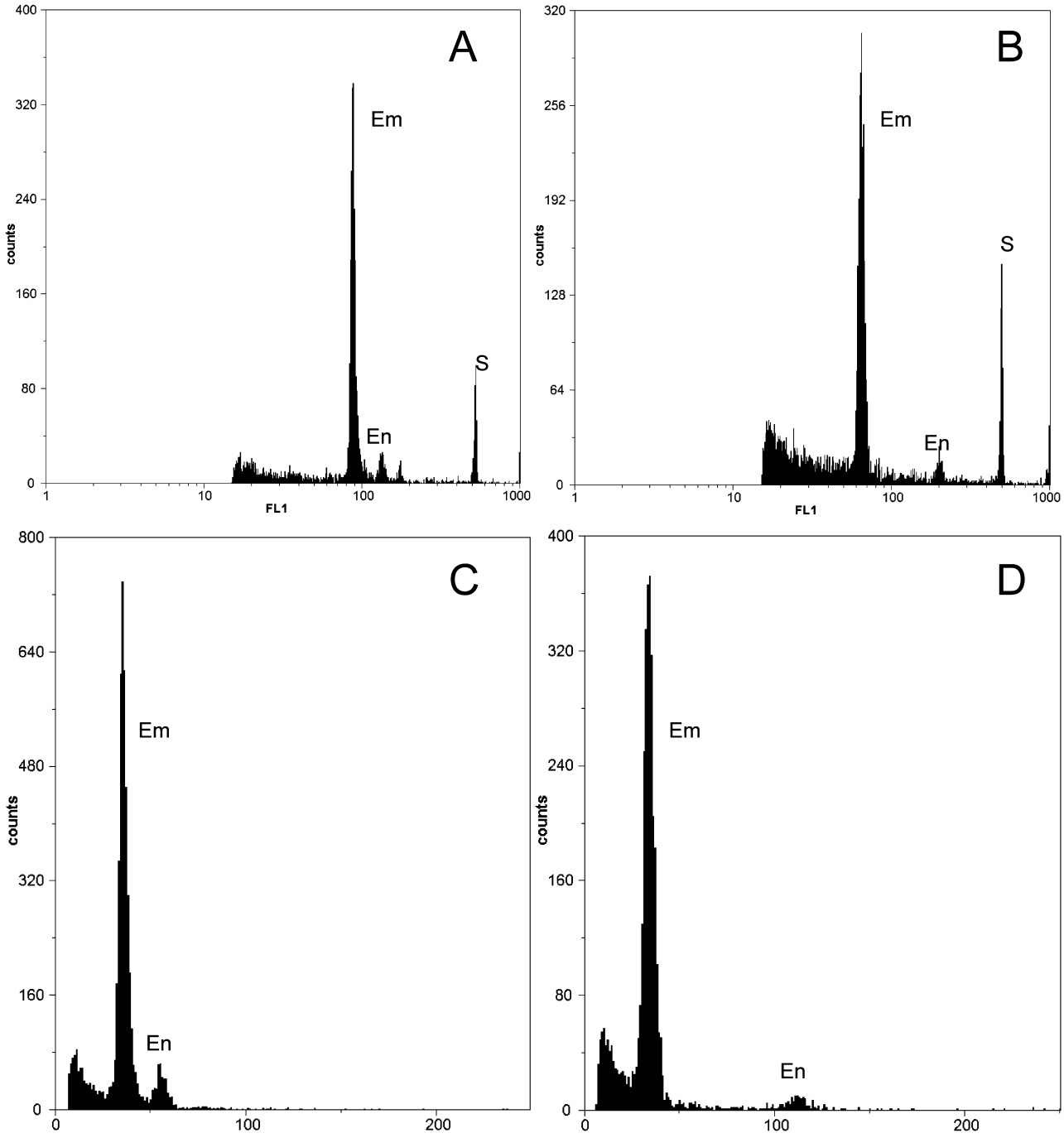

Figure 1. Flow cytometric seed screen (FCSS) of single seeds with embryos of (A) regular sexual (*Potentilla alba* L., Ptl8204, peak index 1.52) and (B) apomictic origin (*Potentilla recta* L., Ptl8205, peak index 3.13) performed using the Partec CyFlow Ploidy Analyzer and 4′-6-diamidino-2-phenylindole (DAPI) staining. The histograms in (C, D) show results for seeds with embryos of zygotic (*Potentilla grandiflora* L., Ptl2892, peak index 1.57) and parthenogenetic origin (*Potentilla multifida* L., Ptl2741, peak index 3.32) performed using the Partec PA and DAPI staining. A lack of information about the embryo ploidy precluded inference of the origin of the embryo sac (meiotic versus apomeiotic). Em, embryo peak; En, endosperm peak; S, internal standard *Pisum sativum* peak. A distinct G2 peak is visible for the embryo signal (right to the endosperm) in (A).

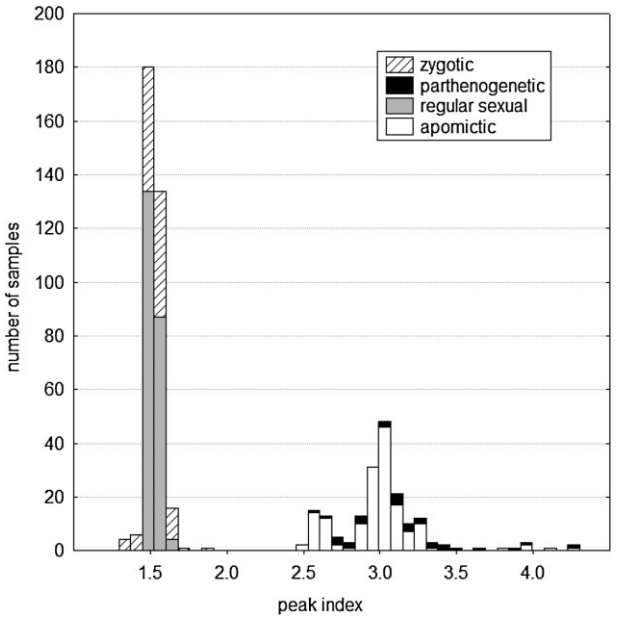

