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Different genome sizes of Western and Eastern *Ficaria verna* lineages shed light on steps of *Ficaria* evolution

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Abstract The genus *Ficaria* is now considered to comprize eight Eurasian species. The most widespread European species is the tetraploid *F. verna* Huds. The present study provides evidence for the existence of two main lineages of *F. verna* that differ considerably in their genome size by about 3 pg. A Western *F. verna* lineage west of river Rhine displays a mean genome size (2C-value) of 34.2 pg and is almost precisely codistributed with the diploid *F. ambigua* Boreau (20 pg) north of the Mediterranean. The remaining part of Europe appears to be occupied by the Eastern *F. verna* lineage solely (mean genome size of 31.3 pg) which codistributes in South-Eastern Europe with the diploid *F. calthifolia* Rchb. (15 pg). There is little overlap at the boundary of Western and Eastern *F. verna* lineages with the occurrence of a separate intermediate group in The Netherlands (mean genome size of 33.2 pg) that appears to result from hybridization of both lineages. On the basis of these observations and further considerations we propose development of *F. ambigua* and *F. calthifolia* south of the Alps with subsequent divergence to populate their current Western and Eastern European ranges, respectively. The Western *F. verna* lineage is proposed to originate from autotetraploidization of *F. ambigua* (precursor) with moderate genome downsizing and the Eastern *F. verna* lineage from autotetraploidization of *F. calthifolia* (precursor).

Keywords *Ficaria verna*, *Ficaria calthifolia*, *Ficaria ambigua*, genome size, flow cytometry, evolution

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Introduction

The Central European area north of the Pyrenees and Alps and north of the French and the Adriatic coast contains three species of the Genus *Ficaria*, i.e. *F. verna* Huds. (*F. verna* Huds. subsp. *verna*), *F. ambigua* Boreau (*F. verna* Huds. subsp. *fertilis* [A.R.Clapham ex Laegaard] Stace), and *F. calthifolia* Rchb. (*F. verna* Huds. subsp. *calthifolia* [Rchb.] Rchb. ex Nyman). The latter two are diploid (2*n*=16) and fertile whereas *F. verna* is tetraploid (2*n*=32) and largely sterile. *F. verna* propagates mainly by axillary tubercles that are absent from the two fertile diploid species (Damboldt 1974, Gill et al. 1972, Greilhuber 1974, Löve & Löve 1982, Marsden-Jones 1935, 1937, Veldkamp 2015, Sell 1994, Tutin et al. 1993).
North of the Mediterranean area *F. ambigua* and *F. calthifolia* are geographically largely separated, however, south of the Alps they overlap in the Italian Penisula. *F. calthifolia* is a south-eastern species that reaches from the Italian Peninsula to Northern Greece, the Black Sea and the Southern Caucasian area and extends west- and northwards to Southern Poland and East and South Germany (Saxony, Brandenburg, Franconia). In contrast, *F. ambigua* is a western species that extends from Italy (Apennines, Sardinia, Elba, Piedmont) and the Iberian Peninsula northwestwards via France and Belgium to Great Britain and Ireland, and has isolated populations in Northern Denmark (Limfjord area, Sealand Odde) and Southwestern Norway (Jalas & Suominen 1989, Laegaard 1966, Sell 1994, Tutin et al. 1993, Veldkamp 2015).
F. verna covers most parts of the extra-Mediterranean European land mass northwards from the Pyrenees, Alps, the Adriatic coast and Northern Greece to Great Britain/Ireland, Norway, Sweden, Finland and further eastwards to the Black Sea coast and Kazakhstan. In Northern Italy (Lombardia and Istria) there is some local overlap with *F. ambigua* and *F. calthifolia* (Jalas & Suominen 1989, Tutin 1993). There are also records from Spain and even North Africa is mentioned (López González 1986).
In the course of flow cytometric (FCM) studies on the nuclear DNA content of European *Ficaria* taxa six specimens with *Ficaria verna* phenotype from Northern Poland and two from Austria displayed significantly lower genomic DNA content than western individuals and were tentatively interpreted to represent tetraploid *F. calthifolia* plants (Zonneveld 2015). This led us to a more systematic analysis on the genome size of *F. verna* throughout Central and Northern Europe. We also included in this survey *F. calthifolia* specimens from its northwestern boundary (Germany) and *F. ambigua* from the isolated population in

Northern Denmark to further supplement and validate the data set.

Nuclear DNA content can conveniently be measured by FCM using the fluorescent dye propidium iodide that intercalates stoichiometrically in the DNA double helix. Whereas most species in the genus *Ficaria* have the same chromosome number, differences in genome size (DNA 2C-value) have proven to be very effective in delimiting infrageneric divisions of *Ficaria* taxa (Zonneveld 2015). Genome size differences of 1 picogram (pg) obtained with FCM equal to a difference of nearly 10^9 base pairs. Hence, genome size data can supplement DNA sequence data that often rely on just a few 1,000 base pairs (Greilhuber 1998, 2005, Leitch et al. 1998, Ohri 1998, Vesely et al. 2011, Zonneveld et al. 2001, 2005, Zonneveld 2008, 2009, 2015).

