Karyotype Variation of
*Honckenya peploides* on the Island of Surtsey

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2016
Karyotype Variation of *Honckenya peploides* on the Island of Surtsey

by Audrey Pace

30 ECTS report
LÍF 039L Research project in Biology for foreign students

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June 2016
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Bibliographic information:
Audrey Pace, 2016, Karyotype Variation of *Honckenya peploides* on the Island of Surtsey, Faculty of Life and Environmental Sciences, University of Iceland.

Printing: Háskólaprent ehf., Fálkagata 2, 107 Reykjavík
Reykjavík, Iceland, June 2016
Abstract

In this study chromosome numbers of several individuals of *Honckenya peploides* from Surtsey and other locations across the northern hemisphere were obtained. Metaphase chromosomes were extracted from meristem tissue in root tips, stained with a fluorochrome and counted from photos that were taken at 1000x magnification. Some plants from this study displayed the formerly recorded somatic chromosome number of $2n = 68$, while many specimens appeared to have lower chromosome numbers. This would indicate the presence of lower ploidys within the species, which seems possible and likely for a pioneer plant, with great dispersal abilities and genetic variety, such as *H. peploides*.

Yfirlit

Í þessari rannsókn var samansafnað fjölmörgum eintökum af tegundinni *Honckenya peploides* frá Surtsey og hlutum norðurheimskautsins og litningar þeirra taldir. Metafasa litningar voru teknir úr meristem vef úr rótarendum, litaðir í flúorkrómi og myndir teknar í 1000x stækkun. Sumar plöntur voru með litningatöluna $2n=68$, en margar plöntur voru med færri litninga. Þetta myndi benda til lágri ploidya í tegundinni, og þetta er líklegt fyrir frumkvöðlaplöntu eins og H. peploites vegna þess hve auðveldlega plantan dreifist og hefur einnig auðugt genamengi.
Acknowledgements

I sincerely thank Prof. Kesara Anamthawat-Jónsson, who has become a dear friend to me, for being an exemplary and patient mentor throughout my venture in Iceland.
I furthermore thank Sigurður H. Árnason for initiating this study with his former work and for establishing the collaboration, that allowed this wide comparison of *Honckenya peploides* from across the northern hemisphere.
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1 Introduction

1.1 Genetic Diversity on Oceanic Islands

In recent years some crucial paradigms in plant genetics and population diversity, especially in populations inhabiting oceanic islands, have been undergoing re-evaluation. The Theory of Biogeography (MacArthur & Wilson 1967) implies that the species number on an island decreases with decreasing size of island habitats and with increasing distance to larger landmasses, due to higher extinction rates and larger migration barriers. Furthermore, founder effects, such as bottlenecks or genetic drift, were assumed to generally cause a loss of genetic variation due to reduction in population size (Frankham 1997).

However, whilst regarding populations of the sea sandwort Honckenya peploides around Iceland, Árnason et al. (2014) observed that genetic diversity of the plant was higher in populations from Surtsey and Iceland, compared to Denmark. This was contrary to initial expectations as the mainland populations are greater in size and age and are spread out in a larger geographic range (Nei et al. 1975, Árnason et al. 2014).

As H. peploides is not the only angiosperm plant species that has shown higher genetic diversity in island populations compared to the ones on the continent (Fernández-Mazuecos et al. 2011), it seems as if the genetic composition of populations on oceanic islands are more complex than previously assumed. Island size and isolation may negatively affect genetic diversity, but the interplay of numerous life history traits, such as breeding systems and seed dispersal, spatio-temporal factors and polyploidy have shown to maintain heterozygosity in colonising arctic plant populations (Árnason et al. 2014). A higher level of fixed heterozygosity is believed to enable polyploids to buffer against inbreeding and genetic drift during severe climatic changes as allelic diversity is maintained, which is a great adaptive advantage especially in the Arctic (Soltis & Soltis 2000, Otto & Whitton 2000, Brochmann et al. 2004).
1.2 Karyotype Variation