Figure 2. Frequency distribution of peak indices observed in 531 flow cytometric samples (representing 632 seeds) from 50 species and six genera of Potentilleae. Different bar designs refer to the regular sexual, apomictic, zygotic, and parthenogenetic origins of the embryos.

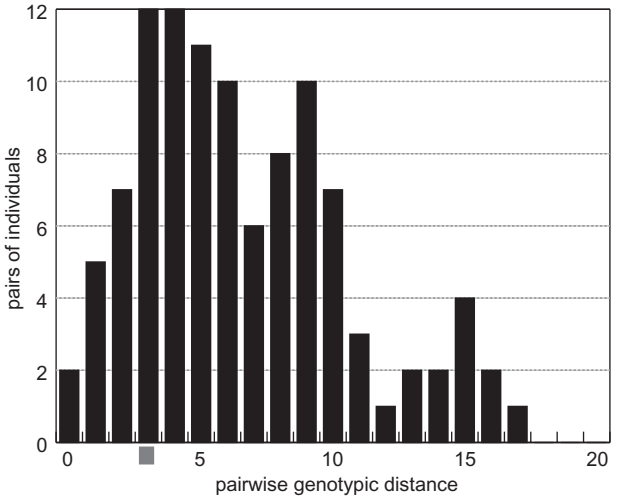

Figure 3. Frequency distribution of pairwise genotypic distances (*x*-axis) between pairs of individuals (*y*-axis). The grey square indicates the threshold value based on the 96.25% repeatability.

both reproductive modes in most series. This contrasted with the sole observation of sexuality (and zygosis) in *Potentilla* series *Tormentillae* Th.Wolf (clade F), *Biflorae* Rydb. (clade I), *Caulescentes* Th.Wolf, *Crassinerviae* Th.Wolf, *Fragariastra* Th.Wolf, *Nitidae* Th.Wolf, and *Speciosae* Th.Wolf (clades G–H). *Argentina*, *Dasiphora*, *Drymocallis*, *Farinopsis*, and *Sibbaldia* also produced embryos from fertilization only.