Materials and Methods

Plant material was collected by the authors and by other botanists who are listed in Zonneveld (2015) and in „Acknowledgements" of this study. Care was taken to ensure correct phenotypical identification of all plants.

Flow cytometric measurement of DNA-2C value

Measurements were conducted as outlined in detail (Zonneveld 2015). In brief, if available, nuclei were extracted from petioles. If these were not available, root tubers or axillary tubercles were used with same results. For the isolation of nuclei, a piece of about 1 cm of the petiole or 0.5 cm of the tuber was chopped together with a piece of *Agave americana* L. '*Aureomarginata*' (2C-value of 15.9 pg) which is used as an internal standard (Galbraith et al. 1983). The chopping was done at room temperature with a new razor blade in a Petri dish in 0.25 ml nuclei-isolation buffer to which 0.25 mg RNase/ml was added (changed after Bharathan et al. 1994). After adding 1.35 ml propidium iodide solution (50 mg PI/l in buffer) the suspension with nuclei was filtered through a 20 µm nylon filter. The fluorescence of the nuclei was measured half an hour and one hour after addition of propidium iodide, using a BD Accuri C6 flow cytometer (BD Accuri) equipped with a 488 nm laser suitable for propidium iodide. Data were analyzed by means of BD Accuri Cflow Plus software provided by the supplier. Plots were first gated to exclude debris on a scatter diagram (Fl2-A vs FL1-A) and counted against FL2-A on a logarithmic scale. The DNA content of the sample was calculated as the sample peak mean, divided by the *Agave* peak mean, and multiplied with the amount of DNA of the *Agave* standard. At least two different samples, with each at least 2000−5000 nuclei, were measured twice for each specimen. Most histograms revealed a coefficient of variation of less than 5%. The standard deviation (σ) was calculated for the DNA content of each specimen using all relevant measurements.

Statistics

Data are presented as histograms using constant binning for all subsets of data performed by the public domain numeric environment GNU Octave Version 4.2.1 (Ubuntu 16.04.; Canonical, London, UK). For determination of the intrinsic distribution of genome sizes, i.e. to eliminate uncertainties due to the measurement process, a maximum likelihood deconvolution algorithm was used (Baumgartner & Drenckhahn 2002). As starting parameters 5 Gauß distributions with means 30, 32, 35, 37 and 39 pg with standard deviation σ=1 and weights of 0.2 each were assumed. Convergence was reached after typically 100 iterations. All samples from the Netherlands were compared with the western and eastern samples using a general non parametric two sample test (BWS-test, Baumgartner et al. 1998). This method tests the null-hypothesis H_0 that two independent samples belong to the same underlying distribution against the alternative hypothesis H_1 that the underlying distributions differ significantly. Furthermore a bootstrapping approach was applied (Shao & Tu 1995).

Results and Discussion

Genome size of Ficaria verna

Diagnostic characters of *F. verna* are axillary tubercles, abortive nutlets, and multisegmental stems (e.g. Sell 1994, Zonneveld 2015, Drenckhahn, 2016). Previous measurements on nuclear DNA content (2C-value, genome size) of *F. verna* from Western Europe (Zonneveld 2015) resulted in a mean of 33.5 pg (σ=1.08, n=46). The mean genome size of 16 specimens from France, Switzerland, Belgium, UK and Ireland was 34.2 pg (σ=0.81). In contrast, our analysis of 30 specimens from Central and Eastern Europe (Germany, Poland, Austria) revealed a significantly lower mean genome size of 31.3 pg (σ=0.42). The genome size of *F. verna* specimens from the Island of Sealand (eastern Denmark), Uppsala (Middle Sweden) and Helsinki (Southern Finland) also belong to the Eastern Lineage with lower mean DNA content (31.5 pg, σ=0.48, n=4). Northern Denmark (32.8 pg, σ=0.42, n=2), South-Western Norway (32.8 pg, σ=1.08, n=7) as well as The Netherlands (33.2 pg, σ=0.94, n=25) can be considered mixed areas with coexistence of the Eastern and Western *F. verna* lineages and intermediates between both lineages (Fig. 2).

