Genome sizes of 227 accessions of *Gagea* (Liliaceae) discriminate between the species from the Netherlands and reveal new ploidies in *Gagea*

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**Abstract**

Nuclear genome size, as measured by flow cytometry with propidium iodide, was used to investigate the relationships within the genus *Gagea* (Liliaceae), mainly from the Netherlands. The basic chromosome number for *Gagea* is \( x = 12 \). The inferred ploidy in the Dutch and German accessions varies from diploid to decaploid. Consequently there is a large range of genome sizes (DNA 2C-values) from 14.9 to 75.1 pg. Genome sizes are evaluated here in combination with the results of morphological observations. Five species and the hybrid *G. × megapolitana* are reported. Apart from 14 diploid *G. villosa*, six plants of *G. villosa* with an inferred tetraploidy were found. For the 186 Dutch accessions investigated 85 turned out to be the largely sterile *G. pratensis* (inferred to be pentaploid). Inferred tetraploid and hexaploid *G. pratensis* were found in 30 and 20 localities, respectively. In one locality an inferred decaploid (10×) plant was found that could represent a doubled pentaploid *G. pratensis*. An inferred decaploid *G. pratensis* was never reported before. The genome size of *Gagea × megapolitana* from Germany fitted with its origin as a cross between the two hexaploids *G. pratensis* and *G. lutea*. *Gagea spatheacea* from the Netherlands was inferred to be nonaploid as was recorded from plants across Europe. The aim of the study was to use flow cytometry as a tool to elucidate the taxonomic position of the Dutch *Gagea*.

**Keywords:** *Gagea*, The Netherlands, DNA 2C-value, Genome size, Ploidy level, New decaploid *G. pratensis*

**Background**

The genus *Gagea* Salisb. comprises about 275 species. In the World Checklist for *Gagea* (Govaerts 2006) 594 names were listed. It is a genus of small bulbous plants in the family Liliaceae, endemic to Eurasia and North Africa. A single circumpolar species, a former *Lloydia* is now included in *Gagea* (Peruzzi 2012). The greatest number of species can be found in Kazakhstan in the Tien Shan and Pamir-Alai. This coincides with the greatest richness of *Tulipa* (Zonneveld 2010). In Flora Neerlandica (van Oostrom and Reichgelt 1964) four species are recorded for The Netherlands and in Heukels Flora of The Netherlands (van der Meijden 2005) a fifth is added.

To elucidate the relationships between *Gagea* species, the classical taxonomic traits based on morphological characters, chromosome numbers (Peruzzi 2003, 2012) and sequencing data (Peterson et al. 2008; Zarrei et al. 2009) are here supplemented with data on nuclear DNA content. From only five species genome size was determined earlier (Greilhuber et al. 2000; Vesely et al. 2011; Leitch et al. 2007). Taxonomy of *Gagea* is rather difficult and the main useful characters so far are: the chromosome numbers, the number and type of bulbils, the number and width of the leaves, the presence of red coloration at the base of the leaf, the hairiness of the flower stalk, the shape of the petals and the number of flowers on a scape. Newer investigations are also based on morpho-anatomical data (Peruzzi 2012).
186 different accessions from The Netherlands were measured in an attempt to understand the relationships within the Dutch gageas. These values were compared with an additional 41 taxa from Germany. Nuclear DNA content can conveniently be measured by flow cytometry using propidium iodide, a stoichiometric DNA stain that intercalates in the double helix. Where many species in a genus have the same chromosome number, differences in DNA 2C-value have proven to be very effective in delimiting infrageneric divisions in a number of taxa (Ohri 1998). The evolution of genome size (Greilhuber 1979) has received increased attention during recent years (Greilhuber 2005). The smallest angiosperm genome size reported so far is for Genlisia margarethae Hutch. with $2C = 0.13$ pg (Greilhuber et al. 2006). The record holders for maximum genome size were for eudicots Viscum album L. with $2C = 205.8$ pg and for monocots Paris japonica with $2C = 304.5$ pg (Pellicer et al. 2010). Flow cytometry was successfully used to measure the 2C-value for the genera Hosta Tratt., Helleborus L., Clivia Lindl., Nerine Herb., Agapanthus L’Hér., Galanthus L., Narcissus L., Gasteria Duval., Tulipa L. etc. by Zonneveld (2001, 2003, 2008, 2009, 2010), Zonneveld and Van Iren (2001), Zonneveld and Duncan (2003, 2006), and Zonneveld et al. (2003, 2012). In this paper it is shown that genome size is helpful to discriminate between the species of Gagea from The Netherlands (Fig. 1).

Nuclear DNA content as measured by using flow cytometry may conveniently be used to produce systematic data. It is applicable even in dormant bulbs or sterile plants for the monitoring of the trade in bulbous species. In the case of Gagea, it is difficult to ascribe a plant to a taxon in the often non-flowering state. Genome size is a good way to determine the species and their ploidy. A different genome size infers usually a different ploidy or a different species. However, the reverse is not true: if plants have the same genome size it does not automatically mean that they are the same species, it might be a coincidence.

Based on van den Berg and te Linde (2003) and new observations, morphological descriptions were given for the species, correlating it with the measured genome weights. New ploidies were inferred for Gagea villosa which, apart from 14 diploids, had six plants with an inferred tetraploid amount of DNA and for G. pratensis that was found to have, apart from the tetraploid, the hexaploid and the very common pentaploid accessions, also a genome size inferring decaploidy.

**Methods**

**Plant material**

The plant material used in this study was collected from locations across The Netherlands and Germany as described in Table 1. It was mainly obtained from B. te Linde, Stichting Berglinde, Babberich and a few from L. Duistermaat from NCB Naturalis, Leiden, The Netherlands. Further material came from T. Pfeiffer from the Ernst-Moritz-Arndt-University of Greifswald, Germany. The German plants, supplied with chromosomes counts, were used to infer the ploidy of the Dutch plants. Material of known origin was used. Vouchers will be lodged in the Herbarium of Naturalis Leiden (L). Figures 2, 3, 4, 5, 6, 7, 8 show the spread of the taxa in the Netherlands.