According to the record in the literature (see Supporting information, Doc. S3), apomixis was observed in 20 *Potentilla* spp. Reliable evidence for apomixis was also found for *Alchemilla* and *Aphanes*. The reproductive mode was contradictory for *Tormentilla*, as was the expression of apomixis in *Drymocallis* and *Potentilla aurea* L. Sexuality was documented for 11 *Potentilla* spp. and *Aphanes*, *Argentina*, *Comarum*, *Dasiphora*, *Drymocallis*, *Fragaria*, *Horkeliella* (Rydb.) Rydb., and *Sibbaldia*. In additional six *Potentilla* spp., the reproductive modes co-occurred. Fifteen of the surveyed taxa were also analyzed in the present study.

The combined data (literature and our FCSS data) provide information on reproductive modes for 11 genera of Potentilleae. Sixty-nine *Potentilla* spp. representing 22 series were studied, 26 *Alchemilla* spp., six *Drymocallis* spp., five *Aphanes* spp., five *Fragaria* spp., three *Sibbaldia* spp., two *Argentina* spp., and one species each of *Comarum*, *Dasiphora*, *Farinopsis* and *Horkeliella*. Apomixis, parthenogenesis, and sexuality were observed in all Northern Hemisphere continents. Apomixis and sexuality geographically co-occurred on the continental scale in *Potentilla* (eight sexual/eight apomictic species in Asia; 25/24 in Europe; four/four in North America). Among the other exclusively Northern Hemisphere genera, one to three species were studied from each of the three continents (*Argentina*: one from Asia/one from Europe, one from North America; *Drymocallis*: one/one/three; *Sibbaldia*: one/one/one), except *Dasiphora* for which species from two continents only were studied (one/–/one). One species only (*Horkeliella purpurascens*) was analyzed for reproductive mode from the species-rich North American ivesioid clade (Table 1; see also Supporting information, Doc. S3). For *Alchemilla*, studies focused on Europe (26 species) (Murbeck, 1901; Izmailow, 1986; Izmailow, 1994; Mandryk, 2009). In addition, three *Alchemilla* spp. from North Africa were analyzed (Hjelmquist, 1956). The only two species previously studied from the Southern Hemisphere are from *Aphanes* (Böös, 1917, 1920). The geographical origin of the five wild *Fragaria* spp. analyzed by Nosrati, Price & Wilcock (2010) using controlled crossings could not be attributed because the accessions used as maternal individuals were not specifically noted.

DISCUSSION

The origin of the embryo (zygotic versus parthenogenetic) is inferred from the peak index, whereas inference of the origin of the embryo sac (meiotic versus apomeiotic) requires additional comparison of the