Genome size of Ficaria calthifolia

Ficaria calthifolia is a diploid and fertile species with a monosegmental stem and a rosette of stem leaves. Diploid *F. calthifolia* has recently been reported to also occur in Southern an Eastern Germany (Drenckhahn 2016, Illig & Ristow 2015). Four samples from Bavaria, Brandenburg and Saxony had a mean genome size of 14.9 pg (σ=0.37) and matched the genome size of four specimens from Hungary and Austria (15.1 pg, σ=0.59). Triploid *F. calthifolia* plants are more robust with larger flowers, more fleshy blades, lack of leaflets at flower stalks and largely abortive nutlets (classified as *F. calthifolia* type 2, Drenckhahn 2016). Chromosome counts of one type-2 plant from Würzburg indicated triploidy and 2C-values of 4 different type-2 plants from Würzburg supported these counts (23.7 pg, σ=0.33). At its south eastern distribution range on the island of Lefkas (West Greece) *F. calthifolia* types were detected that are phenotypically indistinguishable from type-2 *F. calthifolia* plants in Würzburg. Specimens collected by G. Dietrich on Lefkas are commercially sold by Sarastro Stauden, Austria. Mean genome size of five type-2 specimens from Lefkas was 23.3 pg (σ=0.30) and, hence, almost identical to type-2 plants from Würzburg. Tetraploid *F. calthifolia*: One specimen from Lefkas (Sarastro Stauden, collected by G. Dietrich) resembled phenotypically diploid *F. calthifolia* plants. The mean genome size of three of these specimens was 31.6 pg (σ=0.30) which indicates tetraploidy. Tetraploid plants with *F. calthifolia* phenotype were also reported from Hungary (Soó & Borhidi 1966).

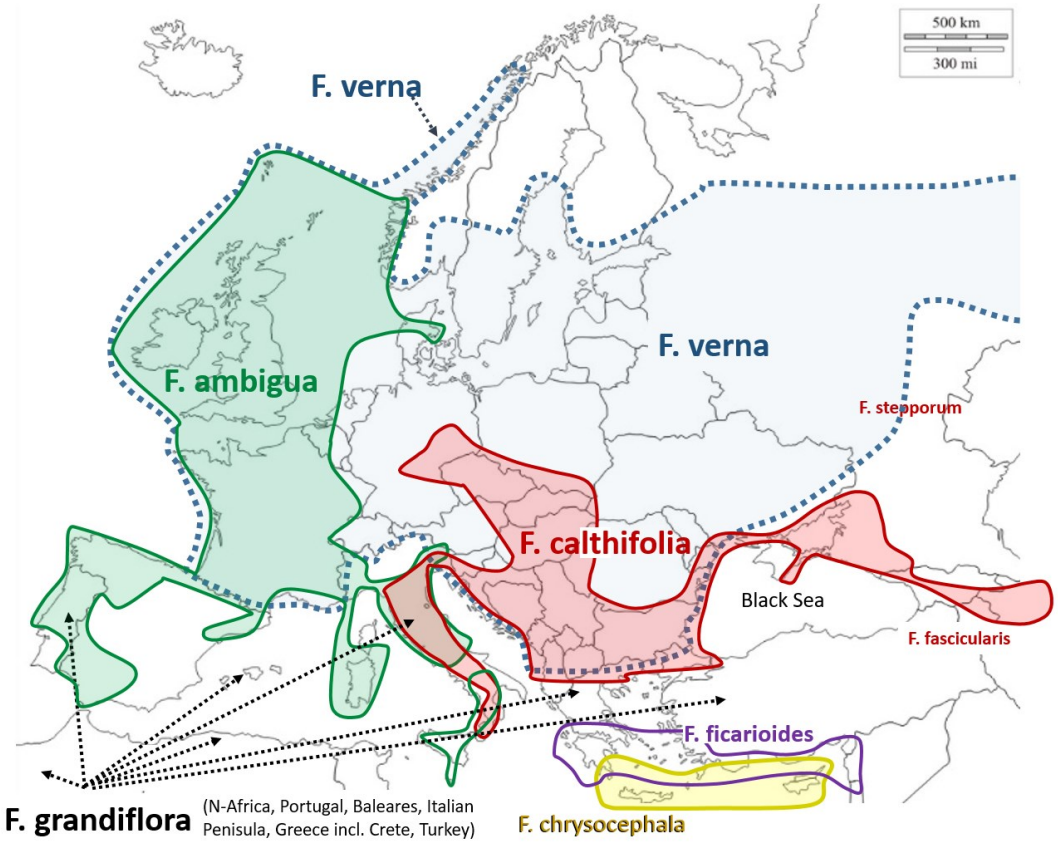

Fig. 1 Distribution of *Ficaria* species in Europe based on Meusel et al. 1965, Jalas & Suominen 1989, Veldkamp 2015. Records outside the main distribution ranges are not shown. The disjunct distribution of the less well characterized *F. grandiflora* was not included in this map, but arrows indicate the main sites of this collective species. *Ficaria grandiflora* is treated in Flora Iberica (López González 1986) as subspecies of *F. ambigua*, whereas in other areas it is defined as tetraploid fertile species with axillary tubercles (Veldkamp 2015). Red colour/line shows distribution of *F. calthifolia.* Boundary lines of the related diploid *F. stepporum* and tetraploid *F. fascicularis* are not shown.