**Flow cytometric measurement of DNA 2C-value**

For the isolation of nuclei, a few cm of leaf or a single bulbil was chopped together with a piece of Agave americana ‘Aureomarginata’ or Agave attenuata L. as an internal standard (see below). The chopping was done with a new razor blade in a Petri dish in 0.25 ml nucleisolation buffer to which 0.25 mg RNase/ml was added (Zonneveld and van Iren 2001). After adding 1.75 ml propidium iodide solution (50 mg PI/l in isolation buffer) the suspension with nuclei was filtered through a 20 μm nylon filter. The fluorescence of the nuclei at 585 nm was measured half an hour and 1 h after addition of propidium iodide excitation, using a BD Accuri C6 flow cytometer 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 2C 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. Two different samples, with each at least 5,000 nuclei, were measured twice for each clone. Most histograms revealed a Coefficient of Variation of less than 5%. The standard deviation was calculated for the DNA content of each species, using all relevant measurements.

**Internal standard and absolute DNA content values**

When measuring nuclear DNA content by means of flow cytometry, it is necessary to chop tissue from the plant of interest together with an internal standard. This standard must be as close as possible to the plants of interest and not overlap with the ploidy area of interest. If they are too close together the peak values interfere with each other. Linearity is checked by comparing the different ploidies as found within leaves and roots of many plants. In this way, variation in signal intensities due to staining kinetics, to light absorption and quenching by sample components, as well as to instrument and other variables, is reduced to a minimum. Agave americana was chosen as internal standard for Gagea. For Gagea minima and G. villosa, with 2C-values that more or less coincided with Agave americana,
Agave attenuata was used. Agave is available year-round, does not mind several weeks without water and, being a large plant, a single specimen can serve a lifetime, thereby further reducing variation in readings. It also has a low background in propidium iodide measurements, and show a single G₀ peak, almost lacking G₂ arrest.

Fresh male human leucocytes [2C = 7.0 pg; 1 pg = 10⁻¹² g = 0.978 × 10⁹ base pairs (Doležel et al. 2003)] were chosen as primary standard (Tiersch et al. 1989). This yields 2C = 15.9 pg for nuclei of Agave americana L. and 8.0 pg for A. attenuata. Based on a published male human genome size of 6.294 × 10⁹ base pairs the nucleus was calculated as containing 6.436 pg (Doležel et al. 2003). However this is based on a human sequence where the size of the very large repeat sequences could not accurately be determined. So in the end the genome size could be closer to 7 pg than now envisioned.

Results

General
Morphologically the species of Gagea are rather difficult to differentiate. They are all small bulbous plants with grass like leaves and mostly yellow flowers. Moreover they are visible above the soil surface only about 2 months a year in early spring. The Dutch Gagea can be divided over four out of 12–14 different sections. G. lutea, G. × pomeranica and G. pratensis belong to section Gagea whereas G. minima, G. villosa and G. spathacea each belong to a separate section. Gageas have been measured from 186 localities in The Netherlands (Tables 1, 2) and they are compared with 41 accessions from Germany. They are shown to comprise six taxa with several inferred ploidies.

Gagea minima (L.) Ker Gawl.-section Minimae
Gagea minima is a small plant with 1 (or 2) narrow 2–3(5) mm wide leaves and 1–3 flowers per scape. G. minima with 2C = 14.9 pg from two localities, together with G. villosa, are the only two inferred diploid species found in The Netherlands.

Gagea villosa (M.Bieb.) Sweet-section Didymobolbos
Gagea villosa is a hairy, largely sterile plant with numerous bulbils. Fourteen accessions of G. villosa from the Dutch provinces of Gelderland, Overijssel and Zuid-Holland are inferred to be diploid with 16.9 pg. Six accessions of G. villosa are inferred to be tetraploid with on average 32.3 pg. This is based on the basic value of 7–8 pg as in the other species (except G. spathacea) and the published counts of 24 and 48 chromosomes (http://www.tropicos.org/gagea).

