**Salix transect of Europe: variation in ploidy and genome size in willow-associated common nettle, *Urtica dioica* L. sens. lat., from Greece to arctic Norway**

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Abstract

Background

The common stinging nettle, *Urtica dioica* L. sensu lato, is an invertebrate "superhost", its clonal patches maintaining large populations of insects and molluscs. It is extremely widespread in Europe and highly variable, and two ploidy levels (diploid and tetraploid) are known. However, geographical patterns in cytotype variation require further study.

New information

We assembled a collection of nettles in conjunction with a transect of Europe from the Aegean to Arctic Norway (primarily conducted to examine the diversity of *Salix* and *Salix*-associated insects). Using flow cytometry to measure genome size, our sample of 29
plants reveals 5 diploids and 24 tetraploids. Two diploids were found in SE Europe (Bulgaria and Romania) and three diploids in S. Finland. More detailed cytotype surveys in these regions are suggested. The tetraploid genome size (2C value) varied between accessions from 2.36 to 2.59 pg. The diploids varied from 1.31 to 1.35 pg per 2C nucleus, equivalent to a haploid genome size of c. 650 Mbp. Within the tetraploids, we find that the most northerly samples (from N. Finland and arctic Norway) have a generally higher genome size. This is possibly indicative of a distinct population in this region.

Keywords

megatransect, genome size, cytotype variation, Urtica

Introduction

During a recent study of willow (Salix spp.) stands on a latitudinal transect across Europe (Cronk et al. 2015) the opportunity arose to sample individuals of Urtica dioica L. ssp. dioica (the common stinging nettle) that frequently co-occurs with willow in riparian habitats (see under Materials and Methods for further details). Urtica dioica is one of the most remarkable plants of Europe. First it possesses a defense, stinging hairs, which are a small marvel of biochemistry and biomechanics. These are highly effective against vertebrate herbivores (Levin 1973, Pollard and Briggs 1984, Pullin and Gilbert 1989, Tuberville et al. 1996). The cell walls of the trichome tip are silicified and brittle (Haberlandt 1914, Barber and Shone 1966, Thurston and Lersten 1969, Thurston 1974, Sowers and Thurston 1979) and break off (like the tip of a glass ampoule) on the slightest mechanical stimulation. The fluid released is a potent and complex mixture of toxins including histamine, oxalic acid and tartaric acid (Emmelin and Feldberg 1949, Fu et al. 2006, Taskila et al. 2000).

Secondly it has an extraordinary biogeographical range, occurring in every corner of Europe, from the shores of the Mediterranean to the Arctic Ocean and from the winter-cold central European plain to the rainswept coasts of western Ireland. Few plants have the ability to grow in such a wide range of climatic conditions. Over this range it is largely native, having spread along its natural habitat of rich alluvial river floodplains. However, it has also become an aggressive ruderal, taking advantage of human disturbance to complete its conquest of Europe through accidental introduction by humans.

Thirdly it is an invertebrate “super-host”. Throughout Europe it provides the food plant for large numbers of specialist and generalist insects, notably in the Lepidoptera, Coleoptera and Hemiptera (Davis 1973, Davis 1975, Davis 1983, Davis 1989, Perrin 1975).

Fourthly, it has exceptional mineral nutrition, being highly phosphate demanding. It ceases growth if phosphate is limiting and responds luxuriantly if phosphate is added, whereas in contrast plants adapted to poor soil scarcely respond to additional phosphate (Pigott 1971, Pigott and Taylor 1964, Taylor 2009). It is not only an indicator of high available phosphate, but it is also a general mineral accumulator, having high concentrations of calcium, nitrogen
and phosphorus in its tissues (Müllerová et al. 2014). This may go some way to explaining its attractiveness to herbivorous invertebrates.