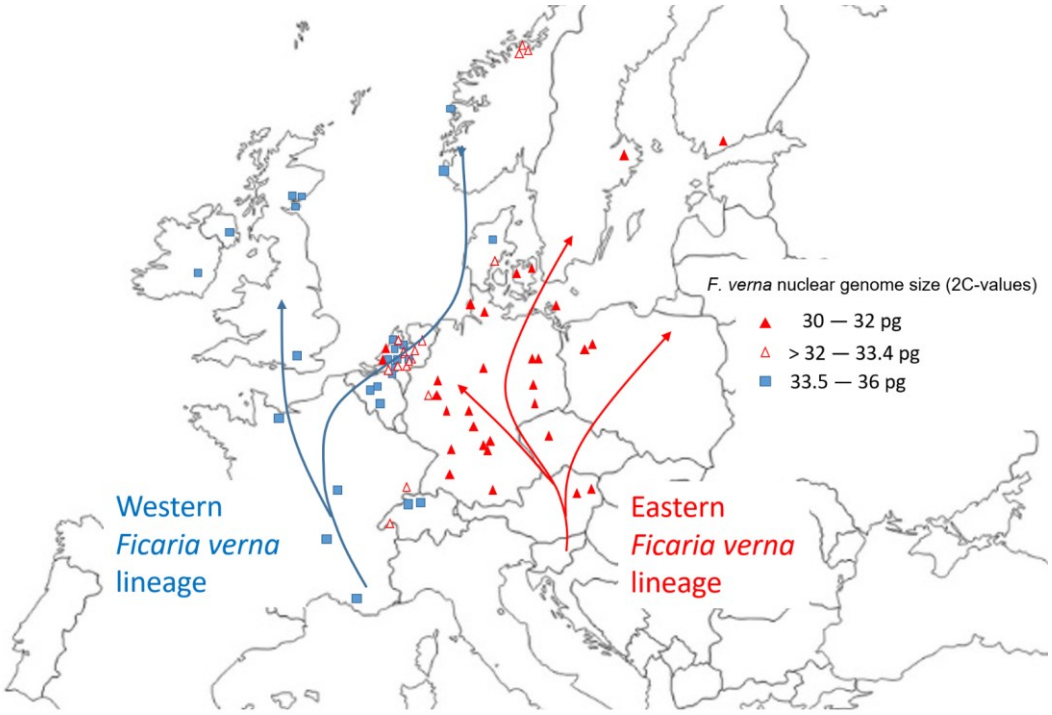

Fig. 2 Location of samples of *F. verna* and their respective genome sizes. Arrows indicate putative postglacial routes of dispersal of a Western *F. verna* lineage with a mean genome size of 34.2 pg and an Eastern *F. verna* lineage with a mean genome size of 31.3 pg. Both tetraploid lineages evolved probably by tetraploidization of *F. ambigua* and *F. calthifolia*, respectively, or precursors of these diploid species.

Genome size of Ficaria ambigua

The genome size of 17 specimens of diploid *F. ambigua* from its Mediterranean (Sardinia) and Western European distribution area (Spain, France, Great Britain, SW Norway) displayed a mean genome size of 20.0 pg (σ=0.44) (Zonneveld 2015). Three specimens collected 2017 in Northern Denmark (Limfjord area) are in full agreement with these data (19.9 pg, σ=0.19). Thus *F. ambigua* appears to be a uniform entity with negligible variation of its genome size troughout its entire distribution range between Norway and the Mediterranean area.

Wstern and Eastern *F. verna* lineages

The data of this study provide evidence for the existence of two main *F. verna* lineages throughout Europe that differ considerably in their genome size by about 3 pg, i.e. a Western *F. verna* lineage (Fig. 3b) west of river Rhine with an mean genome size of 34.2 pg (σ=0.81, n=16) and an Eastern *F. verna* lineage (Fig. 3c) east of river Rhine (Germany, Poland, Austria) with a mean of 31.3 pg (σ=0.42, n=30). Four specimens from Eastern Denmark, Sweden and Finland investigated in this study also belong to the Eastern *F. verna* lineage (31.5 pg, σ=0.48). There is overlap between both lineages in The Netherlands with the occurrence of individuals with intermediate genome size (32–33 pg).

