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Polyploidy and invasion of English ivy (*Hedera* spp., Araliaceae) in North American forests

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Abstract Polyploidy is a common feature of agricultural weeds and natural area invaders. There are few studies comparing related diploid and polyploid exotics, however, and it is unclear what ecological and genetic factors favor the establishment of weedy polyploids. This research characterizes the geographic distribution and phenotypic characteristics of diploid *Hedera helix* and tetraploid *Hedera hibernica*, European species that are invading North American forests. To confirm the taxonomic affinity of invasive plants, we sequenced five non-coding cpDNA regions for 108 individuals (105 populations) as well as reference samples representing all species in the genus *Hedera*. Because diploid *H. helix* and tetraploid *H. hibernica* are poorly distinguished by morphology and DNA sequence, we used flow cytometry to determine their distribution (585 individuals). More than 90 % of sampled plants had cpDNA sequences identical or similar to *H. helix* sensu lato and *H. hibernica*. Diploid *H. helix* was dominant on the U.S. east coast (78.5 % of sampled plants) while tetraploid *H. hibernica* was dominant on the U.S. west coast (72.2 % of sampled plants), mirroring the species' occurrence in maritime versus continental climates of Europe. Moreover, for sympatric occurrences in the Pacific Northwest, *H. hibernica* was larger and more frequently reproductive than *H. helix*. In a 2-year garden experiment, tetraploid *H. hibernica* had substantial architectural differences compared to diploid *H. helix*, including larger (but less numerous) leaves and thicker (but less branched) stems. Field experiments are needed to evaluate "pre-adaptation" (directional ecological filtering) and other factors mediating the invasion of *H. helix* and *H. hibernica*.

Keywords Cytogeography · Flow cytometry · Genome duplication · Horticulture · Phylogeography · Triploidy

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Introduction

Polyploidy is common in flowering plants and frequently associated with the propensity to invade agricultural systems and natural habitats (Bennett et al. 1998; Broz et al. 2009; Chapman et al. 2004; Czarnecki 2010; Pandit et al. 2006, 2011; Verlaque et al. 2002). Several mechanisms may underlie this association (te Beest et al. 2012). For example, triploids exhibit increased allocation to clonal growth because the presence of a third, unmatched chromosome complement in meiosis renders plants sterile, or nearly so (Bruneau and Anderson 1988; Marchant and Macfarlane 1980; but see Lui et al. 2005). Tetraploids are typically fertile but have altered growth patterns and life-history traits because of increased cell size and gene dosage effects (Levin 1983, 2002; Ramsey

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2011). Both triploids and tetraploids exhibit increased allelic diversity and diminished inbreeding depression in comparison to diploids (Bingham 1980; Otto and Whitton 2000). Thus, polyploids may be more likely to survive demographic bottlenecks and persist in disturbed habitats than diploids. Moreover, polyploids may exhibit rapid ecological adaptation due to multisomic inheritance, intergenomic recombination, and accelerated epigenetic processes (Parisod et al. 2010; Soltis and Soltis 2009; te Beest et al. 2012).

Critical analysis of the relationship between invasiveness and polyploidy will require study of polyploid complexes, in which one may directly compare closely-related diploid and polyploid taxa in field conditions (Kubátová et al. 2008; Schlaepfer 2010; Treier et al. 2009). Here we investigate invasive diploid and tetraploid ivies (*Hedera* spp., Araliaceae) in North American forests. Ivies are native to Eurasia and North Africa but cultivated worldwide as a groundcover and garden ornamental (Ackerfield and Wen 2002, 2003; Reichard 2000; Rose 1996). In the U.S. and Canada, two species are reported to invade forests: the diploid *H. helix* ($2n = 2x = 48$) and its autotetraploid sister species, *H. hibernica* ($2n = 4x = 96$) (Clarke et al. 2006). These taxa harbor identical cpDNA and nDNA haplotypes and are frequently called "English ivy" (Green et al. 2011). *Hedera helix* and *H. hibernica* exhibit subtle morphological differences related to trichome orientation and leaf shape—in practice, the species are difficult to distinguish in the field (Sulgrove 1984). In Europe, *H. helix* and *H. hibernica* were used for decoration since ancient times, and by the 1700s, both species were established in the nursery trade (McAllister and Rutherford 1990; Rose 1996). Herbarium records of naturalized ivies date to the 1870s (Georgia; deposited at the University of Florida herbarium) on the U.S. east coast and the 1930s (California; deposited at Washington State University herbarium) on the U.S. west coast. Invasive ivy is particularly prominent in the Pacific Northwest and the central Atlantic Seaboard (Reichard 2000; Thomas 1998), which are focal areas for this research.

This study combines field surveys, molecular analyses, and garden experiments to investigate the relationship between polyploidy and forest invasion on the east and west coasts of North America. First, we use non-coding cpDNA sequence to confirm the taxonomic affinities of invasive North American populations. Second, we employ flow cytometry to determine the geographic distribution and abundance of diploid *H. helix* and tetraploid *H. hibernica*. Third, we leverage co-occurrence of the two species in the Pacific Northwest to compare their size and reproductive status while growing in sympatry. Finally, we compare the growth and architecture of field-collected *H. helix* and *H. hibernica* in a 2-year common garden experiment.

Materials and methods

Geographic sampling

We sampled 585 plants from 131 naturalized populations along the eastern seaboard (Florida to New York) and west coast (southern California to northern Washington state) of North America. Sample sites included woodlots, parks, and natural areas in both urban and rural environments (Table 5 in Appendix). To minimize the risk of collecting multiple samples from the same individual, harvested stems were separated by at least 20 m. Approximately 95 % of plants were sampled in the juvenile state in the forest understory. We obtained *Hedera* reference samples from the American Ivy Society (Deerfield, NJ, USA), a nonprofit organization that maintains ivy collections for taxonomic research and cultivar development. Nomenclature of American Ivy Society accessions is based on the classification developed by Hugh McAllister and Alison Rutherford, who have handled the stocks, performed chromosome counts, and in many cases provided original specimens from field collections (Green et al. 2011; McAllister and Rutherford 1990; Rutherford et al. 1993).

DNA extraction, sequencing, and analysis

The taxonomy of *Hedera* is complex, with most species delineated by subtle differences in leaf shape, trichome morphology, and/or ploidy level (Ackerfield and Wen 2002, 2003; Rutherford et al. 1993). Naturalized populations may be comprised of a mixture of ornamental and "wild-type" specimens, further complicating species identification efforts. To confirm that invasive populations are comprised primarily of the European species *H. helix* and *H. hibernica*, we sequenced non-coding cpDNA regions that previous research had shown to distinguish major

species groups in *Hedera* (Green et al. 2011) for a subsample of field-collected plants. We preferentially selected heavily-invaded forest sites for analysis, sequencing 108 plants from 105 naturalized populations as well as 21 *Hedera* reference specimens obtained from the American Ivy Society. The reference specimens represented all 13 species in the genus *Hedera* (Table 1).

Genomic DNA was isolated from invasive plants and reference samples using the DNeasy plant kit (Qiagen, Valencia, CA). Approximately 60 mg of fresh tissue was used per extraction. Universal primers were used to amplify five non-coding chloroplast regions, including the *psbA-trnH* intergenic spacer, *psbB-psbH* intergenic spacer, *rpL16* intron, *rpoB-trnC* intergenic spacer, and *ycf6R-trnC* intergenic spacer (Shaw et al. 2005). Amplification and sequencing were performed as described by Green et al. (2011). Sequences were manually edited with Sequencher™ (v. 4.1; Gene Codes, Ann Arbor, MI) and manually aligned with MacClade (v. 4.08, Sinauer Associates, Sunderland, MA). Indels were retained for analysis when insertion/deletion regions could be unambiguously reconstructed using outgroups and did not contain microsatellites, defined as tandems of simple sequence (1–4 bp) repeated ten or more times (Queller et al. 1993). DNA sequences produced for this study can be found in GenBank accessions JN994638–JN995185 (specimens from naturalized populations) as well as HQ220374–HQ220376, HQ220380–HQ220389, HQ220391–HQ220400, HQ220438–HQ220440, HQ220444–HQ220453, HQ220455–HQ220464, HQ220534–HQ220536, HQ220540–HQ220549, HQ220551–HQ220560, HQ220566–HQ220568, HQ220572–HQ220581, HQ220583–HQ220592, HQ220598–HQ220600, HQ220604–HQ220613, and HQ220615–HQ220624 (reference specimens from the American Ivy Society).