Taxonomically *Urtica dioica* is part of a complex of closely related taxa and subtaxa (Grosse-Veldmann and Weigend 2015), which includes *U. dioica* subsp. *subinermis* (R. Uechtr.) Hand & Buttler, *U. dioica* subsp. *sondenii* (Simmons) Hyl. and *U. dioica* subsp. *pubescens* (Ledeb.) Domin (Table 1). In addition there are a number of related European perennial nettles that are sometimes confused with *Urtica dioica*, although they are distinctive. These include: *Urtica gracilis* Aiton (the American stinging nettle), *Urtica kioviensis* Rogow. and *Urtica membranacea* Poir. (Table 1; nomenclature follows Euro+Med PlantBase 2006). Most of these taxa are diploid (Table 1) (typically 2n=26) except for *U. dioica* subsp. *dioica* (common nettle), which is reported as largely, but not completely, tetraploid (Table 1).

| Name | Notes | Representative chromosome counts |
|------|-------|----------------------------------|
| *U. dioica* L. subsp. *dioica* | The common stinging nettle | 2n=26 (Kolník M. and Goliašová, in Mráz 2006); 2n=48 (Májovsky et al. 1987); 2n=48, 52 (Skalińska et al. 1974); 2n=48, 52 (Lippert 2006); 2n=52 (Löve and Kjellqvist 1974); 2n=52 (Corsi et al. 1999) |
| *U. dioica* subsp. *subinermis* (R. Uechtr.) Weigend | | 2n=24/26 (Lippert 2006) |
| *U. dioica* subsp. *sondenii* (Simmons) Hyl. | | 2n=26 (Geltman 1984) |
| *U. dioica* subsp. *pubescens* (Ledeb.) Domin | Syn. *U. galeopsifolia* | 2n=26 (Geltman 1984); 2n=26 (McAllister 1999) |
| *U. gracilis* Aiton | Syn. *U. dioica* subsp. *gracilis* (Alton) Selander | 2n=26, 52 (Woodland et al. 1982) |
| *U. kioviensis* Rogow. | | 2n=26 (Kolník M. and Goliašová, in Mráz 2006) |
| *U. membranacea* Poir. | | 2n=22 (Corsi et al. 1999) |

Two types of cytological diversity have been found in *Urtica dioica* subsp. *dioica*. One is the reported difference in tetraploid chromosome number between 2n=48 and 2n=52 (Skalińska et al. 1974). Such a discrepancy could be due to miscounts, but the repeated reports of both numbers leads to a suspicion that both numbers do exist in nature.

There is also the difference in ploidy level. The possibility must be entertained that counts for *Urtica dioica* of 2n=26 (diploid) refer to one of the infraspecific taxa and not to *U. dioica*.
subsp. dioica. However there are numerous counts that are candidates for genuine diploid \textit{U. dioica} subsp. \textit{dioica}. For instance Kolnik and Golliašová (in Mráz 2006), reported a chromosome count of \(2n=26\) for \textit{Urtica dioica} from Závod, Slovakia. Because of the problematic taxonomy of this group it is very important that herbarium voucher specimens are collected in conjunction with any study.

Genome size estimates have also been made for \textit{Urtica dioica} (see Bennett and Leitch 2012 and additional data not yet incorporated into the database), and these results (Table 2) are also indicative of cytotype diversity. Nevertheless, the same cautionary taxonomic considerations apply as well as technological issues arising from the estimation of genome size (e.g. Doležel et al. 2007, Greilhuber et al. 2007, Pellicer and Leitch 2014).

| Taxon name given in study | 2C-value (pg) | Chromosome number if available (2n) | Origin of material | Comment* | Reference |
|---------------------------|--------------|----------------------------------|-------------------|----------|-----------|
| \textit{U. dioica}        | 1.22         | n/a                              | Canada            | Estimated using FC:PI with LB01 or MgSO\(_4\) buffer and Solanum lycopersicum L. ‘Stupické polní rané’ (2C=1.96 pg) as calibration standard. | Bainard et al. 2011 |
| \textit{U. dioica}        | 3.1          | 52                               | UK                | Estimated using Fe with Senecio vulgaris (PBI population (2C=3.16 pg) as calibration standard. | Mowforth 1986 |
| \textit{U. dioica}        | 2.34         | n/a                              | Germany           | Estimated using FC:PI with Galbraith buffer. Calibration standard unclear. | Barow and Meister 2003 |
| \textit{U. dioica}        | 2.16         | n/a                              | West Balkans, Central Bosnia, Serbia Macedonia | Estimated using FC:PI with Galbraith buffer and Petunia hybrid ‘PxPC6’ (2C=2.85 pg) as calibration standard. | Pustahija et al. 2013 |