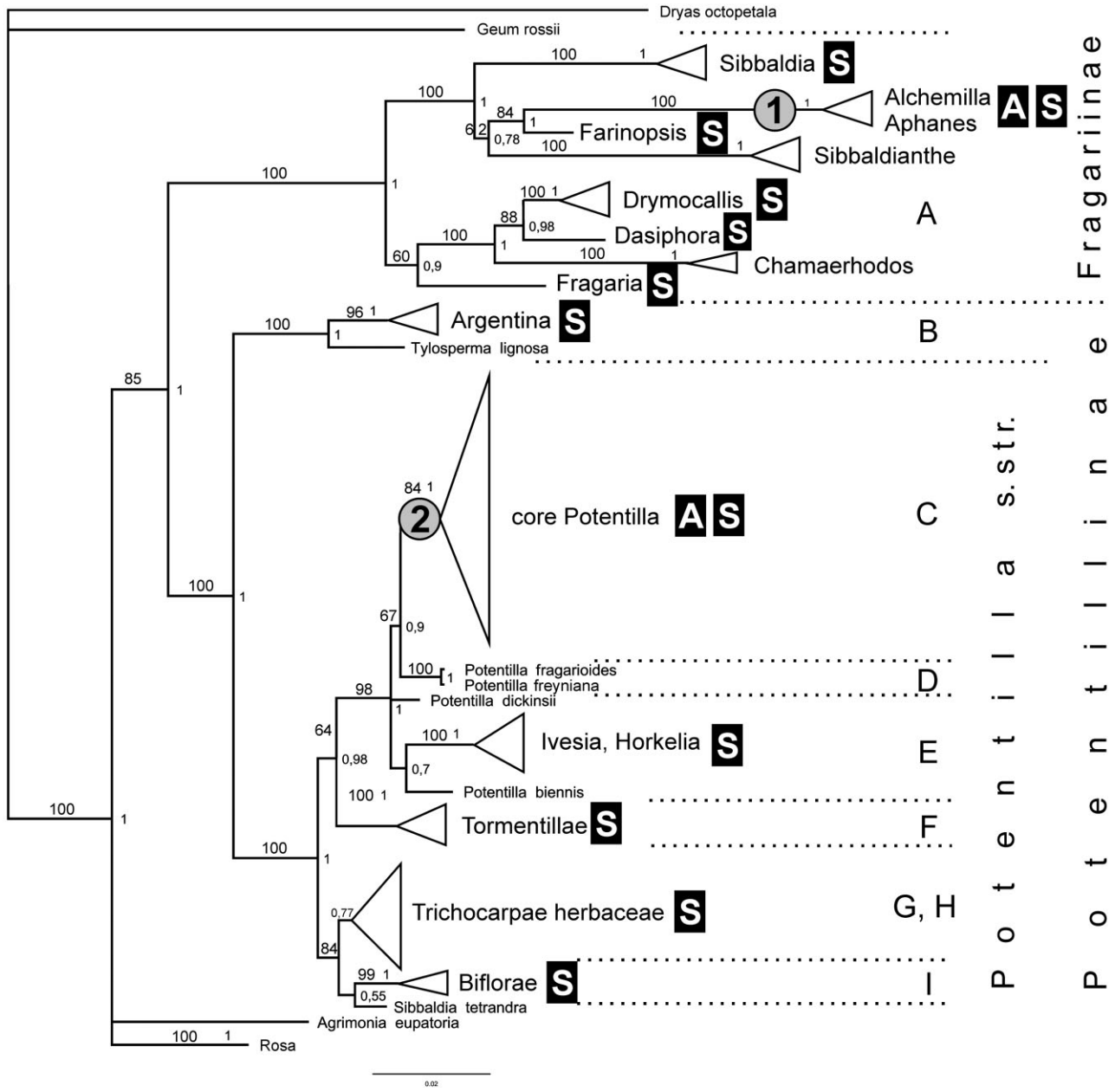

Figure 4. Phylogenetic distribution of reproductive modes in Potentilleae. The white letters stand for seed production via sexuality (S) and apomixis (A) in particular lineages based on the present study (Table 1) and data in the literature (see Supporting information, Doc. S3). The phylogenetic distribution of reproductive modes suggests that apomixis arose once in subtribe Fragariinae (at the position marked by the grey dot numbered '1') and at least once ('2') in Potentillinae. The tree is a 50% percent majority rule tree constructed using maximum parsimony analysis and the combined plastid intergenic spacers *trnS-ycf9*, *trnL-trnF* and *trnC-ycf6* (fig. 1 in Dobeš & Paule, 2010) with bootstrap values above the branches and posterior probabilities at the nodes. Clades are designated by letters A–I. The phylogenetic position of *Aphanes* is based on that reported in Gehrke *et al*. (2008).