Table 1 *Hedera* reference taxa used in cpDNA sequencing

Taxon	Origin	Accession no.
H. algeriensis	Northern Africa	AIS-88-188
H. azorica	Azores	AIS-82-254
H. azorica	Azores	AIS-82-259
H. canariensis	Canary Islands	AIS-94-052
H. colchica	Middle East	AIS-94-058
H. cypria	Cyprus	AIS-03-079
H. helix subsp. *helix*	Europe	AIS-87-139
H. helix subsp. *helix*	Europe	AIS-83-063
H. helix subsp. *rhizomatifera*	Spain	AIS-04-053
H. helix subsp. *caucasigena*	Caucasus	AIS-90-079
H. hibernica	Coastal Europe	AIS-06-023
H. iberica	Iberian Peninsula	AIS-4003
H. maderensis	Madeira	AIS-91-097
H. maroccana 'Spanish Canary'	Northern Africa	AIS-88-009
H. maroccana	Northern Africa	AIS-88-008
H. nepalensis var. *sinensis*	Central Asia	AIS-88-259
H. nepalensis var. *nepalensis*	Central Asia	AIS-88-258
H. pastuchovii	Middle East	AIS-82-118
H. pastuchovii	Middle East	AIS-88-264
H. rhombea	Eastern Asia	AIS-88-260
H. rhombea	Eastern Asia	AIS-88-149

Ploidy analysis

In *Hedera*, related diploid and polyploid species are in some cases poorly distinguished by morphology and DNA sequence data (Green et al. 2011). We thus used flow cytometry to determine the ploidy level of the 585 sampled plants. Approximately 5 g of fresh leaf tissue was placed in 2 mL of buffer (3.6 g HEPES, 2 mL of a 0.5 M solution of EDTA, 6.0 g KCL, 1.2 g NaCl, 102.7 g sucrose, 2 mL Triton X-100, 1 mL ß-mercaptoethanol, and 0.1 g spermine in 1.0 L distilled water) and chopped for approximately 1 min by hand with a razor blade. The resulting slurry was then passed through a syringe filter (25 mm Millipore Swinnex filter holder, Fisher Scientific Company LLC, Pittsburgh, PA, SX0002500) fitted with 48 µm nylon mesh (Small Parts Inc., Miami Lakes, FL, B-CMN-48) to remove debris, and then centrifuged for 1 min at 10,000×*g*. The nuclei pellet was resuspended in 490 µL chopping buffer containing 10 µL of a 5 mg/mL solution of propidium iodide (100 ug/mL final concentration) and 0.24 µL RNAse A (3.4 units/mL final concentration). As an internal control, leaves from diploid individuals of an unrelated species, *Chamerion angustifolium* (Onagraceae), were added to each sample prior to chopping.

Samples were run on a FACS Calibur flow cytometer (Becton–Dickinson, Franklin Lakes, NJ) outfitted with a blue argon laser (488 nm wavelength, 15 mW

power) in the Cell Sorting facility in the Flow Cytometry and Immunological Analysis Center, University of Rochester, Rochester, NY. Samples were analyzed for relative fluorescence ("FL2-A"), which was summarized as a frequency histogram using CellQuest™ (v. 5.2.1, Becton–Dickinson). The 2C DNA content of each sample was then inferred as a proportion to the internal control. *Chamerion angustifolium* has an estimated 2C DNA content of 1.45 pg (P. Kron and B. Husband, University of Guelph, pers. comm.), which almost exactly matches the 1C value of *Hedera*. The correlation between DNA content estimates via flow cytometry and actual ploidy level was tested using reference samples of known diploid, tetraploid, hexaploid, and octoploid species from the American Ivy Society (Table 2) as well as two triploid plants for which mitotic chromosome counts had recently been made (T. Ramsey and H. McAllister, unpubl. data). We used linear regression to test the relationship between ploidy and 2C DNA content for 42 reference specimens.

Table 2 DNA content of reference *Hedera* taxa inferred from flow cytometry (one analysis per specimen)

Taxon	Accession no.	Ploidy	2C DNA (pg)
H. azorica	AIS 82-254	2*x*	2.95
H. azorica	AIS 82-259	2*x*	2.95
H. canariensis	AIS 94-052	2*x*	3.07
H. helix subp. *caucasigena*	AIS 90-079	2*x*	3.05
H. helix subsp. *helix*	AIS 83-063	2*x*	2.97
H. helix subsp. *helix*	AIS 94-012	2*x*	2.92
H. helix subsp. *helix*	AIS 87-139	2*x*	2.98
H. helix subsp. *helix*	AIS 91-115	2*x*	3.15
H. helix subsp. *helix*	AIS 90-070	2*x*	2.91
H. helix subsp. *rhizomatifera*	AIS 04-053	2*x*	3.06
H. maroccana	AIS 88-008	2*x*	3.05
H. maroccana 'Spanish Canary'	AIS 88-009	2*x*	2.91
H. nepalensis var. *nepalensis*	AIS 88-258	2*x*	3.04
H. nepalensis var. *nepalensis*	AIS 95-386	2*x*	3.06
H. nepalensis var. *nepalensis*	AIS 90-065	2*x*	3.06
H. nepalensis var. *sinensis*	AIS 02-030	2*x*	2.99
H. nepalensis var. *sinensis*	AIS 88-259	2*x*	3.09
H. nepalensis var. *sinensis*	AIS 82-159	2*x*	3.06
H. rhombea	AIS 88-260	2*x*	2.93
H. rhombea	AIS 88-261	2*x*	3.12
H. rhombea	AIS 88-149	2*x*	3.02
H. helix × *hibernica*	TSR L103	3*x*	4.56
H. helix × *hibernica*	TSR L104	3*x*	4.85
H. helix 'Woerneri'	AIS 79-428	3*x*	4.65
H. algeriensis	AIS 88-188	4*x*	5.90
H. hibernica	AIS 06-024	4*x*	6.55
H. hibernica	AIS 95-119	4*x*	6.39
H. hibernica	AIS 96-063	4*x*	6.33
H. hibernica	AIS 06-023	4*x*	6.33
H. hibernica	AIS 88-057	4*x*	6.29
H. hibernica	AIS 87-180	4*x*	6.14
H. cypria	AIS 03-079	6*x*	8.83
H. iberica	AIS 04-003	6*x*	9.50
H. maderensis	AIS 91-097	6*x*	9.15
H. maderensis	AIS 82-253	6*x*	9.56
H. maderensis	AIS 94-060	6*x*	8.90
H. pastuchovii	AIS 88-264	6*x*	8.32
H. pastuchovii	AIS 88-118	6*x*	8.40
H. colchica	AIS 82-256	8*x*	10.34

Local distribution, size, and reproductive status

Co-occurrence of diploid *H. helix* and tetraploid *H. hibernica* in Washington state enabled us to evaluate the size and reproduction of the two species while growing in sympatry. Studies were conducted in a natural forest habitat (St. Edward State Park) and nearby suburban neighborhoods (Kenmore, WA, north of Seattle). As a Catholic seminary from 1931 to 1977, St. Edward escaped the development that occurred in many other areas of the Puget Sound. Transferred to state ownership in 1978, St. Edward retains ~100 hectares of high-quality Pacific Northwest forest, composed of big-leaf maple, alder, Douglas fir, western red cedar, and western hemlock. Since the 1950s the park has been surrounded by suburban neighborhoods, where ivy is a conspicuous component of landscaping.

At St. Edward State Park, ivies were located by walking parallel east–west transects through the forest at 40 m intervals. Habitat was inspected to the north and south of transect lines, enabling us to locate all reproductive plants and most juvenile patches. In surrounding suburbs, ivy was found by systematically traversing areas of public access, including paved roads, sidewalks, and trails. In total, we located 141 juvenile and reproductive ivies, which were permanently marked

Table 2 continued

Taxon	Accession no.	Ploidy	2C DNA (pg)
H. colchica	AIS 87-001	8*x*	10.48
H. colchica	AIS 94-058	8*x*	10.66
H. colchica	AIS 95-181	8*x*	10.81

and mapped using GPS coordinates and aerial photographs. We estimated total groundcover of juvenile plants as the product of patch length and width. The number of reproductive stems (ramets growing up distinct host trees) were counted in each patch. We used Kruskal–Wallis tests to compare the total groundcover size of *H. helix* and *H. hibernica* while a contingency table was used to compare the proportion of sampled plants that were reproductive.

During the winters of 2003 and 2004, we estimated fruit production of reproductive ivies from the base of their host trees. We used binoculars to count the number of inflorescences on each reproductive ramet (N = 32 ramets in St. Edward State Park and N = 21 ramets in suburbs) and a spotting scope to count the number of developed and undeveloped fruits on ~50 inflorescences per ramet. The proportional fruiting success of ramets was calculated as the ratio of developed fruits to total fruits.