Polyploidy is a frequent and highly important mechanism in the evolution, diversification and speciation of angiosperms (Otto & Whitton 2000, Soltis et al. 2007, Wood et al. 2009, Fehlberg & Ferguson 2012). In former times, each species was thought to merely possess one somatic chromosome number, ploidy level and karyotype structure, as variable chromosome numbers often yielded mitotic or meiotic irregularities, often leading to infertility in the offspring (Levin 2002). With improving plant cytological methods (Anamthawat-Jónsson 2004, Suda et al. 2007) the assessment of ploidy level patterns is increasing, revealing that cytotypic variations among recognised plant species are more common than previously assumed (Frello & Heslop-Harrison 2000, Stuessy et al. 2004, Suda et al. 2007) and have been greatly underestimated (Soltis et al. 2007). In some cases, karyotypic variations result in no apparent phenotypic difference between cytotypes, as significant gene flow is maintained (Ramsey et al. 2008), while polyploidy helps to buffer against environmental stress (Leitch & Leitch 2008). In other cases, modest karyotypic variations have yielded morphological distinctions (Suda et al. 2004, Cires et al. 2009). It has been shown that these can affect flower sex (Suda et al. 2004), pollination style and pollinator species (Thompson & Merz 2008) as well as use of ecological niches (Johnson et al. 2003), which may constrain gene flow in time. This can cause disappearance of one or more cytotypes, or initiate further genetic differentiation among them (Garcia et al. 2008), often leading to reproductive isolation and the formation of cryptic species (Soltis et al. 2007).

1.3 H. peploides on Surtsey

Surtsey is a small volcanic island, located in the Vestmannaeyjar archipelago off the south coast of Iceland. The island arose during an eruption that lasted for 3.5 years (1963-1967) and is one of the youngest islands of its kind (Magnússon et al. 2014, Romagnoli & Jakobsson 2015). Highly restricted
access has kept anthropological disturbances at an absolute minimum, while colonisation and accumulation of flora and fauna of the entire island have been recorded continuously since its emergence (Svavarsson & Walker 2009, Magnússon et al. 2014). The island has undergone major morphological changes, as destructive forces have been eroding the surfaces since they were formed, greatly minimising the island’s size (Magnússon et al. 2014). For these reasons, Surtsey can be seen as a rare and well-protected natural laboratory, that bears great value for succession studies, which can improve our understanding of species composition and development on oceanic islands (Svavarsson & Walker 2009).

First pioneers to the island were shore plants, including H. peploides, that were dispersed by the sea and washed up on the shores of Surtsey (Magnússon et al. 2014). As surrounding landmasses are not too distant, the chances of large, nutrient-rich seeds arriving and establishing on the island were high (Magnússon et al. 2014). After 1977 no new plant species arrived on the island by sea and distribution by birds became the major mode of dispersal (Magnússon et al. 2014). Excreting marine nutrients, sea birds yielded the necessary soil amelioration for further species to propagate, which resulted in the introduction of 75% of the present plant species (Magnússon et al. 2014).

Sea birds and migratory passerine birds are thought to not only contribute to soil quality and long-distance seed dispersal, but also facilitate seed movement within the island (Árnason et al. 2014). Intra-island genetic diversity of H. peploides on Surtsey indicates some degree of population structuring, whereas strong gene flow among populations seems to be inhibiting intraspecific divergence (Árnason et al. 2014). The structuring is considerably distinct among populations of H. peploides on mainland Iceland, where habitats are far apart, connected by highly variable topography and are much older in age than locations on Surtsey (Árnason et al. 2014).