Materials and Methods

Context of study

\textit{Urtica dioica} samples (Table 3) were collected during a survey of willow habitats in a latitudinal transect across Europe: the \textit{Salix} transect of Europe (Cronk et al. 2015). The aim
of this was to survey variation in *Urtica* and one of its constant herbivores, the *Urtica* psyllid, *Trioza urticae*, which were co-sampled. Information on *Trioza* will be the given in separate papers. Herbarium and living *Urtica* samples were collected. The aim of the current work was to investigate the extent of ploidy level and genome diversity within the resultant *Urtica* collection.

Table 3.
Locations of the *Urtica* samples collected in April and June 2015, together with estimated genome size (2C-values) and ploidy levels made from the living material (herb. = only herbarium material available).

| Sample | Latitude (N) | Longitude (E) | Country | River/ location | 2C-value (pg) | Ploidy Level (x) | Living material/Flow cytometry |
|--------|--------------|---------------|---------|----------------|---------------|-----------------|------------------------------|
| 2-4    | 38.902       | 22.31015      | Greece  | R. Sperchios, near Leianokladi, east of Lamia | -             | -               | Herb. only                 |
| 4-4    | 40.032685    | 22.175437     | Greece  | Stream near Kokkinogelia, Thrace               | 2.46          | 4               | Yes                         |
| 5-3    | 41.113317    | 23.273893     | Greece  | At R. Struma, near Lithotopos                  | -             | -               | Herb. only                 |
| 6-5    | 41.412468    | 23.318609     | Bulgaria | R. Struma, near Topolnitsa                     | 2.41          | 4               | Yes                         |
| 7-5    | 42.165622    | 22.998141     | Bulgaria | R. Struma, north of Boboshevo                 | 1.35          | 2               | Yes                         |
| 8-3    | 42.923989    | 23.810563     | Bulgaria | R. Kalnitza, near Botevgrad                    | 2.46          | 4               | Yes                         |
| 11-4   | 44.961981    | 23.190337     | Romania | R. Jiu, north of Rovinari                     | 1.33          | 2               | Yes                         |
| 12-3   | 45.510676    | 22.737225     | Romania | Meadow near Paucinesti, Carpathian region     | -             | -               | Herb. only                 |
| 13-4   | 46.518504    | 21.512839     | Romania | R. Crisul Alb, at Chisineu-Cris               | -             | -               | Herb. only                 |
| 14-6   | 46.700744    | 21.31268      | Hungary | R. Fekete-Koros, near Gyula                   | 2.46/2.46     | 4               | Yes (x2)                    |
| 15-5   | 47.665648    | 21.261768     | Hungary | Drainage ditches near R. Hortobagy, north-east of Balmazujvaros | 2.42          | 4               | Yes                         |
| No. | Latitude  | Longitude | Country | Location Details | Distance | Elevation | Presence |
|-----|-----------|-----------|---------|------------------|----------|-----------|----------|
| 16-7| 48.374291 | 20.725264 | Hungary | R. Bodva, south of Szendro | 2.43 | n.d. | Yes |
| 17-4| 49.463447 | 21.697255 | Poland | R. Panna, at Tylawa | - | - | Herb. only |
| 18-4| 50.470234 | 22.238372 | Poland | Fields north of Rudnik nad Sanem | 2.48 | 4 | Yes |
| 19-7| 50.673994 | 21.823391 | Poland | R. Leg, near Gorzyce | - | - | Herb. only |
| 20-6| 51.775039 | 21.1971 | Poland | R. Pilica, at Warka | 2.42 | 4 | Yes |
| 21-11a| 52.69398 | 21.8529 | Poland | R. Bug, near Brok | 2.48 | 4 | Yes |
| 22-6| 53.55483 | 22.30299 | Poland | Meadow near R. Biebrza at Wasocz, near Szczuczyn | 2.44 | 4 | Yes |
| 23-6| 54.06943 | 23.11745 | Poland | R. Czarna Hancza, near Sejny on road from Suwałki | 2.45 | 4 | Yes |
| 24-11| 54.92583 | 23.7742 | Lithuania | Embankment of River at Kaunas | 2.40 | 4 | Yes |
| 26-15| 56.71141 | 24.25162 | Latvia | Near R. Misa, between Iecava and Kekana | - | - | Herb. only |
| 27-6 & 7| 57.74963 | 24.4023 | Latvia | R. Salaca short distance inland from Salacgriva | 2.40 | 4 | Yes (27-7) |
| 28-10| 58.42257 | 24.44063 | Estonia | Field near Parnu | 2.36 | 4 | Yes |
| 29-7| 59.40289 | 24.93577 | Estonia | R. Pirita at Lagedi near Tallinn | 2.44 | 4 | Yes |
| 30-8| 60.27299 | 24.65843 | Finland | Near Lake Bodom, Espoo, Finland | n.d. | n.d. | Yes |
| 31-12| 61.09965 | 25.6282 | Finland | Drainage flowing into lake Vesijärvi at Paimela near Lahti | 1.33 | 2 | Yes |
| 32-11| 62.04962 | 26.12369 | Finland | Lake near Tolvakka | 1.34 | 2 | Yes |
| 34-6| 64.05074 | 25.52664 | Finland | R. Pyhjoki, at Joutenniva, south of Haapavesi | 1.31 | 2 | Yes |
| 35-8| 64.61287 | 25.53805 | Finland | Tributary of the R. Siljakoki near Mankila | 2.49 | 4 | Yes |
Site selection and sampling