Common garden experiment

We used a 2-year common garden experiment to compare growth characteristics of diploid *H. helix* sampled from seven east coast sites (Annapolis, Battery-Kemble, Georgetown, Meridian, Potomoc, Roosevelt, University of D.C.) versus tetraploid *H. hibernica* sampled from six west coast sites (Camano, Chuckanut, Gladstone, Pacifica, St. Edward, Trinidad) in 2006–2007 (Table 5 in Appendix). Established in June 2008, the garden experiment was performed adjacent to the University of Rochester South Campus (N43°6′, W77°38′; 165 m asl) in a grassy meadow shaded by black walnut and white ash trees. The experiment included 6–10 cuttings from each of 3–6 genotypes per collection site (N = 396 total cuttings). Cuttings were generated as 15–20 cm stem segments with 2–4 leaves and 3–5 nodes, and planted in 350 cm^3 square pots containing peat-perlite mix (Growing Mix 2, Conrad Fafard, Agawam, MA, USA). Cuttings were rooted for 3 weeks under propagation domes; thereafter, pot locations within flats were assigned randomly. The orientation and position of flats were randomized monthly from June through September in 2008 and 2009. During the spring and summer months, plants were irrigated as needed to avoid water stress (2–3 day intervals, depending on ambient temperatures and precipitation) and fertilized once per week. Daily high temperatures in July and August averaged 26.6 °C (range 19.4–33.9°) in 2008 and 26.1 °C (range 19.3–34.4°) in 2009.

To compare pre- and post-vernalization growth, one-half of the cuttings from each genotype were harvested in October 2008. We removed entire specimens from their pots and rinsed soil from the roots and rhizomes. We then pressed roots, stems, and leaves in newspaper between blotter paper and cardboard ventilators. Plants not harvested in October 2008 were buried, potted, in garden soil to a depth of 3–5 cm at a steep angle to mimic the sprawling habit of juvenile ivies. Cold frames were assembled in November 2008 to buffer the experiment from extreme winter conditions. Frames were constructed with 3.5 m cross-linked polyethylene pipe and covered with plastic sheeting (FLEX-O-GLASS 4-year UV Clear Film, Warp Bros., Chicago, IL, USA). Temperatures within cold frames were monitored with radio-transmitting thermometers (WS-9023U base-station plus TX21U-IT 915 MHz temperature sensors, Lacrosse Technology, La Crescent, MN); daily low temperatures averaged −2.8 °C (range −6.7 to 2.9 °C) from January through February 2009. We routinely cleared snow from the cold frames, which were dismantled in late March 2009. At this point, plants were randomized and relocated to flats. In September 2009, remaining plants were harvested and pressed.

After drying, the leaves of each plant were spread onto posterboard with a scale-bar. Posters were photographed on a copy stand using an EOS 10D digital SLR camera with 28 mm lens (Canon USA, Lake Success, NY, USA). Thereafter, we counted and weighed all leaves, and counted, measured, and weighed all stems. To estimate leaf surface area, we imported digital photographs to the ImageJ image analysis software package (Abramoff et al. 2004). Images were converted to grayscale and the threshold function was applied to highlight leaves; we then calculated leaf area using the "analyze particles" function. All statistical analyses were conducted with JMP (v. 9.0; SAS Institute, Cary, NC, USA). We analyzed leaf and stem characters—including leaf number, average leaf surface area, number of stem

branches, and stem density (stem mass/stem length)—by MANOVA, with ploidy, population (nested under ploidy), time-of-measurement (experiment year one or two), and ploidy × time-of-measurement as model effects. Values for all four traits were log-transformed to improve the distribution of residuals. We then used univariate ANOVA to analyze leaf and stem traits individually, specifying ploidy, population, time-of-measurement, and ploidy × time-of-measurement as model effects.

Results

Chloroplast sequence

The aligned cpDNA dataset contained 3,099 base pairs, including 32 variable and 17 parsimony informative sites. Indels were non-overlapping and rarely obscured base pair differences among haplotypes (Table 3). The five non-coding regions contributed similar numbers of variable sites (*psbA-trnH,* 5*; psbB-psbH,* 3; *rpL16,* 10; *rpoB-trnC,* 8; *ycf6R-trnC,* 6). *Hedera* reference samples harbored 15 cpDNA haplotypes (among 21 genotypes). Invasive North American plants had six haplotypes (among 108 genotypes), including three unique haplotypes not present in reference samples (Table 3).

The majority (85.2 %) of cpDNA haplotypes recovered from invasive populations were identical to those found in reference samples of *H. hibernica* and *H. helix* subsp. *helix* (haplotypes *a, b*; Table 3). Two other invasive plants had haplotypes that were a single mutational step removed from reference *H. hibernica* and *H. helix* subsp. *helix* sequences (haplotypes *e*, *f*). Among the remaining invasive specimens, 5.6 % had sequence similar to that of diploid *H. helix* subsp. *caucasignea* (haplotype *c*) while 7.4 % had haplotypes identical to that tetraploid *H. algeriensis* (haplotype *d*). Most of the cpDNA haplotypes recovered from invasive populations were widely dispersed geographically; however, *H. algeriensis* haplotypes were only recovered in southern and central California (Table 5 in Appendix).

Ploidy analysis

Analysis of nuclei fluorescence distinguished reference samples of known diploid, tetraploid, hexaploid, and octoploid species of *Hedera* (Fig. 1). Coefficients of variation for DNA content histograms averaged 2.94 % (range 2.06–4.26 %) in reference samples. Relative fluorescence increased in proportion to ploidy level with minor variation across homoploid taxa; linear regression confirmed a close relationship between accession chromosome number and 2C DNA content (N = 42 genotypes; $R^2 = 0.991$, $P < 0.001$; Fig. 1). For samples from naturalized populations, coefficients of variation averaged 3.10 % (range 1.64–7.02 %), with approximately one-in-twenty samples having values ≥5 %. DNA content of naturalized plants (N = 585) was trimodal with values corresponding to diploid, triploid, and tetraploid cytotypes (Fig. 2). Because cpDNA haplotypes indicate that the vast majority of invasive plants are affiliated to *H. helix* sensu lato and *H. hibernica*, we hereafter interpret diploid plants as "*H. helix*" and tetraploid plants outside of southern California as "*H. hibernica*."

Diploid *H. helix* comprised 39.1 % (229 individuals) of the total collection and were dominant on the east coast (146 of 187 individuals) (Figs. 3, 4; Table 5 in Appendix). Tetraploid *H. hibernica* comprised 56.2 % (329 individuals) of the total collection and were recovered primarily from the west coast (290 of 398 individuals) (Figs. 3, 4; Table 5 in Appendix). There were striking differences in species composition between the central Atlantic Seaboard and the Pacific Northwest, the two principle epicenters of ivy invasion. For example, in Washington, D.C., and adjacent areas of Virginia and Maryland, 97.5 % of sampled ivies were diploid (79 of 81 plants from 11 populations). In the Puget Sound region of Washington state, on the other hand, 16.4 % of sampled ivies were diploid (20 of 122 plants from 21 populations). Triploids comprised 4.4 % (26 individuals) of the total collection and were found primarily on the west coast (Table 5 in Appendix).

Local abundance, size, and distribution

In our studies of Kenmore, Washington state, diploid *H. helix* was observed significantly less often in forest habitat than in suburban areas (forest: 8 *H. helix* vs. 63 *H. hibernica*; suburbs: 18 *H. helix* vs. 47 *H. hibernica*; $\chi^2 = 5.92$, $P = 0.015$). Forest individuals of *H. helix* were found primarily in the northern end of St. Edward State Park while suburban individuals of *H. helix* occurred in equal numbers on the north and south sides

Table 3 Major and minor cpDNA haplotypes recovered from *Hedera* reference samples and North American invasive populations