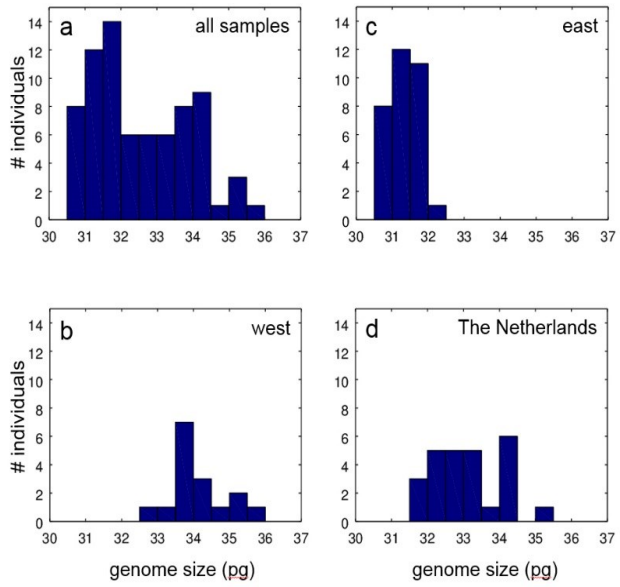

Fig. 3 Frequency distribution (histograms) of genome size of (a) all samples, (b) samples west of river Rhine including British Isles and Southern Norway, (c) samples east of river Rhine (Germany, Poland, Austria, Rest of Scandinavia), and (d) samples from The Netherlands.

The difference of about 3 pg nuclear DNA contents between the linages of Western and Eastern *F. verna* corresponds to the DNA content of about 3×10^9 base pairs or almost the DNA content of 3 average *F. verna* chromosomes. This is an enormous amount of DNA that cannot be explained by different ploidity levels as numerous chromosome counts of *F. verna* specimens throughout Europe confirmed tetraploidy (32 chromosomes) (Dobeš et al. 1996, Gill et al. 1972, Greilhuber 1974, Löve & Löve 1982, Pogan & Wcisło 1975). Triploid, pentaploid and hexaploid *F. verna* individuals were also reported (Anders-Gasser 1985, Marchant & Brighton 1974, Pogan & Wcisło 1975, Tröhler 1976). But these must be very rare because we did not detect any example among the 77 specimens examined in this study displaying a genome size that would match triploid, pentaploid or higher ploidy levels.

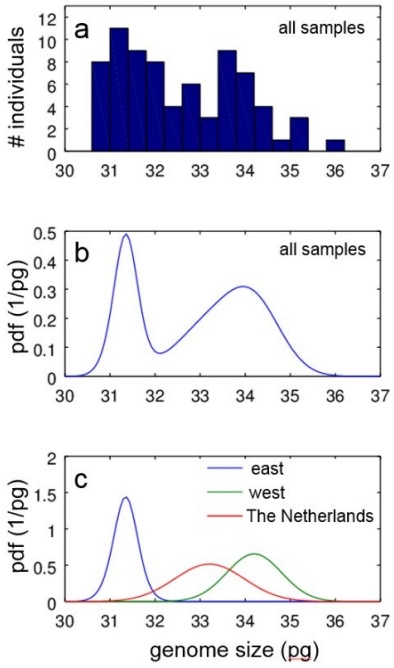

Fig. 4 (a) Histogram of all samples of *F. verna*. (b, c) Probability density function (pdf) of (b) all samples and (c) of samples of the three different geographical regions west of river Rhine (west), east of river Rhine (east) and The Netherlands analyzed by maximum-likelihood deconvolution algorithm. Western and Eastern *F. verna* are well separated as distinct lineages (peaks). Notably, the *F. verna* cohort of The Netherlands is not simply a mixture of eastern and western genome size types but, rather, comprises a separate Gaussian distribution indicative of a hybrid population between Western and Eastern *F. verna* lineages.

Intermediate population in The Netherlands indicates hybridization between Western and Eastern *F. verna* lineages

The histograms in Fig. 3 and 4 are not only suggestive of distinct Western and Eastern *F. verna* lineages of but also suggest that the cohort of The Netherlands is not simply a mixture of both lineages but may comprise an independent third genomic entity intermediate between the Western and Eastern *F. verna* lineages. This possibility was analyzed by the maximum-likelihood deconvolution algorithm of Baumgartner & Drenckhahn (2002). This algorithm revealed three separate Gaussian distributions with mean (μ) peaks at μ_1=31.3 (east), μ_2=34.2 (west) and μ_3=33.2 pg (The Netherlands) and standard deviations of σ_1=0.36, σ_2=0.60 and σ_3=0.77 pg, respectively. This is shown in Fig. 4. Notably, the specimens of The Netherlands do not simply represent a mixture of eastern and western genome size lineages but, instead, have to be considered an intermediate population that resulted to a large degree from hybridization between the Eastern and Western *F. verna* lineages.