Full details of the sites (mainly riverine alluvial habitats), and their selection are given in Cronk et al. (2015). The sites are summarized in Table 3. In all, 42 Salix sites were chosen between Athens (Greece) and Hammerfest (Norway) (Fig. 1). Of these 33 (and one supplementary site) had Urtica dioica present and a herbarium voucher specimen was collected from each of these sites (and in addition one specimen of U. membranacea from Greece). Herbarium voucher specimens are deposited in the herbarium of the Natural History Museum, London (BM). Living specimens were also collected for cultivation in London (Queen Mary University of London), for future experimental work. One living specimen was collected from each site (two from site 27). Of the living specimens collected, 27 survived into cultivation and could be used for flow cytometry (see results). The living specimens were grown in London in a ‘common garden’ (rooftop plant growth facility at Queen Mary University of London, Lat. 51.5234. Long. -0.0423).

Flow cytometry and buffers

Ploidy level (diploid vs tetraploid) was assayed using flow cytometry (as described in Hanson 2005), using a Partec CyFlow flow cytometer with Petroselinum crispum (parsley) ‘Champion Moss Curled’ 2C=4.50 pg (Obermayer 2002) as calibration standard. A range of different flow cytometry buffers were tested (including the Galbraith buffer, the general purpose buffer and the LB01 buffer, Pellicer and Leitch 2014). However, only the ‘CyStain PI Absolute P kit’ buffer (Sysmex UK) gave acceptable flow histograms with CVs routinely less than 3%, so it was chosen for estimating the ploidy level of the 29 Urtica specimens.
Results

The flow cytometry results are given in Table 3. In all, 24 plants have flow cytometry results consistent with tetraploidy whereas five plants, 7-5 (Bulgaria), 11-4 (Romania), while 31-12, 32-11, 34-6 (southern Finland), have results consistent with diploidy. The identity of these diploid plants was checked and confirmed as *U. dioica* sens. lat. As diploidy is often associated with stinglessness, information on the presence of stinging hairs was collected after cultivation in a common garden (Queen Mary University of London, QMUL) for one year (Table 4). Information on flowering time in the common garden is also given. Flowering time shows an overall correlation with latitude (generally with late flowering plants coming from Finland and Norway, although there are some exceptions (Table 4). Voucher specimens of both the original specimens and plants after cultivation (in common garden conditions for one year) are deposited at the Natural History Museum, London (BM).
Table 4.