embryo ploidy and the ploidy of the seed donor (Matzk *et al*., 2000; Dobeš *et al*., 2013a). We could not relate maternal ploidy to the ploidy of seed tissues because the maternal individuals were not available for most accessions. A peak index of approximately 1.5 was observed for most seeds with a zygotically derived embryo and peak indices exhibited a bimodal frequency distribution with maxima at approximately 2.5 and 3.0 for seeds with parthenogenetically derived embryos (Fig. 2). Under the assumption of equal

genome size for the parental individuals, the peak index of 1.5 results from the fusion of reduced female and male gametes (i.e. regular sexuality) (reproductive pathway *M* in Dobeš *et al*., (2013a). Alternatively, a peak index of 1.5 is obtained from the fusion of unreduced gametes, raising the ploidy of the embryo compared to its parents (reproductive pathway *N*). A peak index of 3.0 also characterizes a seed with an embryo derived via haploid parthenogenesis (female meiosis combined with parthenogenesis), in which embryo ploidy is decreasing (reproductive pathway *E* in Dobeš *et al*., 2013a). Although meiosis and zygosis and apomeiosis and parthenogenesis occur independently of each other (Asker, 1970b), their uncoupling will result in monoploidy (haploid parthenogenesis) or in an indefinite increase in ploidy (polyploidization), respectively. Therefore, the combination of either meiosis plus zygosis or apomeiosis plus parthenogenesis is expected to be under strong positive selection (Van Dijk & Vijverberg, 2005). Our data for samples measured with an internal standard revealed that, despite analyses of seven to 36 seeds per accession, the same number of holoploid female genomes was contributed to zygotically and parthenogenetically derived embryos as indicated by the moderate variation in the female genomic contribution within each of the accessions (up to 16.1% and 7.0%, respectively). In accordance with the argument put forward by Van Dijk & Vijverberg (2005), we assume that samples of zygotically and parthenogenetically derived embryos receiving identical numbers of female genomes originated via regular sexuality and apomixis, respectively. In the case of samples measured without an internal standard, we refrain from this interpretation because the number of seeds analyzed per accession was usually low (mostly one to four; on average, three seeds per accession) and variation in the female genomic contribution could not be reliably assessed (because we have not established embryo ploidy). Therefore, for those seeds, we merely distinguished between a zygotic and parthenogenetic origin of the embryo. The invariable observation of peak indices deviating from 2 in both types of analyses, however, indicated that the endosperm generally originated from fertilization.

Taxonomic distribution of reproductive modes

The FCSS data from the present study agree with the data in the literature because consistently regular sexuality (or zygosis) and apomixis (or parthenogenesis), respectively, was inferred for ten and five species out of 19 analyzed in the present and the previous studies. However, there are conflicts for *P. aurea* and *Drymocallis*. Apomixis was suggested for *P. aurea* (Shimotomai, 1935a; Shimotomai, 1935b) and *Drymocallis arguta* (Pursh) Rydb. (Popoff, 1935) based on crossing experiments, whereas regular sexuality was suggested by FCSS (present study; Dobeš *et al*., 2013a). Although progeny tests and controlled pollination are a valid approach (Czapik, 1994), false results may be obtained because of pollen dependence of the realized reproductive mode (Müntzing & Müntzing, 1941; Rutishauser, 1943; Rutishauser, 1949), unintended self-pollination (Schwendener, 1969) or the dominance of maternal markers (Nosrati *et al*., 2010). Alternatively, taxonomic misidentification may explain the discrepancies. The cases of *P. aurea* and *D. arguta* are particularly critical because both species are diploid (Popoff, 1935; Dobeš & Vitek, 2000), a condition not considered to occur in association with apomixis in Potentilleae (Holm & Ghatnekar, 1996b; Holm, Ghatnekar & Bengtsson, 1997).

Apomeiotic (aside from meiotic) embryo sac formation and matroclinal offspring in interspecific crossings are documented for *Potentilla erecta* (L.) Räuschel from *Tormentillae*. However, parthenogenesis has not been observed and unintended selfing in the experimental crossings could not be excluded (Schwendener, 1969). Thus, in *P. erecta*, apomixis is not fully proven and the FCSS suggested sexuality. A full documentation of the apomictic pathway is analogously missing for other representatives of *Tormentillae*, *Potentilla reptans* L and *Potentilla procumbens* Sibth. (Schwendener, 1969) and *Potentilla* × *mixta* Nolte (Czapik, 1975). In the present study, apomixis was dismissed and sexuality confirmed for *P. indica* based on the performed controlled crossing experiment and the progeny survey. Nonsegregation of molecular markers in the offspring observed by Dobeš *et al*. (2013a) can be explained by (a high degree of) homozygosity of the selfed seed donor. Hence, we suggest sexuality for *Tormentillae*. The finding implies that endosperms received a binucleate female contribution (i.e. dismissing apomixis involving a mononucleate female contribution to the endosperm as an alternative explanation for the formation of seeds with 2*n* embryos and 3*n* endosperms: reproductive pathway *O* in Dobeš *et al*., 2013a) and, importantly, that this condition is conserved throughout Potentilleae.