Fig. 1 Scans of petals of Gagea pratensis in the Netherlands. In the left column petals from a fresh inflorescence. On the left the inner petals (left the upper side and on the right the bottom side). On the right the outer petals (left the upper side and on the right the bottom side). In the right column petals from an inflorescence after bloom. On the left the inner petals (left the upper side and on the right the bottom side). On the right the outer petals (left the upper side and on the right the bottom side). The tetraploid gagea is collected in a park near the river Berkel in Almen. The pentaploid (a) gagea is collected in a churchyard in Wassenaar. (All gageas in the western part of the Netherlands are of this type). The pentaploid (b) is collected in a road verge near Fromberg. The pentaploid (c) is collected in a park in Zuutphen. (These large gageas resemble Gagea megapolitana). The hexaploid gagea is collected in a park near the river IJssel in Deventer.
| Species           | pg/2C | Average | stdev | Locality                  | coll. nr   | Locality (counties NL) |
|-------------------|-------|---------|-------|---------------------------|------------|------------------------|
| Gagea minima diploid |       |         |       |                           |            |                        |
| Gagea minima      | 14.8  | 14.9    | 0.6   | Leyduin, Bloemendaal      | BtL11-0092 | N Holland              |
| Gagea villosa     | 16.5  | 17.0    | 0.4   | Doddendaal                | BtL11-053  | Gelderland             |
| Gagea villosa     | 16.7  |         |       | Deventer                   | BtL11-085  | Overijssel             |
| Gagea villosa     | 16.7  |         |       | Zutphen, Hanzehof         | BtL11-003  | Gelderland             |
| Gagea villosa     | 16.8  |         |       | Elten                      | BtL11-001  | Germany                |
| Gagea villosa     | 16.8  |         |       | Eys                        | BtL11-002  | Limburg                |
| Gagea villosa     | 16.8  |         |       | Zevenaar                   | BtL11-084  | Gelderland             |
| Gagea villosa     | 16.9  |         |       | Zeist                      | BtL11-037  | Utrecht                |
| Gagea villosa     | 17.0  |         |       | Lent                       | BtL11-055  | Gelderland             |
| Gagea villosa     | 17.0  |         |       | Valkenburg                 | BtL11-077  | Gelderland             |
| Gagea villosa     | 17.2  |         |       | Zevenaar                   | BtL11-078  | Gelderland             |
| Gagea villosa     | 17.3  |         |       | Zalk                       | BtL11-017  | Gelderland             |
| Gagea villosa     | 17.4  |         |       | Mijnsherenland             | BtL14-002  | Gelderland             |
| Gagea villosa     | 17.3  |         |       | Spijk                      | BtL14-003  | Gelderland             |
| Gagea villosa     | 17.2  |         |       | Amerongen                  | BtL14-004  | Gelderland             |
| Gagea villosa tetraploid |     |         |       |                           |            |                        |
| Gagea villosa     | 31.5  | 32.3    | 0.7   | Heelsum                    | BtL11-052  | Gelderland             |
| Gagea pratensis   | 32.1  | 32.8    | 0.4   | Deventer                   | BtL11-085  | Gelderland             |
| Gagea pratensis   | 32.1  |         |       | Kootwijk                   | BtL11-073  | Gelderland             |
| Gagea pratensis   | 32.2  |         |       | Almen 2                    | BtL11-066  | Gelderland             |
| Gagea pratensis   | 32.2  |         |       | Legden                     | BtL13-028  | Gelderland             |
| Gagea pratensis   | 32.3  |         |       | Almen 1                    | BtL11-040  | Gelderland             |
| Gagea pratensis   | 32.3  |         |       | Rhienderen 1               | BtL11-067  | Gelderland             |
| Gagea pratensis   | 32.4  |         |       | Suderas, Wichmond          | BtL13-053  | Gelderland             |
| Gagea pratensis   | 32.4  |         |       | Bussloo, begraafplaats t.o Zutphenboer | BtL13-087 | Gelderland             |
| Gagea pratensis   | 32.5  |         |       | Babberich                  | BtL11-014  | Gelderland             |
| Gagea pratensis   | 32.6  |         |       | Voorst                     | BtL11-062  | Gelderland             |
| Gagea pratensis   | 32.7  |         |       | Gietelo                    | BtL13-002  | Gelderland             |
| Gagea pratensis   | 32.7  |         |       | Ravenswaarden              | BtL13-009  | Gelderland             |
| Gagea pratensis   | 32.7  |         |       | Keppel, klein              | BtL13-050  | Gelderland             |
| Gagea pratensis   | 32.8  |         |       | Almen                      | BtL11-012  | Gelderland             |
| Gagea pratensis   | 32.9  |         |       | Trent 2                    | Tre11-xx   | Germany                |
| Gagea pratensis   | 32.9  |         |       | Gintg                      | Gin11-xx   | Germany                |
| Gagea pratensis   | 33.0  |         |       | Meppen, stadswal           | BtL13-077  | Germany                |
| Gagea pratensis   | 33.0  |         |       | Subzow                     | Sub11-18   | Germany                |
| Gagea pratensis   | 33.0  |         |       | Elbe Jasebeck              | BtL13-043  | Germany                |
| Gagea pratensis   | 33.0  |         |       | Deventer, Brinckgrave      | BtL13-084  | Overijssel             |
| Gagea pratensis   | 33.1  |         |       | Almen 3                    | BtL11-074  | Gelderland             |
| Gagea pratensis   | 33.2  |         |       | Zirchow                    | Zir11-xx   | Germany                |
| Species                  | pg/2C | Average | stdev | Locality               | coll. nr   | Locality (counties NL) |
|--------------------------|-------|---------|-------|------------------------|------------|------------------------|
| Gagea pratensis          | 33.2  |         |       | Deventer, Brinkgrave   | BtL13-015  | Overijsel              |
| Gagea pratensis          | 33.3  |         |       | Beek                   | BtL11-045  | Gelderland             |
| Gagea pratensis          | 33.3  |         |       | Klarenbeek             | BtL11-061  | Gelderland             |
| Gagea pratensis          | 33.3  |         |       | Trent                  | Tre11-xx   | Germany                |
| Gagea pratensis          | 33.3  |         |       | Kampens dl             | BtL13-035  | Overijsel              |
| Gagea pratensis          | 33.4  |         |       | Gossel, oud kerkhof    | BtL13-083  | Gelderland             |
| Gagea pratensis          | 33.7  |         |       | Bingerden 1a           | BtL11-075  | Gelderland             |
| Gagea pratensis          | 33.7  |         |       | Gietelo                | BtL13-002  | Gelderland             |
| Gagea pratensis pentaploid |     | 37.9    | 39.9  | Cortenoever 1          | BtL11-087  | Gelderland             |
| Gagea pratensis          | 37.9  |         |       | Leyduin                | BtL11-041  | N. Holland             |
| Gagea pratensis          | 38.3  |         |       | Ressen                 | BtL11-056  | Gelderland             |
| Gagea pratensis          | 38.5  |         |       | Bingerden 2            | BtL11-094  | Gelderland             |
| Gagea pratensis          | 38.5  |         |       | Bemmel 1               | BtL11-033  | Gelderland             |
| Gagea pratensis          | 38.6  |         |       | Zwolle 2               | BtL11-015  | Overijsel              |
| Gagea pratensis          | 38.6  |         |       | Hattem, Heezenberg     | BtL13-089  | Gelderland             |
| Gagea pratensis          | 38.7  |         |       | Angerlo                | BtL11-027  | Gelderland             |
| Gagea pratensis          | 38.7  |         |       | Ravenswaarden          | BtL11-058  | Gelderland             |
| Gagea pratensis          | 38.7  |         |       | Den Haag, Westerdijnpark | BtL13-031 | Z. Holland             |
| Gagea pratensis          | 38.8  |         |       | Castricum, kerkhof     | BtL13-085  | N. Holland             |
| Gagea pratensis          | 38.9  |         |       | Didam                  | BtL13-048  | Gelderland             |