Long-distance seed dispersal events from various source populations, likely including Heimaey, the Reykjanes peninsula and the southern coast of Iceland, have provided great influx of genetic material, increasing the effective population size and growth rates of populations of H. peploides on Surtsey, whilst
maintaining allelic richness among the species (DLUGOSCH & PARKER 2008, ÁRNASON et al. 2014). Multiple colonising events are said to countervail the loss of allelic richness and, followed by gene flow, have previously lead to degrees of genetic diversity, that exceeded those in source populations (DLUGOSCH & PARKER 2008).

Genetic diversity may furthermore have been promoted by variations among populations in different source regions (ELLSTRAND & SCHIERENBECK 2000). During the expansion and dispersal from mainland Europe the species was exposed to diverse habitats. Different conditions might have selected for genotypes, which are less favorable on the continent, promoting divergence from mainland source populations (LE CORRE & KREMER 1998).

In conclusion this shows, that populations of *H. peploides* on Surtsey do not only bare a long and variable colonisation history, but also have great potential to diverge in time if gene flow decreases (ÁRNASON et al. 2014).

### 1.4 Objectives

The aim of this study was to shed light on karyotypic differences among individuals of *Honckenya peploides* and to distinguish possible variations on a subspecies level. This was achieved by (i) determining chromosome numbers of plants from Surtsey and several other locations across the northern hemisphere and (ii) assessing differences in karyotype composition. This study may contribute to understanding the origin and evolution of the species - a major aspect of plant conservation - whilst contributing to the thorough studies that have been performed on the island of Surtsey.
2 Material and Methods

2.1 Study Species

The sea sandwort *Honckenya peploides* (L.) Ehrh. (Caryophyllaceae) is a perennial, gynodioecious, maritime hemicryptophyte with circumpolar distribution, mainly inhabiting dunes, drift lines and seashores. *H. peploides* is an early coloniser, that arrived on Surtsey in the year 1967 (FRIÐRIKSSON & JOHNSEN, 1968), just a few years after the island had arisen, and is presently one of the most common plant species on the island (MAGNÚSSON et al. 2014). Its fleshy leaves are wide at the base, stand opposite and form an acute angle with the stem, allowing sand to accumulate, wherefore the species contributes to soil anchorage during colonisation of barren areas (GAGNÉ & HOULE 2002, PHILIPP & ANDERSEN 2014). The plant emerges each spring from overwintering buds on the rhizomes, producing daughter clones in the form of mats or clumps, that may remain attached to the parent plant by connections longer than two metres (SÁNCHEZ-VILAZ et al. 2010). In addition to the ability of reproducing asexually, *H. peploides* bears white, axillary flowers for sexual reproduction. The pistillate or female flowers have a normally developed gynoecium, small petals and non-functional, staminodie-like anthers (MALLING 1957, PHILIPP & ANDERSEN 2014). They produce six to ten millimeter big capsules with large seeds (MALLING 1957). Staminate flowers on the other hand have short styles, larger petals and well-developed anthers, producing and delivering pollen grains (MALLING 1957). Some of these staminate flowers have the ability to produce capsules with low seed numbers and are therefore categorised as hermaphrodites (MALLING 1957, PHILIPP & ANDERSEN 2014).

Varying chromosome numbers have been reported for *H. peploides* so far (2n = 34, 66, 68, 70), whereas 2n = 68 is the most common count (Table 1). Two tetraploid subspecies have been recognised among the genus. These are *Honckenya peploides* (L.) Ehrh., which occurs from Norway to Portugal and *Honckenya peploides* subsp. *diffusa*, which is mainly found in the Arctic. The diploid somatic chromosome number (2n = 34) assessed by PROBATOVA et al. (2004)
is thought to represent the subspecies *H. oblongifolia* which occurs in the North West Pacific area, Russia and Japan, implying the existence of differing ploidy levels among this small genus. Physiological and phenotypic variations as well as life history characteristics of different cytotypes remain largely unexplored.