Haplotype	cpDNA region																		Reference taxa	Representation within U.S. naturalized populations
	psbA-trnH					*psbB-psbH*			*RPL16*											
	1 1 9	2 3 7	2 4 9	2 5 7	2 9 2	2 1 0	2 2 9	3 4 1	0 0 1	0 6 3	1 3 2	1 4 9	1 7 4	2 5 5	4 0 1	4 7 1	7 6 9	7 8 2		
a	T	A	G	A	T	T	–	A	T	A	A	A	–	G	C	A	C	T	*H. helix* subsp. *helix, H. hibernica*	70.4 %
b	T	A	G	A	T	T	–	A	T	A	A	A	–	T	C	A	C	T	*H. helix* subsp. *helix, H. hibernica*	14.8 %
c	T	A	G	A	G	T	–	A	T	T	A	C	–	G	C	A	T	T	–	5.6 %
d	T	A	T	A	G	T	–	A	T	A	A	C	–	G	C	A	C	T	*H. algeriensis*	7.4 %
e	T	A	G	A	T	T	–	A	T	A	A	A	–	G	C	A	C	T	–	0.9 %
f	T	A	G	A	T	T	–	A	T	A	A	A	–	G	C	A	C	T	–	0.9 %
g	T	A	G	A	T	T	–	A	T	A	A	A	–	G	C	A	C	T	*H. helix* subsp. *helix*	0
h	T	A	G	A	T	T	–	A	T	A	A	A	–	G	C	A	C	T	*H. colchica*, *H. pastuchovii*, *H. nepalensis* var. *sinensis*	0
i	T	A	G	A	T	T	T	A	T	A	A	A	–	G	C	A	C	T	*H. rhombea*	0
j	T	A	G	A	T	T	T	A	T	A	A	A	–	G	C	C	C	T	*H. nepalensis* var. *nepalensis*	0
k	T	C	G	A	G	T	–	A	T	A	A	C	–	G	C	A	C	T	*H. helix* subsp. *caucasigena*	0
l	T	C	G	A	G	T	–	A	T	A	A	C	T	G	C	A	C	T	*H. cypria*	0
m	T	A	G	A	G	T	–	A	T	A	A	C	–	G	T	A	C	T	*H. maderensis*, *H. azorica*	0
n	T	A	G	A	G	T	–	A	C	A	A	C	–	G	T	A	C	T	*H. azorica*	0
o	A	A	G	A	G	–	–	A	T	A	T	C	–	G	C	A	C	T	*H. maroccana* 'Spanish Canary'	0
p	A	A	G	A	G	–	–	A	T	A	T	C	–	G	C	A	C	T	*H. maroccana*	0
q	A	A	G	C	G	–	–	A	T	A	A	C	–	G	C	A	C	T	*H. helix* subsp. *rhizamotifera*, *H. iberica*	0
r	A	A	G	C	G	–	–	T	T	A	A	C	–	G	C	A	C	G	*H. canariensis*	0

Table 3 continued

Haplotype	cpDNA region														Reference taxa	Representation within U.S. naturalized populations
	rpoB-trnC								*ycf-trnC*							
	0 7 1	3 3 4	3 5 8	5 4 4	5 6 5	7 3 8	7 9 2	8 0 9	1 5 2	2 6 6	2 9 5	3 1 5	4 3 7	5 4 2		
a	A	T	T	A	G	G	C	C	–	T	T	T	T	T	*H. helix* subsp. *helix, H. hibernica*	70.4 %
b	A	T	T	A	G	G	C	C	–	T	T	T	T	T	*H. helix* subsp. *helix, H. hibernica*	14.8 %
c	A	T	T	–	G	G	C	C	–	T	T	G	C	T	–	5.6 %
d	A	T	T	A	G	G	C	C	–	T	T	G	C	T	*H. algeriensis*	7.4 %
e	–	T	T	A	G	G	C	C	–	T	T	T	T	T	–	0.9 %
f	A	T	T	A	T	G	C	C	–	T	T	T	T	T	–	0.9 %
g	A	T	T	A	G	G	C	C	–	T	–	T	T	T	*H. helix* subsp. *helix*	0
h	A	T	G	A	G	G	C	C	–	T	T	G	C	T	*H. colchica*, *H. pastuchovii, H. nepalensis* var. *sinensis*	0
i	A	T	G	A	G	G	C	C	–	T	T	G	C	T	*H. rhombea*	0
j	A	T	G	A	G	G	C	C	–	T	T	G	C	T	*H. nepalensis* var. *nepalensis*	0
k	A	T	T	–	G	G	C	C	–	T	T	G	C	T	*H. helix* subsp. *caucasigena*	0
l	A	T	T	A	G	A	C	C	–	T	T	G	C	T	*H. cypria*	0
m	–	T	T	A	G	G	C	C	–	T	T	G	C	T	*H. maderensis*, *H. azorica*	0
n	–	T	T	A	G	G	C	C	–	T	T	G	C	T	*H. azorica*	0
o	–	C	T	A	G	G	C	C	–	G	T	G	C	T	*H. maroccana* 'Spanish Canary'	0
p	–	C	T	A	G	G	C	C	T	G	T	G	C	A	*H. maroccana*	0
q	A	C	T	A	G	G	C	A	–	T	T	G	C	A	*H. helix* subsp. *rhizamotifera*, *H. iberica*	0
r	A	–	–	A	G	G	A	C	–	T	T	G	C	A	*H. canariensis*	0

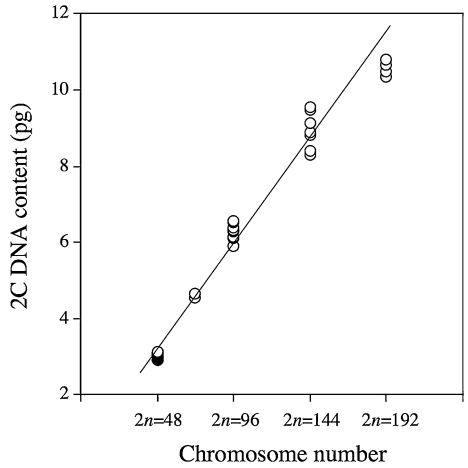

Fig. 1 Linear regression showing relationship between ploidy level and 2C DNA content for known diploid, triploid, tetraploid, hexaploid and octoploid reference samples (Table 2)

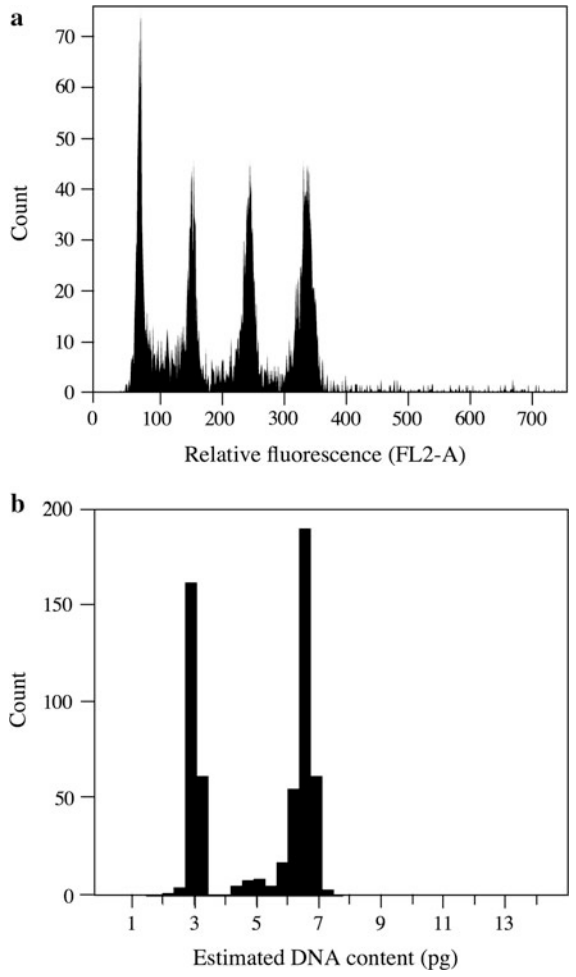

Fig. 2 Relative fluorescence and 2C DNA content values for diploid, triploid, and tetraploid ivies. **a** Flow cytometry output of relative fluorescence (FL2-A) in sample prepared with internal control and equal amounts of diploid, triploid, and tetraploid leaf tissues. **b** Distribution of 2C DNA contents recovered from all North American populations (585 samples)

of the park (Fig. 5). Tetraploid *H. hibernica* represented 77.4 % of sampled plants (87.5 % of sampled forest plants and 70.5 % of sampled suburban plants). Although abundant throughout the study area, *H. hibernica* was found more often in "edge" habitats of St. Edward (adjacent to lakeshore, roads, trails and ball fields) than in "interior" regions (Fig. 5). Triploids, which represented 3.5 % of sampled plants, were restricted to the northwest corner of St. Edward State Park.

Patch size differed significantly between cytotypes (Kruskal–Wallis test, $H = 6.953$, $P = 0.031$). Specimens of *H. helix* were small (mean 18.3 m^2 of ground cover, range 1–150 m^2) while *H. hibernica* plants were often large (mean 117.5 m^2 of ground cover, range 1–1,600 m^2). Triploids were intermediate in size between *H. helix* and *H. hibernica* (mean 63.0 m^2 of ground cover, range 12–200 m^2). Reproductive status exhibited similar differences ($\chi^2 = 16.171$, $P < 0.001$). Of the 80 sampled reproductive plants, only one plant was *H. helix* (3.8 % of all sampled *H. helix* individuals), and this individual had a single reproductive stem. In contrast, 45.5 % of sampled *H. hibernica* were reproductive. Sixty-four percent of reproductive *H. hibernica* patches had two or more reproductive ramets (mean 4.4 reproductive ramets per patch). The proportion of developed fruits on *H. hibernica* plants was low (mean 17.7 %, range 0–64.3 %), however, while the proportion of developed fruits on the single reproductive *H. helix* was high (92.8 %). The sampled triploid patches were not reproductive.