Excluding poorly documented cases and doubtful inferences of apomixis, the assembled literature record (see Supporting information, Doc. S3) and FCSS data (Table 1) suggested sexuality, apomixis (or parthenogenesis), and reproduction via both reproductive modes for 29, 25, and 11 *Potentilla* spp., respectively. Apomixis is confirmed for 14 series from core *Potentilla* (*Argenteae*, *Aureae*, *Chrysanthae*, *Collinae* Zimm., *Graciles* Th.Wolf, *Grandiflorae*, *Haematochroae*, *Multifidae*, *Multijugae* Th.Wolf, *Niveae*

Th.Wolf, *Persicae* Th.Wolf, *Rectae*, *Rivales* Th.Wolf, and *Rupestres*). Apomixis is also documented for *Alchemilla* and *Aphanes* (Czapik, 1996) and has been suggested (Baturin, 2009) but dismissed (Nosrati *et al*., 2010) for *Fragaria*. The combined evidence thus documents sexuality for all studied genera of Potentilleae, except for *Alchemilla*.

The present study substantially improved our knowledge of the taxonomic distribution of reproductive modes in Potentilleae. The genus *Farinopsis*, 29 additional species (from five genera) and seven *Potentilla* series were studied for the first time. Notably, the reproduction of representatives of the phylogenetically old clades G–I has not been explored so far. Nevertheless, our survey unravelled marked differences in taxonomic coverage. Relatively well-studied taxa are *Potentilla* (69 out of 300–500 species), *Sibbaldia* (three out of five or six species), and the monotypic genera *Comarum* and *Farinopsis*. By contrast, only two out of 64 *Argentina* spp. (3.1%) and 26 out of the approximately 1000 *Alchemilla* spp. (2.6%) were studied for reproductive mode so far.

In *Potentilla* series *Argenteae*, *Aureae*, *Collinae*, *Graciles*, *Multifidae*, *Niveae*, and *Rectae*, and in *Alchemilla*, the expression of apomixis is associated with complex patterns of taxonomic differentiation, suggesting an evolutionary significant role of apomixis and polyploidy for diversification (Wolf, 1908; Asker & Fröst, 1970; Soják, 1989; Fröhner, 1995; Dobeš, 1999; Rico *et al*., 2003; Paule, Scherbantin & Dobeš, 2012; Soják, 2012).

Evolutionary origin and geography of apomixis

Apomixis evolves in parallel, spreads as a heritable trait through introgressive hybridization, or is shared by common descent (Van Dijk & Vijverberg, 2005). Subtribes Fragariinae and Potentillinae split from each other approximately 50 Mya (Dobeš & Paule, 2010). This long period of isolated evolution suggests a parallel origin of the apomictic trait in these subtribes. This conclusion is drawn from the derived phylogenetic position of the only genera of Fragariinae expressing apomixis, *Alchemilla* and *Aphanes*, relative to *Dasiphora*, *Drymocallis*, *Comarum*, *Farinopsis*, *Fragaria*, and *Sibbaldia* for which the combined evidence suggested sexuality. Analogously, the sexual taxa of Potentillinae (*Argentina*, series *Biflorae* and *Tormentillae*, *Trichocarpae herbaceae*) diverged much earlier than the core *Potentilla*, which exhibited high potential for apomixis. However, the available data do not allow the distinction between common versus parallel origins of apomixis within Potentillinae. Nuclear DNA markers suggested a sister group relationship of sexual-apomictic *Potentilla norvegica* L., as well as additional members of *Rivales* (Töpel, 2010) and the North American genera *Horkelia/Ivesia* (whereas *Rivales* were placed within the core group in the plastid-based phylogenetic reconstruction). This incongruence of the plastid and nuclear marker based phylogenetic trees implies that apomixis may have been transferred between clade C and clade E via hybridization (Dobeš & Paule, 2010; Töpel *et al*., 2011). Alternatively, apomixis may have originated at the base of the lineage joining clades C–E. These hypotheses need to be confirmed by studies of the reproduction of additional taxa from clade E, in particular representatives of *Horkelia/Ivesia*.