| Gagea pratensis          | 38.9  |         |       | Zwolle, Vecht          | BtL13-081  | Overijsel              |
| Gagea pratensis          | 39.0  |         |       | Loil                   | BtL11-024  | Gelderland             |
| Gagea pratensis          | 39.0  |         |       | Angerlo                | BtL11-026  | Gelderland             |
| Gagea pratensis          | 39.0  |         |       | Babberich 2            | BtL11-072  | Gelderland             |
| Gagea pratensis          | 39.0  |         |       | Den Haag, Marlot       | BtL13-032  | Z. Holland             |
| Gagea pratensis          | 39.0  |         |       | Lisse, Huis te Spekke | BtL13-024  | Z. Holland             |
| Gagea pratensis          | 39.1  |         |       | Haarlem 1a             | BtL11-039  | N. Holland             |
| Gagea pratensis          | 39.1  |         |       | Haarlem, Rhijnhof      | BtL13-042  | Z. Holland             |
| Gagea pratensis          | 39.1  |         |       | Keppel, groot          | BtL13-051  | Gelderland             |
| Gagea pratensis          | 39.2  |         |       | Bemmel                 | BtL11-032  | Gelderland             |
| Gagea pratensis          | 39.2  |         |       | Velp 2                 | BtL11-029  | Gelderland             |
| Gagea pratensis          | 39.2  |         |       | Bemmel 2               | BtL11-032  | Gelderland             |
| Gagea pratensis          | 39.2  |         |       | Noordwijk, Gooweg     | BZ13-023   | Z. Holland             |
| Gagea pratensis          | 39.2  |         |       | Haarlem, Schoetersingel | BtL13-036 | N. Holland             |
| Gagea pratensis          | 39.2  |         |       | Driehuis begraafplaats | BtL13-037  | N. Holland             |
| Gagea pratensis          | 39.2  |         |       | Huize Baak             | BtL13-052  | Gelderland             |
| Gagea pratensis          | 39.2  |         |       | Deventer, Drouweerkolk | BtL13-013  | Overijsel              |
| Gagea pratensis          | 39.2  |         |       | Hettemerwaard          | BtL13-093  | Gelderland             |
| Gagea pratensis          | 39.2  |         |       | Velzen, Beeckenstein   | BtL13-101  | N. Holland             |
| Gagea pratensis          | 39.3  |         |       | Huissen, pastorie      | BtL13-040  | Gelderland             |
| Gagea pratensis          | 39.3  |         |       | Voorhout               | BtL13-022  | Z. Holland             |
| Gagea pratensis          | 39.3  |         |       | Eldrik                 | BtL13-049  | Gelderland             |
| Gagea pratensis          | 39.3  |         |       | Kampen, stadswal       | BtL13-033  | Overijsel              |
| Gagea pratensis          | 39.3  |         |       | Mehr                   | BtL13-027  | Germany                |
| Gagea pratensis          | 39.4  |         |       | Bingerden 1b           | BtL11-076  | Gelderland             |
| Gagea pratensis          | 39.4  |         |       | Kampen 1a              | BtL11-019  | Overijsel              |
| Gagea pratensis          | 39.4  |         |       | Brummen 1              | BtL11-068  | Gelderland             |
| Gagea pratensis          | 39.4  |         |       | Warmond                | BtL13-021  | Z. Holland             |
| Species          | pg/2C | Average | stdev | Locality                           | coll. nr   | Locality (counties NL) |
|------------------|-------|---------|-------|------------------------------------|------------|------------------------|
| Gagea pratensis  | 39.5  | Fromberg|       | BtL11-093                          | Limburg    |
| Gagea pratensis  | 39.5  | Oud-Zevenaar |   | BtL11-023                          | Gelderland |
| Gagea pratensis  | 39.5  | Elten   |       | Elt11-xx                           | Germany    |
| Gagea pratensis  | 39.5  | Drempt  |       | BtL11-042                          | Gelderland |
| Gagea pratensis  | 39.5  | Zutphen, Hanzehof |   | BtL13-001                          | Gelderland |
| Gagea pratensis  | 39.5  | Zutphen, kanaal 16 jan 2013 | | BtL13-003                          | Gelderland |
| Gagea pratensis  | 39.5  | Epse    |       | BtL13-005                          | Gelderland |
| Gagea pratensis  | 39.5  | Wassenaar |     | BtL13-006                          | Z. Holland |
| Gagea pratensis  | 39.5  | Sassenheim |   | BtL13-010                          | Z. Holland |
| Gagea pratensis  | 39.5  | Zutphen, De Hoven | | BtL13-011                          | Gelderland |
| Gagea pratensis  | 39.5  | Oegstgeest | | BtL13-012                          | Z. Holland |
| Gagea pratensis  | 39.5  | Ummuren berm | | BtL13-038                          | N. Holland |
| Gagea pratensis  | 39.5  | Velzen, Kanaalweg | | BtL13-086                          | N. Holland |
| Gagea pratensis  | 39.5  | Achthoven |       | BtL14-005                          | Gelderland |
| Gagea pratensis  | 39.6  | Haarlem  |       | BtLs.n.                            | N. Holland |
| Gagea pratensis  | 39.6  | Hoog-Keppel |   | BtL11-043                          | Gelderland |
| Gagea pratensis  | 39.6  | Zwolle, Zandhove | | BtL13-082                          | Overijssel |
| Gagea pratensis  | 39.6  | Velzen, kerkhof | | BtL13-100                          | N. Holland |
| Gagea pratensis  | 39.6  | sdl Zutphen, Hanzehof | | BtL14-08                           | Gelderland |
| Gagea pratensis  | 39.6  | sdl Zutphen, Hanzehof | | BtL14-008                          | Gelderland |
| Gagea pratensis  | 39.7  | Zutphen  |       | BtL11-022                          | Gelderland |
| Gagea pratensis  | 39.7  | Cortenoever 2 | | BtL11-020                          | Gelderland |
| Gagea pratensis  | 39.8  | Beverwijk, Scheybeek | | BtL13-039                          | N. Holland |
| Gagea pratensis  | 39.8  | Zwolle, Engelse werk | | BtL13-078                          | Overijssel |
| Gagea pratensis  | 39.9  | Hummelo  |       | BtL11-044                          | Gelderland |
| Gagea pratensis  | 39.9  | Leiden, Rhynhof | | BZ12-01                            | Z. Holland |
| Gagea pratensis  | 39.9  | Den Haag 2 |   | BtL11-070                          | Z. Holland |
| Gagea pratensis  | 40.0  | sdl Zutphen, Hanzehof | | BtL14-008                          | Gelderland |
| Gagea pratensis  | 40.0  | sdl Zutphen, Hanzehof | | BtL14-008                          | Gelderland |
| Gagea pratensis  | 40.1  | Rhienderen 2 | | BtL11-067                          | Gelderland |
| Gagea pratensis  | 40.1  | Zutphen, Hanzehof | | BtLs.n.                            | Gelderland |
| Gagea pratensis  | 40.2  | Cortenoever 3 | | BtL11-021                          | Gelderland |
| Gagea pratensis  | 40.2  | Weurt    |       | BtL11-054                          | Gelderland |
| Gagea pratensis  | 40.2  | Heelsum, kerk | | BtL14-011                          | Gelderland |
| Gagea pratensis  | 40.3  | Brummen 2 |   | BtL11-071                          | Gelderland |
| Gagea pratensis  | 40.3  | sdl Zutphen, zwembad | | BtL14-009                          | Gelderland |
| Gagea pratensis  | 40.4  | Doesburg |       | BtL11-028                          | Gelderland |
| Gagea pratensis  | 40.4  | Middachten | | BtL11-046                          | Gelderland |
| Gagea pratensis  | 40.4  | parent Zutphen, zwembad | | BtL14-010                          | Gelderland |
| Gagea pratensis  | 40.5  | Spankeren |       | BtL11-060                          | Gelderland |
| Gagea pratensis  | 40.6  | Olburgen |       | BtL11-047                          | Gelderland |
| Gagea pratensis  | 40.6  | Steenderen | | BtL11-048                          | Gelderland |
| Gagea pratensis  | 40.7  | Zutphen, begraf plaats | | BtL13-054                          | Gelderland |
| Gagea pratensis  | 40.9  | Groessen |       | BtL11-035                          | Gelderland |
| Gagea pratensis  | 40.9  | Oud-Zevenaar | | BtL11-057                          | Gelderland |
| Gagea pratensis hexaploid | 43.8 | 45.6 | 1.1 | Zirchow/U 2 | BtL11-xx | Germany |
| Gagea pratensis  | 44.0  | Trent 2  |       | Tre11-xx                           | Germany    |
| Gagea pratensis  | 44.3  | Vaassen, Canneburg 194/478 | | BtL11-095                          | Limburg    |
Table 1 continued