This was also shown by testing the specimens from The Netherlands against the Western and Eastern *F. verna* lineages as well as by testing resampled groups comprising mixtures of eastern and western individuals. The BWS-test (Baumgartner et al. 1998) yielded for the specimens of

Western Europe (France, Switzerland, Belgium, Great Britain, Ireland, Norway) and The Netherlands a test value of B=6.22 which corresponds to a significance level $\alpha<0.001$. Thus, the null-hypothesis has to be rejected and, instead, *F. verna* plants from The Netherlands have to be considered a separate entity and not simply a mixture of Western and Eastern *F. verna* lineages. The significance level between Eastern *F. verna* and the *F. verna* cohort of The Netherlands turned out to be even much higher (B=39.5).

In order to further test the hypothesis of a separate *F. verna* population in The Netherlands a classical bootstrapping approach was performed (Shao & Tu 1995). For this purpose 16 individuals of the Eastern *F. verna* lineage were randomly selected by a random number generator and combined with 16 randomly selected individuals from the western lineage. This new sample was tested against the specimens from The Netherlands using the BWS-test. This was repeated 20-fold with different ratios (0.5–2) between western and eastern individuals. The average test values for each ratio ranged from 2.5 (corresponding to $\alpha<0.05$) up to 11.3 ($\alpha<0.0001$) further supporting the notion of a separate genome size population in The Netherlands intermediate between Western and Eastern *F.verna* lineages.

Origin of *Ficaria verna*

The haploid set of eight chromosomes in *F. calthifolia* arranged according to their length and telomeric location were classified as chromosomes 1–8 (Greilhuber 1974) and A–H (Pogan & Wcisło 1975), respectively. Chromosome G (7) contains the nucleolus organizing centre (satellite) at the short arm and is responsible for the heterochromatic area (nucleolus) in interphase nuclei. The two diploid species (*F. ambigua, F. calthifolia*) contain two sets of chromosomes A–H and *F. verna* contains four sets of chromosomes, each with A–H characteristics, indicating close relationship between the three *Ficaria* species that coexist north of the Mediterranean area.

This led to the assumption that *F. verna* evolved from polyploidization of one of the two diploid species including some mutations (Pogan & Wcisło 1975). However, experimental tetraploidization of *F. ambigua* did not result in *F. verna*-like plants with axillary tubercles (Nicholson 1983), and tetraploid *F. calthifolia* plants (phenotype) were found in Hungary (Soó & Borhidi 1966) and on the Western Greek island of Lefkas (31.6 pg, this study) so that tetraploidization of *F. calthifolia* does also not appear to be sufficient to create plants with *F. verna* phenotype. Triploid *F. calthifolia* plants (Drenckhahn 2016, Pogan & Wcisło 1975, this study) also retain pheno-typical characteristics of *F. calthifolia*.Therefore, it appears more likely that the common A–H karyotype of *F. verna* and of the two diploid species (*F. calthifolia, F. ambigua*) originates from some common ancestral diploid *Ficaria* species.

However, in the light of the present observation of two geographically separate lineages of *F. verna* it appears more likely that these *F verna* lineages evolved separately, i.e. the Eastern *F. verna* lineage with link to the evolution of *F. calthifolia* and the Western *F. verna* lineage with link to the evolution of *F.ambigua*.

This conclusion is based not only on the more or less precise overlap of the distribution of the Western *F. verna* with *F. ambigua* and the vast geographical overlap of Eastern *F. verna* with *F. calthifolia* but also on the fact that the genome size of the Eastern *F. verna* lineage (31 pg) is more or less double the genome size of *F. calthifolia* (2×15 pg) and that the genome size of Western *F.verna* lineage (34 pg) is signifiantly higher towards double the genome size of F. ambigua (2×20 pg). It should be noted in this context that the pattern of variation in genome size in polyploid hybrids (e.g. the additivity of parental genome sizes) might be obscured by different post-polyploidization processes, in particular by genome downsizing (Leitch & Bennett 2004, Loureiro et al. 2010) so that the genome size of Western *F.verna* lineage below the expected size of 40 pg, might be a result of genome downsizing – given that Western *F. verna* emerged from autotetraploidization of *F. ambigua* precursor. However, allotetraploidization between *F. ambigua* (precursor) and *F. calhifolia* (precursor) should also be taken into consideration which would result in an additive genome size of 34–35 pg, perfectly matching that of Western *F.verna* lineage.

Hypothesis of evolution of Western and Eastern *Ficaria verna* lineages

As the Italian Peninsula and its associated islands are a hot spot of *Ficaria* diversity (this region harbours four of the six European *Ficaria* species, Fig. 1) and, moreover, as *F. calthifolia* and *F. ambigua* broadly overlap on the Italian Peninsula we assume an important role of this central Mediterranean area not only for evolution of the three main northern *Ficaria* species but also for the genus *Ficaria* in general. If one considers an ancestral diploid *Ficaria* taxon as a fertile plant with a multisegmental stem (similar in phenotype to *F. ambigua*) the following steps might have occurred: 1. Spread to Greece with development to diploid *F. ficarioides* (precursor) that subsequently might have given rise to other Eastern Mediterranean *Ficaria* species (e.g. the tetraploid *F. chrysocephala*). 2. Development of separate lineages of *F. calthifolia* and *F. ambigua* that diverged south of the Alps to populate their current Eastern and Western European ranges. 3. Autotetraploidization of *F. calthifolia* precursor to give birth of the Eastern *F. verna* lineage and autotetraploidization of *F. ambigua* precursor to generate the Western *F. verna* lineage (Fig. 2). Alternatively, allotetraploidization between *F. calthifolia* and *F. ambigua* (precursors) would be another possibilty for the origin of Western *F. verna* (see above).