In ten out of 14 series expressing apomixis (*Argenteae*, *Aureae*, *Chrysanthae*, *Grandiflorae*, *Haematochroae*, *Multifidae*, *Niveae*, *Persicae*, *Rivales*, and *Rupestris*), sexuality co-occurred (Table 1; see also Supporting information, Doc. S3), suggesting an evolutionary close relationship between these reproductive modes. Sexuality promotes the spread of apomixis with respect to mediating its transfer to sexual backgrounds (Adolfsson & Bengtsson, 2007). Alternatively, apomixis may originate from the asynchronous expression of genes controlling the sexual pathway in allopolyploids (Carman, 1997), a hypothesis that recently found experimental support (Hojsgaard *et al*., 2014). A hybrid origin was suggested for several polyploid *Potentilla* spp. expressing apomictic elements (Matfield, Jones & Ellis, 1970; Asker, 1970a; Soják, 2010b; Paule *et al*., 2011; Paule *et al*., 2012). Although hybridization cannot be considered as an exclusive causal factor, it appears reasonable to conclude that its high propensity in the *Potentilla* core group has contributed to the presence of apomixis.

The survey uncovered that there has been a strong focus on accessions of European provenance (88 species versus 21, 15, three, and two species studied from Asia, North America, Africa, and South/Central America, respectively). These numbers conflict with the geographical distribution of species diversity. Especially, *Alchemilla* and *Potentilla* are still understudied in their respective Asian centres of species diversity. In addition, the reproductive modes of *Drymocallis* are poorly explored in its North American diversity centre, and the North American ivesioid genera were studied for a single species only.

There might be an association between reproductive mode and geographical distribution because genera expressing apomixis (*Alchemilla*, *Aphanes*, and *Potentilla*) exhibited large distribution ranges, whereas the extent of ranges varied widely for purely sexual genera. The ranges of North American ivesioids and (mainly) Asian genera *Argentina* and *Farinopsis*, which are (almost) restricted to single continents, thus contrasted with the Northern Hemisphere distribution ranges of *Drymocallis*, *Dasiphora*, *Fragaria*, and *Sibbaldia*. This pattern was also