| Species                  | pg/2C | Average | stdev | Locality                                | coll. nr  | Locality (counties NL) |
|--------------------------|-------|---------|-------|-----------------------------------------|-----------|------------------------|
| Gagea pratensis          | 45.0  |         |       | Bronkhorst, slotheuvel                  | BtL11‑049 | Gelderland              |
| Gagea pratensis          | 45.1  |         |       | Deventer, De Worp                       | BtL13‑004 | Overijsel               |
| Gagea pratensis          | 45.1  |         |       | Culemborg, Stroomrug                    | BtL13‑008 | Gelderland              |
| Gagea pratensis          | 45.2  |         |       | Brummen, Engelenburg                    | BtL13‑017 | Gelderland              |
| Gagea pratensis          | 45.3  |         |       | Oosterbeek, gazon                       | BtL11‑051 | Gelderland              |
| Gagea pratensis          | 45.4  |         |       | Meppen begraafplaats                    | BtL13‑072 | Germany                |
| Gagea pratensis          | 45.4  |         |       | Deventer, Blauwijk                      | BtL13‑014 | Overijsel               |
| Gagea pratensis          | 45.4  |         |       | Marle, uiterwaardgrasland               | BtL13‑090 | Overijsel               |
| Gagea pratensis          | 45.7  |         |       | Brummen, Ganzenei                       | BtL13‑091 | Gelderland              |
| Gagea pratensis          | 45.8  |         |       | Meppen stadswal                         | BtL13‑071 | Germany                |
| Gagea pratensis          | 46.0  |         |       | Elst, Johan de Wittstraat               | BtL11‑030 | Gelderland              |
| Gagea pratensis          | 46.0  |         |       | Altenkirchen                            | Alt11‑19  | Germany                |
| Gagea pratensis          | 46.1  |         |       | Olst, gemeentehuis                      | BtL13‑016 | Overijsel               |
| Gagea pratensis          | 46.6  |         |       | Ravenswaarden                           | BtL13‑099 | Gelderland              |
| Gagea pratensis          | 47.2  |         |       | Empe                                    | BtL11‑063 | Gelderland              |
| Gagea pratensis          | 47.4  |         |       | Brummen 3                               | BtL11‑064 | Gelderland              |
| Gagea pratensis          | 47.9  |         |       | Brummen 4                               | BtL11‑069 | Gelderland              |
| Gagea pratensis          |       |         |       |                                         |           |                        |
| G. pratensis decaploid   |       | 75.0    | 75.8  | 1.1                                     |           |                        |
| G. pratensis             |       | 76.6    |       |                                         |           |                        |
| Gagea × megapolitana     | 46.7  | 46.8    | 0.1   | Gingst 2                                | Gin11‑xx  | Germany                |
| Gagea × megapolitana     | 46.8  |         |       | Meppen                                  | BtL13‑071 | Germany                |
| Gagea lutea hexaploid    | 41.7  | 42.7    | 0.6   | Trent 1                                 | DEs.n.    | Germany                |
| Gagea lutea              | 41.9  |         |       | Haarlem, Spaem en Hout                  | BtL11‑039 | N. Holland              |
| Gagea lutea              | 42.1  |         |       | Epe, Dinkel                             | BtL13‑030 | Germany                |
| Gagea lutea              | 42.1  |         |       | Vreden, Berkel                          | BtL13‑029 | Germany                |
| Gagea lutea              | 42.1  |         |       | Miste                                   | BtL13‑103 | Gelderland              |
| Gagea lutea              | 42.3  |         |       | Roden                                   | BtL11‑081 | Drenthe                |
| Gagea lutea              | 42.3  |         |       | Kelmis, Hohntal                         | BtL11‑079 | Belgie                 |
| Gagea lutea              | 42.4  |         |       | Zuidlaren                               | BtL11‑089 | Drenthe                |
| Gagea lutea              | 42.4  |         |       | Griebenow                               | DE‑s.n.   | Germany                |
| Gagea lutea              | 42.6  |         |       | Meppen, stadswal                        | BtL13‑070 | Germany                |
| Gagea lutea              | 42.6  |         |       | Bron Berkle                             | Bbe11‑xx  | Germany                |
| Gagea lutea              | 42.6  |         |       | Bredevoort                              | BtL11‑011 | Gelderland              |
| Gagea lutea              | 42.8  |         |       | Haarlem 1c                              | BtL11‑038 | N. Holland              |
| Gagea lutea              | 42.8  |         |       | D Meppen                                | BtL13‑026 | Germany                |
| Gagea lutea              | 42.8  |         |       | Millingerwaard                          | BtL14‑001 | Z. Holland              |
| Gagea lutea              | 42.9  |         |       | Gesher                                  | Ges11‑xx  | Germany                |
| Gagea lutea              | 42.9  |         |       | Haarlem, Spaem en Hout                  | BtL11‑38  | N. Holland              |
| Gagea lutea              | 42.9  |         |       | Elbe, Jasebeck                          | BtL13‑044 | Germany                |
| Gagea lutea              | 43.2  |         |       | Aerdenhout                              | BtL13‑066 | N. Holland              |
| Gagea lutea              | 43.4  |         |       | Winterswijk, Vreehorst weg              | BtL13‑055 | Gelderland              |
| Gagea lutea              | 43.8  |         |       | Bronkhorst                              | BtL11‑005 | Gelderland              |
| Gagea lutea              | 43.9  |         |       | Schoorl                                 | BtL13‑094 | N. Holland              |
| Gagea lutea var. glauca  | 41.9  | 42.3    | 0.3   | Anloo, grasland                         | BtL13‑064 | Drenthe                |
| Gagea lutea var. glauca  | 41.9  |         |       | Veenhof, Berrm                          | BtL13‑057 | Drenthe                |
| Gagea lutea var. glauca  | 42.0  |         |       | Groningen, Noorderplantsoen             | BtL13‑045 | Groningen               |