**Table 1**: List of former reports on the somatic chromosome number of *H. peploides* from different locations.

<table>
<thead>
<tr>
<th>Location</th>
<th>2n</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iceland</td>
<td>66, 68, 70</td>
<td>LÖVE &amp; LÖVE (1950, 1956)</td>
</tr>
<tr>
<td>Disko, Greenland</td>
<td>68</td>
<td>MALLING (1957)</td>
</tr>
<tr>
<td>Svalbard</td>
<td>66</td>
<td>FLOVIK (1940)</td>
</tr>
<tr>
<td>Baltic Sea, Germany</td>
<td>68</td>
<td>MALLING (1957)</td>
</tr>
<tr>
<td>Denmark</td>
<td>68</td>
<td>MALLING (1957)</td>
</tr>
<tr>
<td>Tromsø, Norway</td>
<td>66</td>
<td>FLOVIK (1940)</td>
</tr>
<tr>
<td>Moneron Island, Russia</td>
<td>34</td>
<td>PROBATOVA (2004)</td>
</tr>
<tr>
<td>Sitka, Alaska</td>
<td>68</td>
<td>MALLING (1957)</td>
</tr>
<tr>
<td>Montreal, Canada</td>
<td>68</td>
<td>MALLING (1957)</td>
</tr>
<tr>
<td>Arctic Canada</td>
<td>68</td>
<td>LÖVE &amp; LÖVE (1982)</td>
</tr>
</tbody>
</table>

**2.2 Locations**

Individual plants used in this study had been raised from seeds and grown in pots in the Plant Genetics research laboratory growth room at the University of Iceland. Seeds were obtained through a correspondence with several institutions around the world, which was initiated by S.H. Árnason. In this study the karyotype was analysed of individuals from four sites around Iceland (Surtsey, Heimaey, Stokkseyri and Seltjarnarnes), Kołobrzeg, Poland (Figure 1a), Cold Bay, Alaska and Miquelon Island, Canada (Figure 1b).
Figure 1a&b: Maps showing the origin of individuals of *Honkenya peploides* used in this study. 1a: (A) Surtsey (Hp 08, 09, 21, 29, 30), (B) Heimaey (Hp 01, 03, 04, 05, 07, 19, 25, 27), (C) Sokkseyri, Iceland (Hp 11, 17, 20), (D) Seltjarnarnes, Iceland (Hp 02), (E) Kolobrzeg, Poland (Hp 16). 1b: (F) Cold Bay, Alaska (Hp 10), (G) Miquelon Island, Canada (Hp 06).

Source: maps.google.de
2.3 Enzymatic Root Tip Squash Method

To receive actively dividing cells appropriate for karyotyping, root tips were used. These were harvested during mid-day by picking the newly grown, lucent tips (0.5 to 2 cm long) directly from the soil. Root tips were then placed into 15 mL vials, filled with distilled ice water. The vials were kept on ice and stored at 4°C for approximately 27 hours. This pre-treatment step was conducted in order to synchronise mitosis and arrest as many metaphase cells as possible. It poses as a chemical-free alternative to other techniques of pre-treatment (ANAMTHAWAT-JÓNSSON pers. comment). The root tips were then dried on tissue paper and transferred into 1.5 mL tubes filled with freshly mixed fixative (1:3 glacial acetic acid : absolute ethanol). After resting at room temperature for at least 2 hours the root tips were stored at -32°C.