observed for the reproductively differentiated phylogenetic lineages of *Potentilla*. Although most studies were carried out on European material, the combined evidence suggests that apomixis is geographically widespread in *Potentilla* (Table 1; see also Supporting information, Doc. S3). The representatives of clade C are distributed throughout the Northern Hemisphere (Rydberg, 1898; Wolf, 1908; Kurtto, Lampinen & Junikka, 2004; Dobeš & Paule, 2010; Soják, 2012). Their wide geographical distribution contrasts with the mostly restricted and often disjunct distribution ranges of species assigned to clades G–I (Dobeš & Paule, 2010), for which exclusive sexuality is documented. The early (12.3–19.4 Mya) diversification of clades G–I versus the rapid recent radiation (2.7–8.1 Mya) of core *Potentilla* (Dobeš & Paule, 2010) raises the question of the evolutionary significance of apomixis with respect to colonization abilities (i.e. migration and establishment in novel habitats). The distribution of apomixis coincides with repeated intercontinental migrations inferred for the core group (Dobeš & Paule, 2010). Apomicts often cover larger distribution ranges and ecologically more extreme habitats than their sexual relatives, a phenomenon known as geographical parthenogenesis (Vandel, 1928). Geographical parthenogenesis suggests the increased abilities of apomicts to colonize new areas and novel ecological niches, including high altitudes and latitudes (Hörandl, Cosendai & Temsch, 2008; Hörandl, 2009). This phenomenon may also be relevant in *Potentilla*, which shows a wide distribution in ecologically extreme areas and habitats (Wolf, 1908; Meusel, Jäger & Weinert, 1965; Kurtto & Eriksson, 2003). Ecogeographical distribution patterns suggesting geographical parthenogenesis recently emerged for *Potentilla rigoana* Th.Wolf in the Apennines *et al*. (Dobeš *et al*., 2013c) and *Potentilla puberula* Krašan in the European Alps (Hülber, Scheffknecht & Dobeš, 2013), as well as for the arctic-alpine *P. crantzii* (J. Paule, F. Kolár & C. Dobeš, unpubl. data). The causality of the ecogeography and the radiation of apomicts, however, is still poorly understood and remains to be investigated in conjunction with other phenomena associated with apomixis, such as polyploidy (Bierzychudek, 1985), hybridization (Asker, 1970a; Czapik, 1975; Gregor, Rollik & Weising, 2002; Paule *et al*., 2012) or habitat preferences (Vrijenhoek, 1984). FCSS is a suitable technique for collecting the required data on reproductive modes, even at fine ecogeographical scales and for the diverse representatives of Potentilleae.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Doc. S1. Plant material studied: accessions are ordered by taxon names, followed by an internal material number, the geographical origin, collector/collecting institution (including collection date and a collection number if available), and voucher number(s). Sampling sites are provided in addition as geographical coordinates.

Doc. S2. Descriptive statistics of the flow cytometric measurement of 783 seeds from six genera and 50 species of the Potentilleae. 'Device' is the flow cytometer and DNA-specific stain used. 'Standard' is the used internal biological standard. Taxa names and accessions are provided as used in the manuscript. N_{seeds} is the number of seeds measured in a sample (each row represents a single measurement or sample). 'Count', 'mean' and 'cv' are the number of particles registered, the mean fluorescence, and the variation coefficient, respectively, calculated for the embryo, the endosperm, and the standard. The peak index (i.e. endosperm: embryo ratio) was calculated from the respective fluorescence means. The 'female contribution' is the calculated ploidy of the embryo (provided as number of holoploid genomes *n*) for apomictically-derived seeds and the percentage of the embryo genome contributed by the maternal plant, respectively. The last column gives the reproductive origin of the embryo. Measurements are arranged in ascending order by taxon names, accession number, and device.

Doc. S3. Reproductive modes observed in the tribe *Potentilleae* derived from the record in the literature. Data are provided at the level of species for *Potentilla* and segregate genera. Data for *Fragaria* and *Alchemilla*/*Aphanes* are provided at the genus level. The modes apomixis (Apo), sexuality (Sex), and co-occurrence of modes within single accessions (Apo-Sex) are provided at the species level. 'Mode accepted' provides the reproductive pathways accepted in accordance with the criteria outlined in the Material and methods. The columns 'Embryology', 'Crossings', and 'FCSS' differentiate between evidence drawn from embryological studies, controlled crossing experiments, and FCSS. 'Traditional' and 'Molecular' refer to the use of morphological/karyological characters and molecular markers, respectively, as inherited traits. The exclamation mark ('!') indicates the documentation of the sporo-/gametogenesis, as well as the embryogenesis in embryological studies and FCSS; otherwise, a single reproductive element only was observed. Doubtful and poorly documented claims for apomixis are indicated by a question mark ('?'). Taxa are ordered alphabetically, within the genus *Potentilla* by series. Synonyms used in the original article and revised names are provided in parentheses. The column 'Clade' assigns taxa to the major phylogenetic lineages of the Potentilleae [1]. The column 'origin' provides the geographical origin of the studied material on the level of continents (Af, Afrika; As, Asia; CSAm, Central and South America; E, Europe, N, northern hemisphere; NAm, North America). In the case where collection sites have not been cited in the original publication, the distribution range of the species according to [2–4] is given (indicated by an asterisk). Series refer to [2]. Taxa underlined were analyzed within the present study using the FCSS.