| Species                  | pg/2C | Average | stdev | Locality                                      | coll. nr      | Locality (counties NL) |
|--------------------------|-------|---------|-------|-----------------------------------------------|---------------|------------------------|
| *Gagea lutea var. glauca* | 42.1  | Zeegse  |       | BtL11-090                                     | Drenthe       |                        |
| *Gagea lutea var. glauca* | 42.1  | Bellingwolde, berm | | BtL13-067                                     | Groningen     |                        |
| *Gagea lutea var. glauca* | 42.2  | Midwolda, Ernemaborg | | BtL13-047                                     | Groningen     |                        |
| *Gagea lutea var. glauca* | 42.2  | Eext Berrm | | BtL13-058                                     | Drenthe       |                        |
| *Gagea lutea var. glauca* | 42.2  | Westeresch | | BtL11-080                                     | Drenthe       |                        |
| *Gagea lutea var. glauca* | 42.3  | Sterrenbospark | | BtL11-088                                     | Groningen     |                        |
| *Gagea lutea var. glauca* | 42.3  | Gieten   |       | BtL11-009                                     | Drenthe       |                        |
| *Gagea lutea var. glauca* | 42.3  | Doetinchem, Zunpe 16 jan 2013 | | BtL13-007                                     | Gelderland    |                        |
| *Gagea lutea var. glauca* | 42.4  | Midlaren |       | BtL13-059                                     | Drenthe       |                        |
| *Gagea lutea var. glauca* | 42.8  | Wedde, Huis Te Wedde, onder linde | | BtL13-069                                     | Groningen     |                        |
| *Gagea lutea var. glauca* | 42.8  | Appingedam, Ekenstein | | BtL13-046                                     | Groningen     |                        |
| *Gagea lutea var. glauca* | 43.0  | Eext, Brink | | BtL13-056                                     | Drenthe       |                        |
| *Gagea lutea var. glauca* | 42.9  | Naumburg |       | BtL14-012                                     | Germany       |                        |
| *Gagea × pomeranica pentaploid* |       |         |       |                                               |               |                        |
| *Gagea × pomeranica*      | 34.2  | Vitsense | 0.6   | Vt11-11                                       | Germany       |                        |
| *Gagea × pomeranica*      | 34.3  | Zirchow/U |      | ZIU11-xx                                      | Germany       |                        |
| *Gagea × pomeranica*      | 34.3  | Zirchow/U 2 |     | ZIU11-xx                                      | Germany       |                        |
| *Gagea × pomeranica*      | 34.4  | W. Baggendorf |   | WBa11-15                                      | Germany       |                        |
| *Gagea × pomeranica*      | 34.8  | Zirchow/U 2 |     | ZIU11-xx                                      | Germany       |                        |
| *Gagea × pomeranica*      | 35.1  | Semlow 2 |       | Sem11-xx                                      | Germany       |                        |
| *Gagea × pomeranica*      | 35.2  | Semlow   |       | Sem11-xx                                      | Germany       |                        |
| *Gagea × pomeranica*      | 35.2  | Poseritz |       | Pos11-12                                      | Germany       |                        |
| *Gagea × pomeranica*      | 35.3  | Semlow 2 |       | Sem11-xx                                      | Germany       |                        |
| *Gagea × pomeranica*      | 35.5  | Zirkow/R |       | ZIR11-12                                      | Germany       |                        |
| *Gagea × pomeranica*      | 36.0  | Semlow   |       | Sem11-xx                                      | Germany       |                        |
| *Gagea spathacea nonaploid* |     |         |       |                                               |               |                        |
| *Gagea spathacea*         | 45.4  | Ootmarsum, de Voort | 0.8  | BtL13-063                                     | Z. Holland     |                        |
| *Gagea spathacea*         | 45.4  | Zeegse   |       | BtL11-091                                     | Drenthe       |                        |
| *Gagea spathacea*         | 45.5  | Losser   |       | BtL11-082                                     | Overijsel     |                        |
| *Gagea spathacea*         | 45.9  | Samerot, eiken-haagbeukenbos | | BtL13-074                                     | Germany       |                        |
| *Gagea spathacea*         | 46.1  | Vasse, beekoever | | BtL13-076                                     | Overijsel     |                        |
| *Gagea spathacea*         | 46.1  | Brummen, Ganzenei, stroomrug | | BtL13-092                                     | Gelderland    |                        |
| *Gagea spathacea*         | 46.3  | Amen     |       | BtL14-006                                     | Drenthe       |                        |
| *Gagea spathacea*         | 46.4  | Bentheim, langs pad | | BtL13-073                                     | Germany       |                        |
| *Gagea spathacea*         | 47.0  | Roden, Havezate | | BtL11-081                                     | Drenthe       |                        |
| *Gagea spathacea*         | 47.0  | Peizermade, bosrand | | BtL13-096                                     | Drenthe       |                        |
| *Gagea spathacea*         | 47.2  | Roden, Havezate | | BtL13-097                                     | Drenthe       |                        |
| *Gagea spathacea*         | 47.3  | Wüllen, eiken-haagbeukenbos | | BtL13-068                                     | Germany       |                        |
| *Gagea spathacea*         | 47.5  | Nietap   |       | BtL13-098                                     | Drenthe       |                        |
| *Gagea spathacea*         | 47.6  | Bentheim, gazon | | BtL13-041                                     | Germany       |                        |
| *Gagea spathacea*         | 47.7  | Ootmarsum, weilandrand | | BtL13-075                                     | Overijsel     |                        |
| *Gagea spathacea*         | 47.9  | Varik, eikenbos | | BtL13-065                                     | Drenthe       |                        |
| *Gagea spathacea*         | 48.1  | Peize    |       | BtL13-095                                     | Drenthe       |                        |