An alternative origin of Western and Eastern *F. verna* lineages from a common *F. verna* precursor followed by splitting in the western and eastern geographic routes with different genome down- or upsizing is considered less likely.

Another obvious question is why the Western *F. verna* lineage is restricted to the Atlantic area and appears to be basically absent from Continental Europe east of the Rhine valley. One possible explanation would be that expansion during postglacial time of Eastern *F. verna* was faster or earlier than expansion of the western lineage and led to occupation of the land mass east of river Rhine before the Western *F. verna* lineage had reached that area. As rivers are important distribution vectors of plant material (i.e. tubercles, plant fragments, seeds) the Eastern *F. verna* lineage would have been in strategic advantage because once having reached the Bohemian / Franconian area plant material could have been translocated over long distances by rivers into the Baltic- and North Sea area (rivers Elbe and Oder / Odra) as well as into the Rhine basin via river Main.

Concluding remarks

As outlined above, the two *F. verna* lineages, that differ by their genome size, are geographically almost completely separated and this we assume to result from separate origin of these lineages. As *F. verna* proliferates mainly vegetatively both linages can be considered two different

clones. At sites where both lineages merge, specimens with intermediate genome size (32–33.5 pg) were encountered, i.e. in The Netherlands, Western Germany (one case), Southern Rhine valley (one case), Northern Denmark and Norway. These genome size intermediates indicate some kind of hybridization between both lineages (facultative sexuality of *F. verna,* has been shown by Mardsen-Jones & Turrill 1952). This seems to be most obvious in The Netherlands where *F. verna* constitutes a separate entity with a typical Gaussian distribution of intermediate genome size. The *F. verna* group of The Netherlands might represent the northern end of a Central European hybridization zone between Western and Eastern *F. verna* lineages that extends southwards along the Rhine area to the Western Alps.

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References

Anders-Gasser I (1985) Cytotaxonomische Untersuchungen an *Ranunculus*. Mitt. Naturforsch. Ges. Bern (Neue Folge) 42: 79–185

Baumgartner W, Drenckhahn D (2002) An expectation–maximisation algorithm for the deconvolution of the intrinsic distribution of single molecule's parameters. Computers and Chemistry 26: 321–326

Baumgartner W, Weiß P, Schindler H (1998) A nonparametric test for the general two-sample problem. Biometrics 54: 1129-1135

Bharathan G, Lambert GM, Galbraith DW (1994) Nuclear DNA contents of monocotyledons and related taxa. Am. J. Bot. 81:381-386

Damboldt J (1974) *Ranunculus ficaria* Linneaus 1753. In Hegi (ed.) Illustrierte Flora von Mitteleuropa, 2. Auflage, Band III, Teil 3: 244–246. Paul Parey, Berlin-Hamburg

Dobeš C, Kiehn M, Vitek E (1996) Beiträge zur Gefäßpflanzen-Flora von Österreich: Chromosomenzählungen III. Verh. Zool.-Bot. Ges. Österreich 133 (1996): 301–318

Drenckhahn D (2016) Morphologie und Jahreszyklus von *Ficaria calthifolia* Rchb.– eine neu etablierte Sippe in Deutschland. Forum geobotanicum 7: 1-17

Galbraith DW, Harkins KR, Maddox JM, Ayres NM, Sharma DP, Firoozabady E (1983) Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science 220:1049–1051

Gill JBB, Jones BMG, Marchant CJ, Mcleish J, Ockendon DJ (1972) The distribution of chromosome races of *Ranunculus ficaria* L. in the British Isles. Ann. Bot. (Oxford) 36: 31–47

Greilhuber J (1974) Ein Chromosomensatz von *Ranunculus ficaria* ssp. *calthifolius*. Mitt. Bot. Arbeitsgem. Oberösterr. Landesmus. Linz. 6: 3–6

Greilhuber J (1998) Intraspecific variation in genome size: A critical reassessment. Ann. Bot. (Oxford) 82, Suppl. A: 27–35

Greilhuber J (2005) Intraspecific variation in genome size in angiosperms, identifying its existence. Ann. Bot. (Oxford) 95: 91–98

Illig H, Ristow M (2015) *Ficaria calthifolia* RCHB. (syn. *Ranunculus ficaria* subsp. *calthifolius* [RCHB.] ARCANG.) – über einen Neufund für Deutschland. Verh. Bot. Ver. Berlin Brandenburg 147: 13-49

Jalas J, Suominen J (red.) (1989) Atlas Florae Europaeae. 8. *Nymphaeaceae* to *Ranunculaceae*: 184–188, t. 1833–1838. Akateeminen Kirjakauppa, Helsinki.