Growth and architecture in the experimental garden

Garden-grown specimens of diploid *H. helix* and tetraploid *H. hibernica* differed substantially in growth and architecture. MANOVA (Wilks' $\Lambda = 0.049$, $F_{56,1468} = 30.482$, $P < 0.0001$) indicated significant effects of ploidy ($F_{4,377} = 158.032$, $P < 0.0001$), population ($F_{44,1444} = 3.855$, $P < 0.0001$), and year

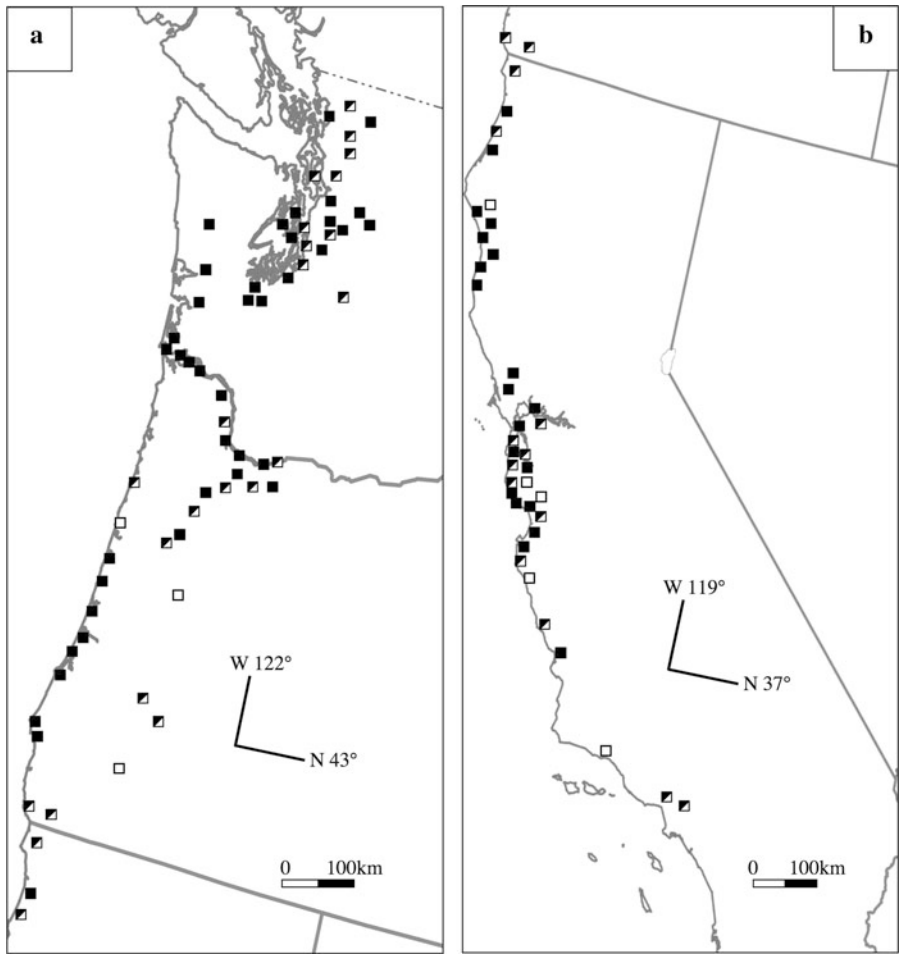

Fig. 3 Sampled populations of *Hedera* in **a** the Pacific Northwest and **b** California. *Boxes* indicate ploidy of populations as diploid *H. helix* (*open*), tetraploid *H. hibernica* (*filled*), or mixed (*half-filled*)

($F_{4,377} = 391.381$, $P < 0.0001$) on plant traits; however, there was no significant interaction effect for ploidy × year ($F_{4,377} = 0.804$, $P = 0.5231$). Univariate ANOVA indicated significant ploidy effects for individual leaf and stem traits after correction by sequential Bonferroni (Rice 1989; Table 4). In general, tetraploid *H. hibernica* had larger—but numerically fewer—leaves and stems than diploid *H. helix*. For example, in the second year of the experiment, diploid plants produced a mean of 67.0 leaves, each averaging 9.2 cm^2 in surface area, while tetraploid plants produced a mean of 42.0 leaves, each averaging 16.4 cm^2 in surface area (Fig. 6a). Similarly, diploid plants had highly branched stems, with an average stem density of 0.015 g/cm, while tetraploids produced moderately branched stems, with an average stem density of 0.022 g/cm (Fig. 6b). Population-level variation was evident for all traits, suggesting that plant architecture is affected to some degree by genic factors as well as by ploidy (Fig. 6; Table 4).

Discussion

Geographic origins of invasive populations

North American populations are dominated by taxa of northern and central Europe, i.e., diploid *H. helix* subsp. *helix* and tetraploid *H. hibernica* (Tables 1, 3). These sister species are parapatrically distributed in their native range (Green et al. 2011; McAllister and Rutherford 1990). *Hedera hibernica* is a primarily maritime species that occurs on coastal cliffs and low- to mid-elevation forest along the Atlantic coast of the UK, the Netherlands, Belgium, France, and Spain. *Hedera helix* subsp. *helix* occurs in woodlands,

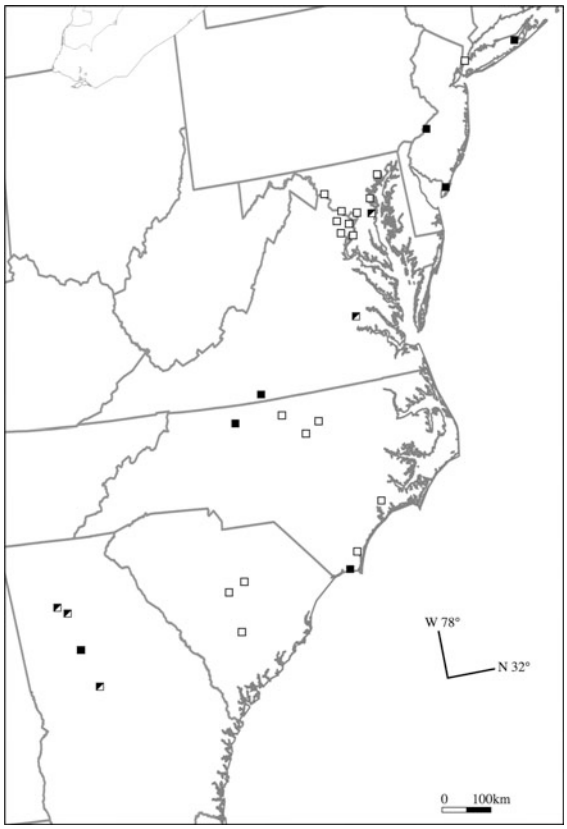

Fig. 4 Sampled populations of *Hedera* on the U.S. Atlantic Seaboard. *Boxes* indicate ploidy of populations as diploid *H. helix* (*open*), tetraploid *H. hibernica* (*filled*), or mixed (*half-filled*)

ravines, and rocky slopes of inland and high elevation areas across western, central and eastern Europe, including eastern Britain. Both species also occur in disturbed habitats (roadsides, hedges, old walls, and buildings) and are cultivated in Europe as phenotypically-modified ornamentals (typically derived as sports on vegetatively propagated material) as well as wild-type specimens (Rose 1996; Sulgrove 1984).

Naturalized *H. hibernica* is probably derived from *H. hibernica* 'Hibernica,' a natural variant of the species in the UK that is widely planted in continental Europe and North America. *Hedera hibernica* 'Hibernica' has somewhat larger and more shallowly-lobed leaves than is typically observed in wild populations (McAllister and Rutherford 1983, 1990). We are unaware of molecular markers that identify *H. hibernica* 'Hibernica,' however, and the leaf features thought to be characteristic of this cultivar are affected by plant age and growth environment (A. Green, T. Ramsey, and J. Ramsey, unpubl. data). Population studies are needed to evaluate *H. hibernica* in its native range to better understand the species' phenotypic and genetic variability and evolutionary relationship to *H. helix* subsp. *helix*.

More than five percent of the sampled North American ivies had cpDNA haplotypes similar to *H. helix* subsp. *caucasigena* (Table 3). This species occurs in low- to mid-elevation forests across Turkey and the Caucasus, and is often lumped with *H. helix* subsp. *helix* into a broadly defined *H. helix* sensu lato (Rose 1996; Sulgrove 1984). *Hedera helix* subsp. *caucasigena* occurs in disturbed areas and is cultivated locally though not to the extent of *H. helix* subsp. *helix* and *H. hibernica* (Rutherford et al. 1993; Shishkin 1973).