All were measured against *Agave americana*, but for *G. minima* and *G. villosa* *A. attenuta* was used.

BtL B. te Linde, stdev standard deviation, coll.nr collection number.
**Gagea pratensis** (Pers.) Dumort.-section Gagea

*Gagea pratensis* is a glabrous plant with up to four flowers per scape. Characteristic are the two nude egg-like, horizontal bulbils. *Gagea pratensis* can be found in The Netherlands with four different inferred ploidies. They can be recognized in that the tetraploid has the leaf sheath circling the stem halfway, the pentaploid three-quarter and the hexaploid and the decaploid completely. They are shown in Figs. 9, 10, 11, 12 and 13. The tetraploids (30 accessions) have a DNA 2C-value (nuclear DNA content) of on average 32.8 pg, the pentaploids (85 accessions) have on average 39.9 pg and the hexaploids (20 accessions) have on average 45.6 pg. The pentaploids could be hybrids between the tetraploid and the hexaploid cytotypes. Even a decaploid with 75.8 pg has been found. The pentaploid form of *G. pratensis* is by far the most common *Gagea* in The Netherlands with 39.5 pg from 85 out of 186 localities. The same ploidy is counted in all 7 populations of *G. pratensis* from Mecklenburg (Germany) (Henker 2005). Therefore it seems most likely that the decaploid plant is derived from the frequently found pentaploid *G. pratensis* that has in this case doubled its genome. As often in polyploids, DNA might have been lost and a similar loss is found in the hexaploid *G. pratensis* but not in the lower ploidies. The inferred decaploid plants have not been reported before for *G. pratensis*. Being pentaploid in most cases, it comes as no surprise that *G. pratensis* is considered to be sterile (van der Meijden 2005). Taxa with anorthoploid chromosome sets

![Graph](image-url)
often show a highly irregular meiosis. An exception are large plants from Zutphen, NL that are fully fertile and differ morphologically with a large basal leaf and 4–8 flowers to a stem. They have a genome size similar to pentaploid *G. pratensis*, but look more like *G. × megapolitana* Henker (Henker 2005). Out of 50 germinated seeds, five seedlings measured from the Zutphen locality had the same genome size as their parents. This is peculiar for a pentaploid. Earlier analysis of seedlings of the triploid Hosta ‘Sum and Substance’ show different, but lower genome sizes in the seedlings (Zonneveld and Pollock 2012). Pfeiffer et al. (2013) report also that some pentaploid populations of *G. pratensis* are partially fertile. None of the calculated genome sizes of the possible hybrids between *G. lutea* and *G. pratensis* would fit the plants from Zutphen. Hence more research is required to explain these results.

*Gagea lutea* (L.) Ker Gawl.-section Gagea

*Gagea lutea* is a glabrous plant with leaves of more than 1 cm wide and up to seven flowers per stem. The bulbs have a diameter of 0.75–1.5 cm and form numerous bulbils.

Apart from hexaploid *G. pratensis* also *G. lutea* is inferred to be hexaploid with $2C = 42.7$ pg, collected in 22 localities. *Gagea lutea* var. *glauca* (a synonym of *G. 
**Gagea lutea** (L.) Ker-Gawler
**Gagea lutea** var. **Glauc a** L. Klein

"Gagea lutea" (L.) Ker-Gawler differs in its glaucous leaves, slightly larger petals, lower fertility and the anthropogenic habitats it grows in. The flowering time of the glaucous forms starts about 2 weeks later when transplanted in the garden. "Gagea var. Glauc a" is restricted to the northern part of the Netherlands and is found in localities separate from the green-leaved form. However, with 42.3 pg for 16 different accessions there is no significant difference in genome size.

**Gagea spathacea** (Hayne) Salisb.-section Spathaceae

"Gagea spathacea" is a glabrous plant with 1–3 flowers per stem and is usually found in fairly moist places. "Gagea spathacea" is only present as a nonaploid plant across (Eastern) Europe (Westergaard 1936; Henker 2005; Pfeiffer et al. 2012). It is observed in about 70 localities in The Netherlands, of which material was collected at 12 localities with an average of 2C = 46.5 pg. This implies a low basic (Cx) value of 5.2, instead of 7.4–8.4 for the other three species. The virtually sterile "G. spathacea" (Pfeiffer et al. 2012) seems to be a nearly monoclonal plant able to occupy a significant range by dispersal of bulbils (Pfeiffer et al. 2011; 2013).

**Hybrid species**

Hybridization and polyploidy are amongst the most important evolutionary mechanism in plants. The parents can be deduced by comparing the genome size of possible parents and their offspring. If parents have say 20 and 30 pg then their offspring will mostly have 25 pg. In more complicated allopolyploids the contribution of
each parent can often be calculated. In *Gagea* inferred polyploids run from triploid to decaploid (Peruzzi 2003) whereby several species show different ploidies (Henker 2005). Three different hybrids have been described that have the same parents, *G. lutea* and *G. pratensis* but combining different ploidies. These three hybrids are here discussed under the names as found in the literature. They are not found in The Netherlands, but two of the hybrids were obtained from Germany (Table 1). The hybrids mostly occur in anthropogenically disturbed sites like churchyards, parks and marginally used meadows. Their parent species are found in forests (*G. lutea*) and forest edges (*G. pratensis*). Based on maternal inheritance of the plastids *G. pratensis* provide the female gametes for *G. × pomeranica* and *G. megapolitana* (Peterson et al. 2009).