To receive good slides suitable for karyotyping the condensed metaphase chromosomes must be well spread and undamaged. This was achieved by enzymatic digestion (rather than acid hydrolysis). First the root tips were submerged in enzyme buffer (0.1 M citric acid monohydrate, 0.1 M trisodium citrate dehydrate, distilled water) for 20 min to dilute off the fixative. The buffer was replaced once during this period of time. Root tips were then placed on acid-cleaned slides, which had been washed in chromium trioxide in 80% sulphuric acid for at least 3 hours, rinsed thoroughly and then stored in 96% EtOH at 12°C. The root tips were trimmed to approximately 1 to 3 mm, then the buffer was removed with tissue paper and 19 μL of enzyme (1 g cellulase from Onozuka R10 and 1.2 mL pectinase from Sigma P7416) were added. The root tips were placed in the incubator at 37°C for 10 min (very thin roots only 9 min) for digestion. After incubation the enzyme was reduced with tissue paper. A drop of acetic acid (45%) was added, reduced with tissue paper, then another drop was added. After resting for 5 min the drop was reduced and reapplied once more. The size of the drop was reduced before extracting the cells under the stereo-microscope. This was achieved by teasing the cloudy meristem tissue above the tip with a needle and tweezers, releasing the meristem cells into the acetic acid suspension. Surplus root tissue was removed and the suspension slightly mixed with a needle. The needle and tweezers were cleaned in 70% ethanol in between applications. A clean cover slip was
placed on the slide and gently tapped vertically with a needle to scatter the cells. By manually pressing the cover slip firmly between filter paper the cells were squashed. Slides were then frozen in liquid nitrogen to fixate the chromosomes. After popping the cover slip, the slides were let to dry and stored at 12°C in an air-tight storage box.

Slides suitable for karyotyping were stained with DAPI (4,6-Diamidino-2-phenylindole) (ca. 100 μL, 1 min), a fluorochrome that binds to A-T-rich regions of DNA. After placing a cover slip on the slides, they were examined under the fluorescence microscope (Nikon Eclipse E8000, magnification 1000x) and images were saved (Nikon DIGITAL CAMERA DXM1200F using Nikon ACT-1 as capturing programme). Chromosomes of several cells of each individual were counted and evaluated by an overall impression of the single photos.

Somatic chromosome numbers were obtained by counting the chromosomes from several cells for each individual. The clearest photos were chosen to display the results.
3 Results

Suitable photographs of metaphase cells were obtained from all individuals except for Hp 03 from Heimaey and Hp 20 from Seltjarnarnes (Table 2). The following figures show images of cells containing chromosomes and nuclei. Yellow numbers indicate the number of chromosomes counted in the corresponding cell, while arrows point out satellite chromosomes.

3.1 Surtsey, Iceland

Cells from three individuals (Hp 08, 09 and 29) from Surtsey island contained more than 60 chromosomes (Figures 2, 3 and 5). Two individuals display lower numbers. A somatic chromosome number of 2n = 44/46 was counted for Hp 21 (Figure 4) and 2n = 58 for Hp 30 (Figure 6).
Table 2: List of all individuals of *H. peploides*, sampling locations and obtained chromosome numbers (2n).

<table>
<thead>
<tr>
<th>Location</th>
<th>Plant ID</th>
<th>2n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surtsey</td>
<td>Hp 08</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Hp 09</td>
<td>60/62</td>
</tr>
<tr>
<td></td>
<td>Hp 21</td>
<td>44/46</td>
</tr>
<tr>
<td></td>
<td>Hp 29</td>
<td>60/62</td>
</tr>
<tr>
<td></td>
<td>Hp 30</td>
<td>58</td>
</tr>
<tr>
<td>Heimaey, Iceland</td>
<td>Hp 01</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Hp 03</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Hp 04</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Hp 05</td>
<td>60/62</td>
</tr>
<tr>
<td></td>
<td>Hp 07</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Hp 19</td>
<td>66/68</td>
</tr>
<tr>
<td></td>
<td>Hp 25</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Hp 27</td>
<td>56+</td>
</tr>
<tr>
<td>Seltjarnarnes, Iceland</td>
<td>Hp 02</td>
<td>68</td>
</tr>
<tr>
<td>Stokkseyri, Iceland</td>
<td>Hp 11</td>
<td>64/66</td>
</tr>
<tr>
<td></td>
<td>Hp 17</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>Hp 20</td>
<td>-</td>
</tr>
<tr>
<td>Miquelon Island, Canada</td>
<td>Hp 06</td>
<td>52/54</td>
</tr>
<tr>
<td>Cold Bay, Alaska</td>
<td>Hp 10</td>
<td>60/62</td>
</tr>
<tr>
<td>Kolobrzeg, Poland</td>
<td>Hp 16</td>
<td>64/66</td>
</tr>
<tr>
<td>unknown</td>
<td>Hp 13</td>
<td>44/46</td>
</tr>
<tr>
<td></td>
<td>Hp 28</td>
<td>50</td>
</tr>
</tbody>
</table>
Figure 2: Hp 08, Surtsey.
Figure 3: Hp 09, Surtsey.
Figure 4: Hp 21, Surtsey.
Figure 5: Hp 29, Surtsey.
Figure 6: Hp 30, Surtsey.
3.2 Heimaey, Iceland