Laegaard S (1966) *Ranunculus ficaria* ssp. *fertilis* in Denmark. Bot. Tidskr. 61: 295–297

Leitch IJ, Bennett MD (2004) Genome downsizing in polyploid plants. Biol. J. Linn. Soc. 82: 651–663.

Leitch IJ, Chase MW, Bennett MD (1998) Phylogenetic analysis of DNA C-values provides evidence for a small ancestral genome size in flowering plants. Ann. Bot. 82 (Oxford), Suppl. A: 85–94

Löve Á, Löve D (1982) Reports. In: Löve Á (red.), IOPB chromosome number reports LXXXVI. Taxon 31: 583–587

López González G (1986) *Ranunculus* L. in Castroviejo S et al. (eds.) Flora Iberica Vol. I: 298-301. Madrid, Real Jardín Botánico

Loureiro J, Trávníček P, Rauchová J, Urfus T, Vít P, Štech M, Castro S, Suda J (2010) The use of flow cytometry in the biosystematics, ecology and population biology of homoploid plants. Preslia 82: 3–21

Marchand CJ, Brighton CA (1975) Cytological diversity and triploid frequency in a complex population of *Ranunculus ficaria* L. Ann. Bot. (London) 38: 7–15

Marsden-Jones EM (1935) *Ranunculus ficaria* L.: life history and pollination. J. Linn. Soc. (London) 50: 39–55

Marsden-Jones EM (1937) Pollination of *Ranunculus ficaria* L. by insects. J. Bot. 75: 133–141

Marsden-Jones EM, Turrill WB (1952) Studies on *Ranunculus ficaria*. J. Genetics 50: 522–534

Meusel H, Jäger EJ, Weinert E (1965) Vergleichende Chorologie der zentraleuropäischen Flora, Karten: 168. Fischer, Jena

Nicholson GG (1983) Studies on the distribution and the relationship between the chromosome races of *Ranunculus ficaria* L. in S.E. Yorkshire. Watsonia 14: 321–328

Ohri D (1998) Genome size variation and plant systematics. Ann. Bot. (Oxford) 82, Suppl. A: 750–812

Pogan E, Wcisło H. (1975) Studies in *Ranunculus ficaria* L. III. Karyotype analysis. Acta Biol. Cracov., Ser. Bot. 18: 79–102

Sell PD (1994) *Ranunculus ficaria* L. sensu lato. Watsonia 20: 41–50

Shao J, Tu D (1995) The Jackknife and Bootstrap. Springer, Berlin

Soó R, Borhidi A (1966) Über einige Formenkreise der ungarischen und karpatischen Flora IV. *Ficaria*. Ann. Univ. Sci. Budapest Rolando Eötvös, Sect. Biol. 8: 297–300

A. Tröhler (1976) Zytotaxonomische Untersuchungen an *Ranunculus ficaria* L.Mitt. Naturforsch. Ges. Bern (Neue Folge) 33: 21- 28

Tutin TG, Burghes NA, Chater AO, Edmondson JR, Heywood VH, Moore DM, Valentine DH, Walters SM, Webb DA (1993) Flora Europaea, Ed. 2, Vol. 1: 281.Cambridge University Press

Veldkamp JF (2015) De nomenclatuur van Speenkruiden (*Ficaria verna* Huds. s.l., Ranunculaceae). Gorteria 37: 84–116

Vesely P, Bures P, Smarda P, Pavlicek T (2011) Genome size and DNA base composition of geophytes: the mirror of phenology and ecology? Ann. Bot. (Oxford) 109: 65–75

Zonneveld BJM, Iren F van (2001) Genome size and pollen viability as taxonomic criteria: Application to the genus *Hosta*. Plant Biol. 3: 176–185

Zonneveld BJM (2008) The systematic value of nuclear DNA content for all species of *Narcissus* L. (*Amaryllidaceae*). Pl. Syst. Evol. 275: 109–132

Zonneveld BJM (2009) The systematic value of nuclear genome size for all species of *Tulipa* L. (*Liliaceae*). Pl. Syst. Evol. 281: 217–245

Zonneveld BJM, Leitch IJ, Bennett MD (2005) First nuclear DNA amounts in more than 300 angiosperms. Ann. Bot. (Oxford) 96: 229–244

Zonneveld BJM (2015) Genome sizes of *Ficaria* Huds. (*Ranunculaceae*) indicate eight separate species. Gorteria 37:118–139