**Gagea × pomeranica R.Ruthe**

The pentaploid *G. × pomeranica* (R.Ruthe) Henker with two genomes of the tetraploid *G. pratensis* and three genomes of the hexaploid *G. lutea* (Peterson et al. 2009). However, in the case of *G. × pomeranica*, 11 accessions were obtained from Germany that had on average a nuclear DNA content of 34.9 pg. This differs considerably (2.2 pg) from the calculated genome size of 37.1 pg, based on the basic values for *G. lutea* and *G. pratensis*. One explanation could be that this hybrid is an old one and has lost DNA. Another possibility is that other species are involved. Pfeiffer et al. (2013) have shown that backcrosses of the hybrid, mostly with the fully fertile hexaploid *G. lutea* as pollen parent are possible. However, backcrosses of *G. × pomeranica* (34.9 pg) with *G.
lutea (42.6 pg) might give higher 2C-values not lower, but these were not observed.

**Gagea × marchica** Henker, Kiesew., U.Raabe, Rätzel

Recently another sterile pentaploid hybrid was described as *G. marchica* Henker et al. (2012). It is described as falling morphologically between the pentaploid *G. (×) pomeranica* and the hexaploid *G. (×) megapolitana* with 57, 59 but probably 60 chromosomes. If it is supposed to be the reversed hybrid (compared to the parents of *G × pomeranica*) between hexaploid *G. pratensis* and tetraploid *G. lutea* the problem arises that a tetraploid *G. lutea* has not been reported so far (Pfeiffer et al. 2013).

**Gagea × megapolitana** Henker

A third hybrid with the same parents is the hexaploid *G. × megapolitana* Henker with three genomes of the hexaploid *G. pratensis* and three genomes of the hexaploid *G. lutea* (Peterson et al. 2009). It was obtained from two localities in Germany with on average 2C = 46.8 pg. In the world checklist for monocots (Govaerts 2006) *G. megapolitana* is accepted as a species. However Peterson et al. (2009) have clearly shown that it is a hybrid between the hexaploids *G. pratensis* and *G. lutea*. The genome size provides a firm argument for this hybridity and confirm the suggestion of Peterson et al. (2009) for the parents and the ploidy of *G. × megapolitana*. 

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![Distribution map of Gagea pratensis in the Netherlands.](image)

*Fig. 6* The distribution of pentaploid *Gagea pratensis* in the Netherlands.
Conclusions

Five species and different inferred ploidies are recorded for The Netherlands, as summarized in Table 2, some of the latter for the first time. Inferred decaploidy in *G. pratensis* was not demonstrated earlier. *G. minima* has an inferred diploid size. *G. minima* was only recently (1994) recognized as a new species for the Netherlands (Diemeer 2005). It is not clear whether it reached Haarlem by itself or was imported with lime trees from abroad. After all, Linnaeus lived there for 3 years only a kilometer away. The nearest known locality is 300 km away in Germany. *G. pratensis* is inferred to have four cytotypes: tetraploid, pentaploid, hexaploid and deca-ploid. Remarkable is the high number, 85 out of 186 accessions, of the pentaploid cytotype. Although it is largely sterile, bulbs seem to be a very effective way for vegetative multiplication, just as found for *G. spathacea* (Pfeiffer et al. 2012). *Gagea lutea* is only found in an inferred hexaploid form. The nonaploidy reported for *G. spathacea* would suggest a low basic genome size. This is corroborated by the fact that *G. spathacea* belongs to a section different from the others. Flow cytometry could provide the correct identification in most cases. It is a taxonomic and diagnostic tool that is applicable even in the case of dormant bulbs or sterile plants, and therefore has applications for conservation monitoring. Future research of the Dutch gageas could focus on combining chromosome counts and flow cytometry of the same samples, especially in the case of *G. villosa*. The fertility of the pentaploid *G. pratensis*
The term ‘inferred ploidy’ indicates that the ploidy is derived from the genome size and not based on chromosome counts. It is preferred to the proposed term ‘DNA ploidy’ (Suda et al. 2006) as this seems more ambiguous. Inferred decaploidy is found for the first time in G. pratensis. The hybrid G. × megapolitana, is only collected in Germany so far. The largest genome contains roughly $60 \times 10^9$ more base pairs than the smallest. A difference of 1 pg amounts to a difference of nearly $1 \times 10^9$ base pairs, so far exceeds a single taxonomic character.

Table 2 Summary of genome sizes in pg (2C), number of accessions and inferred chromosome numbers of species of Dutch and German gageas

| Species                  | Average pg/2C | Inferred ploidy | Chromosome number | Number of accessions |
|--------------------------|---------------|------------------|-------------------|----------------------|
| Gagea minima (L) Ker Gawl. | 14.9          | Diploid          | $2x = 24$         | 2                    |
| Gagea villosa (M.Bieb.) Sweet | 17.0          | Diploid          | $2x = 24$         | 14                   |
| Gagea villosa (M.Bieb.) Sweet | 32.3          | Tetraploid       | $4x = 48$         | 6                    |
| Gagea lutea (L) Ker Gawl. | 42.7          | Hexaploid        | $6x = 72$         | 22                   |
| Gagea lutea var. glauca L.Klein | 42.3          | Hexaploid        | $6x = 72$         | 16                   |
| Gagea spathacea (Hayne) Salisb. | 46.7          | Nonaploid        | $9x = 108$        | 17                   |
| Gagea pratensis (Pers.) Dumet. | 32.8          | Tetraploid       | $4x = 48$         | 30                   |
| Gagea pratensis (Pers.) Dumet. | 39.9          | Hexaploid        | $6x = 72$         | 85                   |
| Gagea pratensis (Pers.) Dumet. | 45.6          | Decaploid        | $10x = 120$       | 2                    |
| G. × pomeranica R.Ruthe | 34.9          | Hexaploid        | $5x = 60$         | 11                   |
| G. × megapolitana Henker | 46.8          | Hexaploid        | $6x = 72$         | 2                    |
needs further investigation. Sequencing of the forma *glauca* of *G. lutea* could reveal if it is a separate species or not.

**Authors' contributions**

BtL and LJB collected the plants, provided morphological and biogeographical data and corrected the manuscript. BZ did the flow cytometry and drafted the manuscript. BtL provided and made all figures. All authors read and approved the final manuscript.

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**Compliance with ethical guidelines**

**Competing interests**

The authors declare that they have no competing interests.
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