Most cells from individuals originating from Heimaey contained more than 60 chromosomes. For Hp 05 (Figure 9) and 25 (Figure 12) roughly 62 chromosomes were counted. Cells from Hp 01 (Figure 7) and Hp 19 (Figure 11) appeared to have a somatic chromosome number of 2n = 68. Two individuals (Hp 04 and 07) displayed a lower set of chromosomes (Figures 08 and 10). No suitable slides could be obtained for Hp 03.

Figure 7: Hp 01, Heimaey.
Figure 8: Hp 04, Heimaey.
Figure 9: Hp 05, Heimaey.
Figure 10: Hp 07, Heimaey.
Figure 11: Hp 19, Heimaey.
Figure 12: Hp 25, Heimaey.
Figure 13: Hp 27, Heimaey.
3.3 Seltjarnarnes, Iceland

Cells from the individual originating from Seltjarnarnes (Hp 02) contained 68 chromosomes (Figure 14). One satellite was observed, chromosomes were slightly clumped.

Figure 14: Hp 02, Seltjarnarnes.
3.4 Stokkseyri, Iceland

One individual from Stokkseyri (Hp 11) appeared to have 68 chromosomes (Figure 15). For Hp 17, a somatic chromosome number of \(2n = 56\) was counted (Figure 16). Chromosomes were not very well spread.

Figure 15: Hp 11, Stokkseyri.
Figure 16: Hp 17, Stokkseyri.
3.5 Canada

The individual from Miquelon Island, Canada, (Hp 06) displayed a smaller set of chromosomes (2n = 52/54) (Figure 17). Chromosomes overlapped a little and were spread out, so the number was hard to determine.
3.6 Alaska

Cells from the individual from Cold Bay, Alaska, (Hp 10) seemed to contain 60 to 62 chromosomes (Figure 18). One satellite could be spotted.

Figure 18: Hp 10, Cold Bay, Alaska.
3.7 Poland

For the individual from Kołobrzeg, Poland, (Hp 16) a somatic chromosome number of $2n = 64$ to 66 was counted (Figure 19). These chromosomes were spread widely.

Figure 19: Hp 16, Kołobrzeg, Poland.
3.8 Location unknown

For two individuals no collecting location could be determined. Both showed smaller sets of chromosomes. For Hp 13 a somatic chromosome number of $2n = 44/46$ was estimated (Figure 20). The chromosomes of Hp 28 were slightly overlapping and estimated to approximately $2n = 50$ (Figure 21).

Figure 20: Hp 13, location unknown.
Figure 21: Hp 28, location unknown.
4 Discussion

The somatic chromosome numbers of 20 individuals of *Honckenya peploides* from seven known and two unknown locations across the northern hemisphere were obtained from microscopic photographs. Results ranged from 2n = 44 to 68 (Table 2). Chromosome numbers of different cells of one individual sometimes varied and were often hard to determine, due to overlapping and clumped chromosomes on the one hand and spreading chromosomes on the other. Clumping occurs if the cover slip is not sufficiently tapped with a needle. Spreading on the other hand is caused if the slide has been tapped and then pressed too much. This yields problems with the correct determination of chromosomes, as part of the chromosomes might have moved away from the others and is therefore not counted. Furthermore, some chromosomes were overly digested by the enzyme, creating merging chromosomes, that appear viscous and slimy and are hard to tell apart.