Urtica phenotype in common garden (London). Fl. (flowering) time refers to category of flowering performance in 2016; 1 = early flowering (flowering before 16 May); 2 = mid-June (flowering by 10 June); 3 = late June (21 June); 4 = early July (2 July); 5 = late or not flowering (not flowering by early July). Stinging hairs refers to the typical number of stinging hairs per leaf; 1 = <10; 2 = 10-50; 3 = 50-100; 4 = >100. Numbers are given for: adaxial surface (first number)/abaxial surface (second number).

| Accession | Fl. time | Stinging hairs | Notes |
|-----------|----------|----------------|-------|
| 4-4       | 1        | 3/3            | Well-armed. |
| 6-5       | 2        | 2/3            | Moderately well-armed. |
| 7-5 (diploid) | 4   | 1/3      | Tall plant with rather narrow leaves but abundant stinging hairs on undersides of leaves. Non-stinging hairs very short. |
| 8-3       | 5        | 1/3            | Moderately well-armed. |
| 11-4 (diploid) | 3    | 1/1       | Leaves largely stingless except on petiole. Shortly pubescent on veins and stems. |
| 14-6      | 1        | 3/4            | Well-armed. |
| 15-5      | 2        | 1/1            | Leaves largely stingless, except on petiole. Shortly pubescent on veins. |
| 16-7      | 2        | 3/4            | Well-armed. |
| 18-4      | 2        | 3.4            | Well-armed. |
| 20-6      | 2        | 3/4            | Well-armed. |
| 21-11     | 2        | 2/3            | Moderately well-armed. |
| 22-6      | 3        | 3/4            | Well-armed. |
| 23-6      | 2        | 3/4            | Well-armed. |
| 24-11     | 2        | 1/1            | Largely stingless except on petiole and midrib. Pubescent on veins. |
| 27-7      | 3        | 3/4            | Well-armed. |
| 28-10     | 3        | 1/1            | Largely stingless except on petiole, pubescent with rather long hairs on veins. |
| 29-7      | 2        | 3/4            | Well-armed. |
| 30-8      | 5        | 2/3            | Moderately well-armed. |
| 31-12 (diploid) | 3 | 1/3       | Moderately armed below, other pubescence of rather sparse very short hairs. |
| 32-11 (diploid) | 3 | 1/1       | Leaves very largely unarmed below, stinging hairs mainly on inflorescence, petiole and stem, otherwise similar to previous, but stems and veins covered with longer non-stinging hairs. |
At the tetraploid level, some variation in the estimated genome sizes was detected, with the northern populations tending to have higher 2C-values compared with the more southerly ones (Table 3; Fig. 2). To confirm that this intraspecific variation was genuine rather a technical artefact, leaves from the two individuals showing the largest difference in 2C-value (i.e. 28-10, 37-6) were co-processed. This resulted in two distinct peaks in the flow histogram (Fig. 3), indicative of biologically real difference in C-values at the tetraploid level.

### Table 3

| Sample | Chromosome | Pubescence | Remarks |
|--------|------------|------------|---------|
| 34-6 (diploid) | 4 | 1/3 | Moderately well-armed; other pubescence of very short hairs. |
| 35-8 | 3 | 3/4 | Well-armed. |
| 37-6 | 5 | 2/3 | Moderately well-armed. |
| 38-11 | 4 | 2/3 | Moderately well-armed. |
| 39-16 | 5 | 2/3 | Moderately well-armed. |
| 42-8 | 3 | 3/4 | Well-armed. |
| 42-8 | 4 | 3/4 | Well-armed. |
| FIN-D4 | 5 | 3/4 | Well-armed. |

Figure 2.