We recovered cpDNA haplotypes identical to tetraploid *H. algeriensis* from invasive ivy populations in southern and central California (Table 3; Appendix). This species is native to coastal woodlands and rocky areas of Algeria (Green et al. 2011; Rutherford et al. 1993). Although *H. algeriensis* is endemic to a small geographic region, the species was introduced to the horticultural trade as early as 1832 (Rose 1996). *Hedera algeriensis* is now cultivated, and sometimes naturalized, throughout France, Spain, and the UK, where for many years it was mistakenly labeled as *H. canariensis* (Rutherford et al. 1993). Sometimes referred to as "Algerian ivy," *H. algeriensis* has large, glossy leaves and is reputed to be more drought tolerant than European species like *H. helix* subsp. *helix* and *H. hibernica* (Rose 1996).

Hedera taxa of Macaronesia (*H. azorica*, *H. canariensis*, *H. maderensis*), the western Mediterranean (*H. helix* subsp. *rhizomatiferra*, *H. iberica*, *H. maroccana*), and Asia (*H. nepalensis*, *H. pastuchovii*, *H. rhombea*) were not recovered from North American invasive populations (Table 3). These taxa have only recently entered the nursery trade (McAllister and Rutherford 1983; Rose 1996; Rutherford et al. 1993) and probably have had few opportunities to establish naturalized populations. Moreover, as residents of warm climates, some of these species may not be physiologically adapted to invade north temperate forest habitats.

Flow cytometry and species identification

Flow cytometry enables rapid and repeatable determination of DNA content (Kron et al. 2007). Because

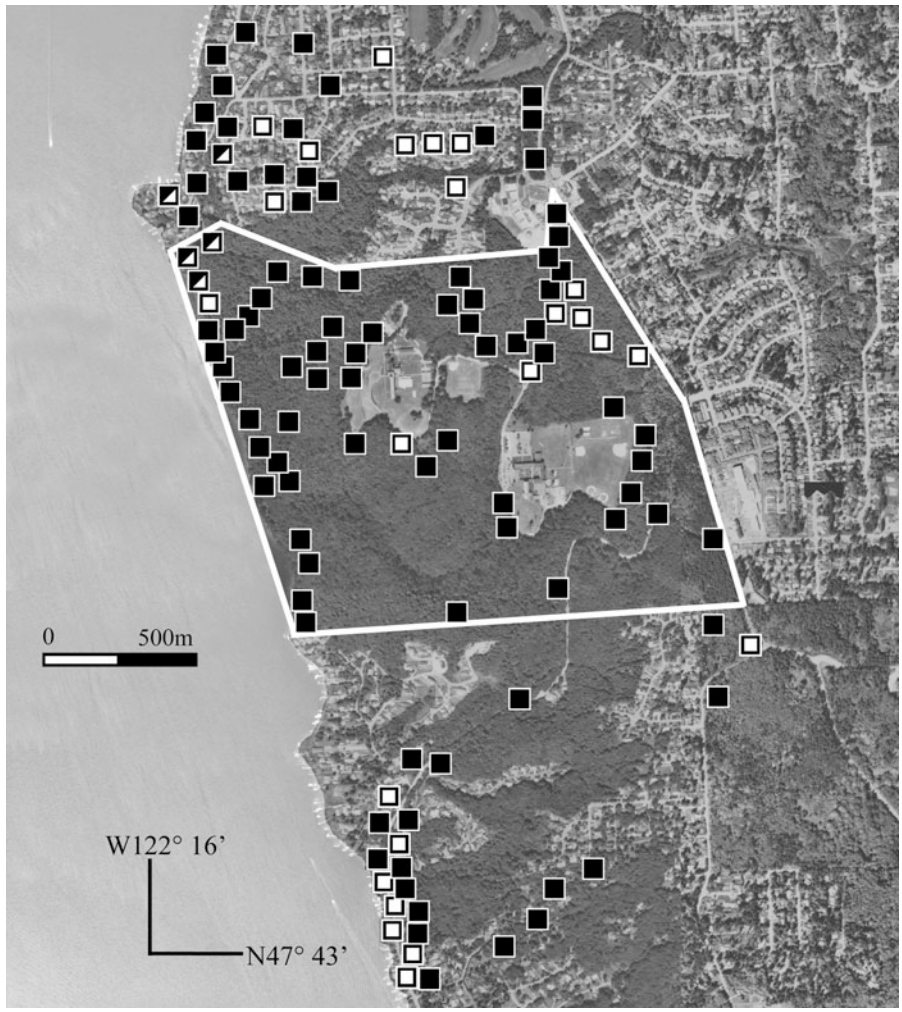

Fig. 5 Spatial distribution of diploid *H. helix* (*white boxes*), tetraploid *H. hibernica* (*black boxes*), and triploids (*half-filled boxes*) in St. Edward State Park, Washington state. Park boundaries are shown by *white lines*

closely-related taxa frequently differ in chromosome number, flow cytometry is well-suited for identifying species and hybrids in cases where morphological differences are subtle or non-existent (Consaul et al. 2008; Mahelka et al. 2005; Ramsden et al. 2006). Naturalized ivy populations are potentially comprised of more species than would co-occur in Europe or Asia, and contain cultivars in addition to wild-type plants. Flow cytometry may provide the most straightforward method to distinguish heteroploid species in these circumstances.

Early studies of genome size inferred striking differences between juvenile and adult tissues of *Hedera* (Kessler and Reches 1977; Schäffner and Nagl 1979). Our work corroborates more recent investigations—based on Fuelgen-staining and flow cytometry—that found similar DNA content between juvenile and adult tissues and a haploid genome size of ~1.50 pg (König et al. 1987; Obermayer and Greilhuber 2000). Moreover, we find a close relationship between DNA content and species ploidy level (Figs. 1, 2). Intraspecific variation was nonetheless evident for several taxa. Reference samples of *H. helix* sensu latu ranged from 2.91 to 3.15 pg, for example, while field-sampled specimens ranged from 2.24 to 3.42 pg. Such variability may reflect incidence of satellite chromosomes in ivy species (Jacobsen 1954) or interference of secondary plant metabolites in DNA staining (Greilhuber et al. 2007; Kron et al. 2007).

High-ploidy species were found to have somewhat less DNA content than would be expected from values

Table 4 Statistical analyses comparing growth and architecture of diploid *H. helix* and tetraploid *H. hibernica* grown in a common garden experiment

Comparison	Result	Effect test	*F*-statistic	*P*
Leaf no.	ANOVA, $F_{14,381} = 90.757$, $P < 0.0001$	Ploidy	$F_{1,381} = 114.400$	<0.0001
		Population [ploidy]	$F_{11,381} = 5.321$	<0.0001
		Year	$F_{1,381} = 993.849$	<0.0001
		Ploidy × year	$F_{1,381} = 0.3730$	=0.5418
Average leaf area	ANOVA, $F_{14,381} = 47.775$, $P < 0.0001$	Ploidy	$F_{1,381} = 433.875$	<0.0001
		Population [ploidy]	$F_{11,381} = 5.954$	<0.0001
		Year	$F_{1,381} = 107.685$	<0.0001
		Ploidy × year	$F_{1,381} = 0.079$	=0.7784
Branch no.	ANOVA, $F_{14,381} = 85.296$, $P < 0.0001$	Ploidy	$F_{1,381} = 52.338$	<0.0001
		Population [ploidy]	$F_{11,381} = 2.597$	=0.0034
		Year	$F_{1,381} = 1026.113$	<0.0001
		Ploidy × year	$F_{1,381} = 0.001$	=0.9753
Stem density	ANOVA, $F_{14,381} = 16.879$, $P < 0.0001$	Ploidy	$F_{1,381} = 148.382$	<0.0001
		Population [ploidy]	$F_{11,381} = 2.005$	=0.0269
		Year	$F_{1,381} = 41.646$	<0.0001
		Ploidy × year	$F_{1,381} = 0.334$	=0.5637