The widely reported chromosome number of 2n = 68 (Table 1) could be observed in this study (Hp 01, 02, 08 and 19). These cells contained nuclei with two and more nucleoli (Figure 7A, B and D, Figure 11B). The number of nucleoli in plants has been positively correlated with the the ploidy level (Levin 2002, Venkatesh et al. 2014, Kim et al. 2015). Two individuals bared two satellite chromosomes (Figure 2B and 7B), while in one other cell there was only one detected (Figure 14). These are chromosomes that carry a secondary constriction, which appears to be attached to the chromosome by a chromatin thread. Satellite chromosomes contain active ribosomal loci and usually appear in homologous pairs.

In three individuals a chromosome number close to 68 was counted (Hp 09, 11 and 16). Hp 11 contained one satellite (Figure 15). Perhaps these two plants display a slightly lower ploidy, but for Hp 09 and 16 it is also likely that the real number is 2n = 68 and chromosomes were counted incorrectly, as they are overly digested (Figure 3) and spread out extremely (Figure 3 and 19).
Conspicuously many cells contained chromosomes ranging around $2n = 56$ to 62 (Hp 05, 06, 10, 17, 25, 27, 29 and 30). Miscounts due to unfavourable root tip squashes are likely in some cases (Hp 17, 25 and 27) but the frequency of this number range indicates a probability of a lower ploidy level. Furthermore, cells in which satellites were spotted (Hp 05 and 10) appeared to contain only one satellite chromosome (Figure 9A and B, Figure 18A). Nucleoli were difficult to estimate for these individuals (Figure 17B), but seemed to be perhaps few more than one or two (Figure 6B, Figure 9C and D).

The rest of the individuals (Hp 04, 07, 13, 21 and 28) displayed chromosome sets of $2n = 44$ to 50. Only one satellite was spotted within this group (Hp 07) and all nuclei that could be found contained one or two nucleoli (Hp 04, 07, 13 and 28).

Results from this study imply that there are more ploidy levels to the tetra- ($2n = 68$) and diploid cytotypes ($2n = 34$) reported so far (Table 1). MALLING (1957) reported a somatic chromosome number of $2n = 68$ for *H. peploides*, after re-examining material from the Baltic Sea. This material he had received from ROHWEDER, who reported chromosome numbers as low as $2n = 48$ in the same material. Observations from this study coincide with those reports, suggesting that there might be a cytotype of *H. peploides* with approximately 48 to 50 chromosomes. Furthermore, present chromosome counts point out the possibility of a cytotype with approximately 58 chromosomes. These outcomes could have arisen by crossing of tetra- and diploid individuals that have merged during the long colonisation history of the species. Dispersal by sea, multiple introductions and distribution by sea birds facilitate *H. peploides* to traverse great distances, which would allow chance to operate on converging individuals that have diverged over time. This might also explain why there is no pattern among chromosome counts from within regions. Recent hybridisation or interim segregation might be acting faster on *H. peploides* yielding variations earlier than expected.

These findings would not be surprising as the species has proven to hold surprising potential in other traits (SÁNCHEZ-VILAZ *et al.* 2010, ÁRNASON *et al.* 2014), with high genetic variation possibly inducing advantageous adaptive mechanisms while leaving space for evolution to act.
In conclusion this study points out the potential that *Honkenya peploides* carries and strongly demands for deeper investigation of karyotype variation of the species. Investigations of available material should be carried on and perhaps expanded, including more individuals from each location, as different ploidy levels could occur (DELAY & BAACK 2012).
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