Scatter plot of genome size (2C-value, pg) values (as given in Table 3), plotted against latitude (Table 3). Only tetraploids (4x) are shown; diploid samples (2x) are not plotted. Note the generally higher genome size of the high latitude samples (see Table 3).
Discussion

The results confirm that the tetraploid is the dominant cytotype in our sample of *U. dioica* but that diploid plants do occur relatively frequently (at least in SE Europe and S. Finland). A more extensive survey of cytotype variation in Romania and Bulgaria, as well as around the Baltic would be of interest. Ploidy level has been shown to correspond with morphological characters (Geltman 1984; Geltman 1986). The possibility must be therefore be examined that the diploid samples here belong to the diploid taxa *Urtica dioica* ssp. *pubescens* (Ledebr.) Domin (synonym: *U. dioica* ssp. *galeopsifolia* (Wierzb. ex Opiz) J. Chrtek), *U. dioica* ssp. *subinermis* (R. Uechtr.) Weigend or *Urtica dioica* ssp. *sondenii* (Simmons) Hyl. These taxa typically lack stinging hairs on the leaves. *U. dioica* ssp. *pubescens* typically has a pubescence of long non-stinging hairs whereas *U. dioica* ssp. *sondenii* is glabrous. We are cautious in assigning any of the individuals studied here to those taxa without further study of the populations, which may not be homogeneous. Of the diploids, only 11-4 and 32-11 can be considered stingless. None of the diploids here are glabrous (although many have an indumentum of very short hairs), ruling out *U. dioica* ssp. *sondenii*. Only 32-11 (with few stinging hairs on leaves and relatively long pubescence) can be considered a reasonable match for *U. dioica* ssp. *pubescens*. However this plant generally resembles the other diploids, 31-12 and 34-6, which vary in stinging hairs and
pubescence. Until the populations from which these plants come can be examined critically we tentatively assign all our samples to the variable *U. dioica* ssp. *dioica*.

The finding of diploids in SE Europe raises the possibility that the widespread tetraploid form of *Urtica dioica* subsp. *dioica*, which has also become a weed, may have originated there, and the diploids may have survived glacial episodes in S. European refugia. The origin of the diploids of S. Finland is as yet unknown, although a phylogeographic analysis might be informative here. Another interesting result is the discovery of intraspecific C-value variation, particularly the generally higher C-values in the far north. This may be indicative of a distinct population of nettles in the north, and again this would benefit from more detailed cytogeographic study.