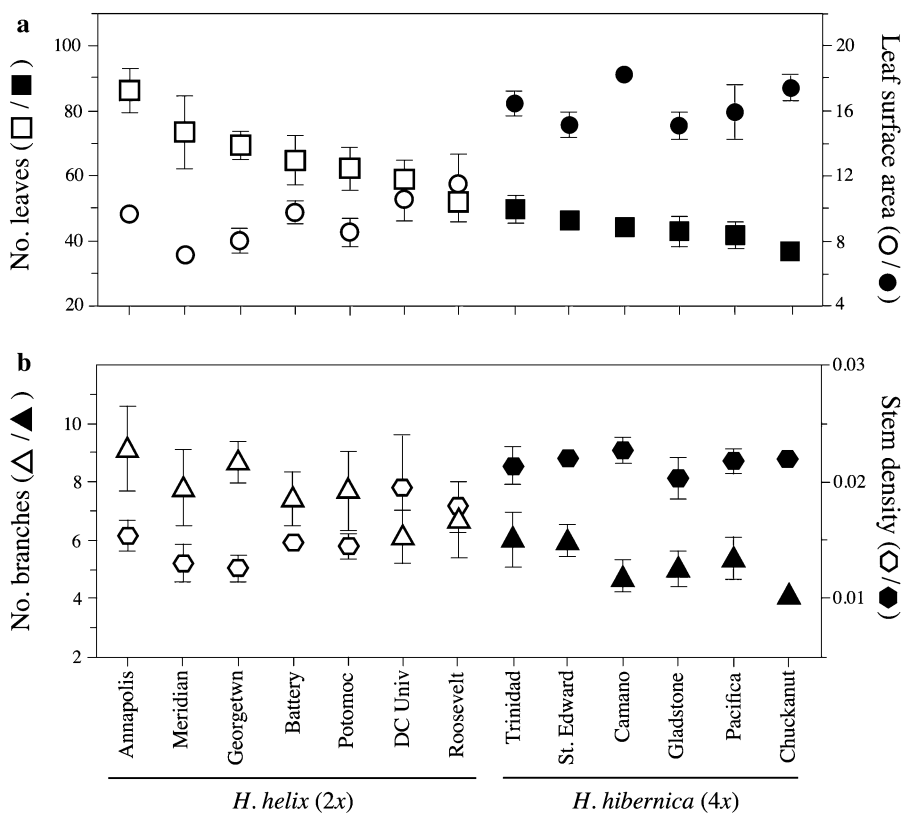

Fig. 6 Growth and architectural traits of diploid *H. helix* (*open icons*) and tetraploid *H. hibernica* (*filled icons*) in the second year of the garden experiment (mean + SE). **a** Foliage characteristics (leaf number, average leaf surface area in cm^2). **b** Stem characteristics (stem branching, stem density in g/cm)

of low-ploidy species (Fig. 1). A decrease in DNA content is sometimes observed in plant species of high chromosome number, a phenomenon attributed to homologous recombination and elimination of specific DNA sequences (Leitch and Bennett 2004). Our genome size estimates for hexaploid and octoploid *Hedera* should be treated with caution, however, due to possible non-linearity of measurements. The internal standard used in this study is targeted towards analysis of diploid and tetraploid species.

Polyploidy and invasion

Recent ecological and phylogeographic studies suggest that polyploid lineages may be predisposed to biological invasion (Amsellem et al. 2001; Barrett and Richardson 1986; Broz et al. 2009; Czarnecki 2010; but see Buggs and Pannell 2007). Giant goldenrod (*Solidago gigantea*) is native to the U.S. but invasive in Europe and Asia. North American populations harbor diploid and tetraploid cytotypes yet Eurasian populations are exclusively tetraploid (Schlaepfer et al. 2008; Schlaepfer 2010). Spotted knapweed (*Centaurea maculosa*) exhibits a similar pattern. While European populations are comprised of approximately equal proportions of diploid and tetraploid cytotypes, invasive North American populations are almost exclusively tetraploid (Treier et al. 2009). Tetraploid *C. maculosa* occurs in drier environments than diploid *C. maculosa*, and is more often polycarpic—successful invasion of tetraploids may thus relate to intrinsic physiological and life-history characteristics of this cytotype (Treier et al. 2009).

In our common garden experiments, we found substantial differences in the growth and architecture of diploid *H. helix* versus tetraploid *H. hibernica*. The robust growth form of *H. hibernica* is stereotypical of recently-derived polyploids (Ramsey and Schemske 1998, 2002) and may reflect increased DNA content and cell size. We hypothesize that the prevalence of *H. helix* versus *H. hibernica* on the east and west coasts of North America reflects ecological adaptation of the species to contrasting climate conditions in their native range (directional ecological filtering; Alexander et al. 2011). While *H. helix* is widespread across inland and high elevation areas of northern and central Europe, *H. hibernica* occurs primarily in coastal habitats of the United Kingdom, France, and Spain (McAllister and Rutherford 1990; Sulgrove 1984; Valcárcel et al. 2003). Lowland areas of western Washington, Oregon, and California have a maritime climate (warm winters, cool summers; Rumney 1968) that may favor tetraploid *H. hibernica*. In contrast, the U.S. east coast—which experiences colder winters and warmer summers because of prevailing west-to-east weather movements—may favor diploid *H. helix*.

The aforementioned scenario is consistent with the observed size and reproductive characteristics of diploid *H. helix* versus tetraploid *H. hibernica* in the Pacific Northwest, a region dominated by the latter species. For example, most of the ivy plants encountered in natural forest habitat (St. Edward State Park) were tetraploid. Juvenile patches of *H. hibernica* had six times more groundcover than patches of *H. helix*, and the vast majority of reproductive plants were *H. hibernica*. There are alternate explanations, however, for the apparent "aggressiveness" of tetraploids on the U.S. west coast and diploids on the U.S. east coast. For example, there may be historical differences in the use of *H. helix* and *H. hibernica* in landscaping, leading to chance differences in the timing of population establishment. Moreover, minority cytotype exclusion (frequency-dependent selection in mixed cytotype populations; Levin 1975) is expected to homogenize the ploidy composition of established populations. It is possible that diploid *H. helix* and tetraploid *H. hibernica* differ in dispersal ability or have alternate rates of ecological adaptation due to their intrinsic genetic characteristics (modes of inheritance, incidence of epigenetic change, etc.). Field experiments are needed to tease apart the factors underlying geographic patterns of cytotype invasion in *Hedera* and other plant genera (te Beest et al. 2012).

Triploid *Hedera*

Triploids are unknown from natural ivy populations in Europe, Asia, and northern Africa (McAllister and Rutherford 1990; Rose 1996; Sulgrove 1984). In recent surveys of the Netherlands, United Kingdom, Belgium, France, Spain, Germany, and Italy we found only diploids and tetraploids among 370 sampled plants in 41 populations (T. Ramsey, A. Green, and J. Ramsey, unpubl. data). Among cultivated ivies, triploidy is known from a single clone (*H. helix* 'Woerneri') that is not widely planted (Rose 1996).

Surprisingly, we found a number of plants with DNA content values intermediate to those of diploid

H. helix and tetraploid *H. hibernica* in North American forests. Chromosome counts made in root tips of three of these plants have confirmed a triploid chromosome number ($2n = 3x = 72$) (T. Ramsey & H. McAllister, unpubl. data). Determination of the origin of triploid ivy is complicated by the low levels of DNA sequence divergence that exist between *Hedera* species (Green et al. 2011). The most likely origin involves spontaneous hybridization between diploid *H. helix* and tetraploid *H. hibernica*, which sometimes occur sympatrically in naturalized populations. Many triploids were found in sites containing both parental species (Table 5 in Appendix). Moreover, morphological features of triploids are generally intermediate to that of *H. helix* and *H. hibernica* (A. Green, T. Ramsey, and J. Ramsey, unpubl. data). The growth and reproductive traits of triploid ivy warrant further study because the material may have low fertility, and thus useful for introduction of "non-invasive" cultivars to horticulture (but see Husband 2004). Triploids may also mediate gene flow between diploid *H. helix* and tetraploid *H. hibernica*, compromising the integrity of the species in their introduced range. We identified several plants with DNA content values intermediate to that expected for triploid and tetraploid *Hedera* (Fig. 2). These individuals could represent aneuploids derived from triploid crosses, but also aberrant tetraploid samples in which secondary metabolites interfered with DNA staining.

Conclusions

Floristic studies have frequently associated polyploidy with weedy and invasive tendencies, while recent population-level studies of knapweeds, goldenrods, and other polyploid complexes have found naturalized populations to be comprised primarily or exclusively of polyploid cytotypes. In contrast, diploid *H. helix* and tetraploid *H. hibernica* are both common invaders in North America but are spatially segregated in a manner reminiscent of their ecogeographic distributions in Europe. We hypothesize that polyploidy has played an indirect role in structuring ivy invasion of North American forests, by creating and/or maintaining ecological differences among closely-related ivy species in the native range: through the process of directional ecological filtering, diploids and tetraploids have come to occupy climatic conditions most similar to their native environs. Sympatric occurrences of diploid *H. helix* and tetraploid *H. hibernica* are nonetheless frequent in parts of the U.S., leading to the formation of triploid F1 hybrids that are unknown from natural populations in Europe. Future studies of invasive ivies will focus on garden and field experiments that test the physiological attributes and growth performance of diploids and tetraploids in alternate climate conditions.

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Appendix

See Table 5.