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**References**

- Bainard J, Husband B, Baldwin S, Fazekas A, Gregory TR, Newmaster S, Kron P (2011) The effects of rapid desiccation on estimates of plant genome size. Chromosome Research 19 (6): 825-842. DOI: [10.1007/s10577-011-9232-5](https://doi.org/10.1007/s10577-011-9232-5)
- Barber DA, Shone MGT (1966) The absorption of silica from aqueous solutions by plants. Journal of Experimental Botany 17 (3): 569-578. DOI: [10.1093/jxb/17.3.569](https://doi.org/10.1093/jxb/17.3.569)
- Barow M, Meister A (2003) Endopolyploidy in seed plants is differently correlated to systematics, organ, life strategy and genome size. Plant, Cell and Environment 26 (4): 571-584. DOI: [10.1046/j.1365-3040.2003.00988.x](https://doi.org/10.1046/j.1365-3040.2003.00988.x)
- Bennett M, Leitch I (2012) Plant DNA C-values database (release 6.0, Dec. 2012). [http://data.kew.org/cvalues/](http://data.kew.org/cvalues/). Accession date: 2016 3 21.
- Corsi G, Garbari F, Maffei F (1999) Il genere *Urtica* L. (Urticaceae) in Italia. Revisione biosistematica. Webbia 53 (2): 193-239. DOI: [10.1080/00837792.1999.10670662](https://doi.org/10.1080/00837792.1999.10670662)
- Cronk Q, Ruzzier E, Belyaeva I, Percy D (2015) *Salix* transect of Europe: latitudinal patterns in willow diversity from Greece to arctic Norway. Biodiversity Data Journal 3: e6258. DOI: [10.3897/bdj.3.e6258](https://doi.org/10.3897/bdj.3.e6258)
• Davis BNK (1973) The Hemiptera and Coleoptera of stinging nettle (Urtica dioica L.) in East Anglia. Journal of Applied Ecology 10 (1): 213-237. DOI: 10.2307/2404726
• Davis BNK (1975) The colonization of isolated patches of nettles (Urtica dioica L.) by insects. Journal of Applied Ecology 12 (1): 1-14. DOI: 10.2307/2401713
• Davis BNK (1983) Insects on Nettles. Naturalists’ Handbook 1. CUP, Cambridge, 65 pp.
• Davis BNK (1989) The European distribution of insects on stinging nettles, Urtica dioica L.: A field survey. Bolletino di zoologia 56 (4): 321-326. DOI: 10.1080/1125000890935658
• Doležel J, Greilhuber J, Suda J (2007) Estimation of nuclear DNA content in plants using flow cytometry. Nature Protocols 2 (9): 2233-2244. DOI: 10.1038/nprot.2007.310
• Emmelin N, Feldberg W (1949) Distribution of acetylcholine and histamine in nettle plants. New Phytologist 48 (2): 143-148. DOI: 10.1111/j.1469-8137.1949.tb05116.x
• Euro+Med PlantBase (2006) Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity. http://ww2.bgbm.org/EuroPlusMed/. Accession date: 2016 3 21.
• Fu HY, Chen SJ, Chen RF, Ding WH, Kuo-Huang LL, Huang RN (2006) Identification of oxalic acid and tartaric acid as major persistent pain-inducing toxins in the stinging hairs of the nettle, Urtica thunbergiana. Annals of botany 98 (1): 57-65. DOI: 10.1093/aob/mcl089
• Geltman DV (1984) Cytotaxonomical studies of the species of the genus Urtica (Urticaceae) in the Flora of the USSR. Bot. Zhurn. SSSR 69 (11): 1525-1530. [In Russian].
• Geltman DV (1986) Systematic and ecological-geographic characteristics of the species from the affinity of Urtica dioica (Urticaceae) in the flora of the U.S.S.R. Bot. Zhurn. SSSR 71: 1480-1489. [In Russian].
• Greilhuber J, Temsch E, Loureiro J (2007) Nuclear DNA content measurement. In: Doležel J, Greilhuber J, Suda J (Eds) Flow Cytometry with Plant Cells: Analysis of Genes, Chromosomes and Genomes. Wiley-VCH, Weinheim, 479 pp.
• Grosse-Veldmann B, Weigend M (2015) Weeding the nettles III: Named nonsense versus named morphotypes in European Urtica dioica L. (Urticaceae). Phytotaxa 208 (4): 239-260. DOI: 10.11646/phytotaxa.208.4.1
• Haberlandt G (1914) Physiological Plant Anatomy (translated by Montagu Drummond). Macmillan and Co., Ltd., London, 777 pp.
• Hanson L (2005) First Nuclear DNA C-values for 18 Eudicot Families. Annals of Botany 96 (7): 1315-1320. DOI: 10.1093/aob/mci283
• Levin D (1973) The role of trichomes in plant defense. The Quarterly Review of Biology 48: 3-15. DOI: 10.1086/407484
• Lippert W (2006) Chromosomenzahlen von Pflanzen aus Bayern und anderen Gebieten. Ber. Bayer. Bot. Ges. 76: 85-110.
• Löve A, Kjellqvist E (1974) Cytotaxonomy of Spanish plants. III.-Dicotyledons. Lagascalia 4 (1): 3-32.
• Majošovský J, Murín A, Hindáková M, Schwarzová T, Uhríková A, Váchová M, Záborský J (1987) Karyotaxonomický pohon flóry Slovenska. Veda, Bratislava, 436 pp.
• McAllister H (1999) Urtica galeopsifolia Wierzb. ex Opiz (Urticaceae) confirmed for Britain by its chromosome number. Watsonia 22 (3): 275-278.
• Mowforth MAG (1986) Variation in nuclear DNA amounts in flowering plants: an ecological analysis. Ph.D. thesis. University of Sheffield, Sheffield, XXX pp.
• Mráz P (2006) Chromosome number and DNA ploidy level reports from Central Europe — 2. Biologia 61 (1): 115-120. DOI: 10.2478/s11756-006-0017-9

• Müllerová V, Hejcmán M, Hejcmanová P, Pavlů V (2014) Effect of fertilizer application on *Urtica dioica* and its element concentrations in a cut grassland. Acta Oecologica 59: 1-6. DOI: 10.1016/j.actao.2014.05.004

• Obermayer R (2002) Nuclear DNA C-values in 30 species double the familial representation in Pteridophytes. Annals of Botany 90 (2): 209-217. DOI: 10.1093/aob/mcf167

• Pellicer J, Leitch I (2014) The application of flow cytometry for estimating genome size and ploidy level in plants. In: Besse P (Ed.) Molecular Plant Taxonomy. Humana Press: 279-307, XX pp.

• Perrin RM (1975) The role of the perennial stinging nettle, *Urtica dioica*, as a reservoir of beneficial natural enemies. Annals of Applied Biology 81 (3): 289-297. DOI: 10.1111/j.1744-7348.1975.tb01644.x

• Pigott CD (1971) Analysis of the response of *Urtica dioica* to phosphate. New Phytologist 70 (5): 953-966. DOI: 10.1111/j.1469-8137.1971.tb02597.x

• Pigott CD, Taylor K (1964) The distribution of some woodland herbs in relation to the supply of nitrogen and phosphorus in the soil. Journal of Animal Ecology 33: 175-185. DOI: 10.2307/2438

• Pollard AJ, Briggs D (1984) Genecological studies of *Urtica dioica* L. III. Stinging hairs and plant-herbivore interactions. New Phytologist 97 (3): 507-522. DOI: 10.1111/j.1469-8137.1984.tb03615.x

• Pullin A, Gilbert J (1989) The stinging nettle, *Urtica dioica*, increases trichome density after herbivore and mechanical damage. Oikos 54 (3): 275-280. DOI: 10.2307/3565285

• Pustahija F, Brown S, Bogunić F, Bašić N, Muratović E, Ollier S, Hidalgo O, Bourge M, Stevanović V, Siljak-Yakovlev S (2013) Small genomes dominate in plants growing on serpentine soils in West Balkans, an exhaustive study of 8 habitats covering 308 taxa. Plant and Soil 373: 427-453. DOI: 10.1007/s11104-013-1794-x

• Rice A, Glick L, Abadi S, Einhorn M, Kopelman N, Salman-Minkov A, Mayzel J, Chay O, Mayrose I (2014) The Chromosome Counts Database (CCDB) - a community resource of plant chromosome numbers. New Phytologist 206 (1): 19-26. DOI: 10.1111/nph.13191

• Skalinska M, Malecka R, Izmailow R (1974) Further studies in chromosome numbers of Polish angiosperms XI. Acta Biologica Cracoviensia, Ser. Botanica 19: 107-148.

• Sowers A, Thurston EL (1979) Ultrastructural evidence for uptake of silicon-containing silicic acid analogs by *Urtica pilulifera* and incorporation into cell wall silica. Protoplasma 101: 11-22. DOI: 10.1007/bf01293431

• Taskila K, Saarinen JV, Harvima IT, Harvima RJ (2000) Histamine and LTC4in stinging nettle-induced urticaria. Allergy 55 (7): 680-680. DOI: 10.1034/j.1398-9995.2000.00635.x

• Taylor K (2009) Biological Flora of the British Isles: *Urtica dioica* L. Journal of Ecology 97 (6): 1436-1458. DOI: 10.1111/j.1365-2745.2009.01575.x

• Thurston EL (1974) Morphology, fine structure, and ontogeny of the stinging emergence of *Urtica dioica*. American Journal of Botany 61 (8): 809-817. DOI: 10.2307/2441617

• Thurston EL, Lersten N (1969) The morphology and toxicology of plant stinging hairs. The Botanical Review 35 (4): 393-412. DOI: 10.1007/bf02858878
• Tuberville T, Dudley P, Pollard AJ (1996) Responses of invertebrate herbivores to stinging trichomes of *Urtica dioica* and *Laportea canadensis*. Oikos 75 (1): 83-88. DOI: [10.2307/3546324](10.2307/3546324)

• Woodland D, Bassett IJ, Crompton C, Forget S (1982) Biosystematics of the perennial North American taxa of *Urtica*. I. Chromosome number, hybridization, and palynology. Systematic Botany 7 (3): 269-281. DOI: [10.2307/2418388](10.2307/2418388)