Table 5 Collection information for naturalized ivy populations

Population	State	County	GPS coordinates	Accession no.	Ploidy (no. samples)	Haplotype	El. (m)
Wildcat Canyon	CA	Contra Costa	N37°57′ W122° 19’	CA07-Y	3*x* (1), 4*x* (6)	*a*	55
Pelican Bay	CA	Del Norte	N41°51′ W124°08′	CA07-NN	2*x* (1), 4*x* (2)	*a*	40
Eureka-Sequoia	CA	Humboldt	N40°46′ W124°08′	CA07-JJ	3*x* (1), 4*x* (9)	*a*	50
Garberville	CA	Humboldt	N40°06′ W123°47′	CA07-HH	4*x* (2)	*a*	180
Philipsville	CA	Humboldt	N40°13′ W123°48′	CA07-II	2*x* (1)	–	95
Pikes Point	CA	Humboldt	N41°08′ W124°09′	CA07-LL	4*x* (4)	*a*	50
Trinidad	CA	Humboldt	N41°03′ W124°08′	CA07-KK	3*x* (1), 4*x* (4)	*a*	55
Griffith	CA	Los Angeles	N34°06′ W118 18′	CA07-A	2*x* (3), 4*x* (5)	*d*	185
Rose Hill	CA	Los Angeles	N34°05′ W118 12′	CA07-B	2*x* (1), 4*x* (1)	–	215
Casper	CA	Mendocino	N39°21′ W123°48′	CA07-CC	4*x* (1)	*f*	20
Litehouse	CA	Mendocino	N39°20′ W123°48′	CA07-DD	3*x* (1), 4*x* (2)	*a*	50
Mackericher	CA	Mendocino	N39°29′ W123°47′	CA07-EE	3*x* (1)	*a*	20
North Highway	CA	Mendocino	N39°43′ W123°48′	CA07-FF	4*x* (1)	–	95
Rockport	CA	Mendocino	N39°44′ W123°49′	CA07-GG	4*x* (1)	*a*	10
Cabrillo	CA	Monterey	N36°15′ W121°48′	CA07-I	2*x* (2), 4*x* (3)	*d*	65
Garrapata	CA	Monterey	N36°29′ W121°56′	CA07-J	4*x* (1)	–	25
Oxton	CA	Monterey	N36°35′ W121°51′	CA07-K	4*x* (3)	*a*	50
Pfeiffer Big Sur	CA	Monterey	N36°14′ W121°46′	CA07-H	2*x* (2)	–	170
Golden Gate	CA	San Francisco	N37°46′ W122°27′	CA07-V	4*x* (10)	*a*	75
Cambria	CA	San Luis Obispo	N35°33′ W121°04′	CA07-G	2*x* (2), 4*x* (1)	*b*	115
Morro Bay	CA	San Luis Obispo	N35°22′ W120 50′	CA07-F	4*x* (1)	*d*	15
Alpine Road	CA	San Mateo	N37°17′ W122°14′	CA07-S	4*x* (1)	–	195

Table 5 continued

Population	State	County	GPS coordinates	Accession no.	Ploidy (no. samples)	Haplotype	El. (m)
Pescadero	CA	San Mateo	N37°17′ W122°15′	CA07-T	4*x* (1)	–	140
Sam McDonald	CA	San Mateo	N37°17′ W122°15′	CA07-U	2*x* (3), 4*x* (1)	*b*	190
San Bruno	CA	San Mateo	N37°41′ W122°22′	CA07-W	2*x* (3), 4*x* (5)	*a*	210
Pacifica	CA	San Mateo	N37°37′ W122°28′	CA07-X	2*x* (1), 3*x* (2), 4*x* (3)	*a*	220
Los Padres	CA	Santa Barbara	N34°25′ W119°34′	CA07-E	2*x* (3)	*b*	30
Big Basin Way	CA	Santa Cruz	N37°08′ W122°08′	CA07-R	2*x* (2), 4*x* (2)	*b*	240
Felton	CA	Santa Cruz	N37°04′ W122°05′	CA07-P	2*x* (1)	–	85
Golf Club	CA	Santa Cruz	N36°59′ W122°02′	CA07-M	2*x* (2), 4*x* (4)	*d*	20
Henry Cowel	CA	Santa Cruz	N37°01′ W122°03′	CA07-O	2*x* (2)	*b*	100
North Escape	CA	Santa Cruz	N37°10′ W122°13′	CA07-Q	4*x* (1)	*a*	140
River	CA	Santa Cruz	N36°59′ W122°02′	CA07-N	4*x* (5)	*d*	10
Santa Cruz	CA	Santa Cruz	N36°58′ W122°02′	CA07-L	4*x* (1)	–	20
Benicia	CA	Solano	N38°04′ W122°11′	CA07-Z	4*x* (1)	–	5
Healdsburg	CA	Sonoma	N38°36′ W122°52′	CA07-BB	4*x* (2)	*d*	30
Santa Rosa	CA	Sonoma	N38°27′ W122°43′	CA07-AA	3*x* (2), 4*x* (1)	*d*	45
Potomac	DC	Arlington	N38°54′ W077°06′	DC06-A	2*x* (11)	*c*	70
Battery-Kemble	DC	District of Columbia	N38°55′ W077°05′	DC06-D	2*x* (5)	*a*	65
Georgetown	DC	District of Columbia	N38°54′ W077°04′	DC06-E	2*x* (9)	*b*	30
Meridan	DC	District of Columbia	N38°55′ W077°02′	DC06-G	2*x* (7)	*a*	55
Rock Creek	DC	District of Columbia	N38°58′ W077°02′	DC06-B	2*x* (2)	*b*	60
Roosevelt	DC	District of Columbia	N38°53′ W077°03′	DC06-F	2*x* (12)	*a*	5
Univ. of DC	DC	District of Columbia	N38°56′ W077°03′	DC06-C	2*x* (5)	*a*	80

Table 5 continued

Population	State	County	GPS coordinates	Accession no.	Ploidy (no. samples)	Haplotype	El. (m)
Macon	GA	Bibb	N32°51′ W083°38′	GA06-A	2*x* (5), 4*x* (7)	*a*	120
Jackson	GA	Butts	N33°17′ W083°57′	GA06-B	4*x* (2)	*a*	210
Decatur	GA	DeKalb	N33°40′ W084°11′	GAO6-D	2*x* (6), 4*x* (4)	*b*	260
Atlanta	GA	Fulton	N33°45′ W084°23′	GA06-C	2*x* (2), 4*x* (4)	*e*	300
Sandy Point	MD	Anne Arundel	N39°00′ W076°23′	MD06-B	2*x* (4)	*a*	0
Annapolis	MD	Anne Arundel	N38°58′ W076°29′	MD06-C	2*x* (12), 3*x* (2)	*c*	5
Baltimore	MD	Baltimore	N39°16′ W076°36′	MD06-A	2*x* (6)	*c*	5
Brunswick	MD	Frederick	N39°18′ W077°37′	MD06-E	2*x* (6)	*c*	70
Winston-Salem	NC	Forsyth	N36°06′ W080°15′	NC06-G	4*x* (3)	*a*	275
Greenfield	NC	New Hanover	N34°12′ W077°56′	NC06-B	4*x* (2)	*a*	0
Wilmington	NC	New Hanover	N34°14′ W077°57′	NC06-A	2*x* (4)	*a*	0
Jacksonville	NC	Onslow	N34°45′ W077°25′	NCO6-C	2*x* (4)	*b*	0
Eno River	NC	Orange	N36°04′ W079 00′	NC06-F	2*x* (4)	*b*	140
Raleigh	NC	Wake	N35°46′ W078°38′	NC06-D	2*x* (2)	*a*	100
Wake	NC	Wake	N35°47′ W078°39′	NC06-E	2*x* (11)	*b*	100
Camden	NJ	Camden	N39°56′ W075°07′	NJ06-B	4*x* (3)	*a*	5
Cape May	NJ	Cape May	N38°56′ W074 57′	NJ06-A	4*x* (4)	*a*	0
Central Park	NY	New York	N40°46′ W073°58′	NY06-B	2*x* (2)	*c*	20
Riverhead	NY	Suffolk	N40°56′ W072°37′	NY06-A	4*x* (3)	*a*	10
Corvallis	OR	Benton	N44°33′ W123°16′	OR07-O	2*x* (3), 4*x* (2)	*a*	70
McDonald	OR	Benton	N44°39′ W123°13′	OR07-P	4*x* (1)	–	105
Gladstone	OR	Clackamas	N45°22′ W122°37′	OR06-H	2*x* (1), 3*x* (1), 4*x* (6)	*a*	50

Table 5 continued

Population	State	County	GPS coordinates	Accession no.	Ploidy (no. samples)	Haplotype	El. (m)
Wilsonville	OR	Clackamas	N45°17′ W122°45′	OR07-T	2*x* (3), 4*x* (2)	*b*	30
Astoria	OR	Clatsop	N46°10′ W123°50′	OR06-A	4*x* (4)	*a*	80
Knappa	OR	Clatsop	N46°10′ W123°35′	OR06-B	3*x* (1)	–	40
St. Helens	OR	Columbia	N45°51′ W122°49′	OR06-D	2*x* (7), 4*x* (3)	*a*	40
Bandon	OR	Coos	N43°07′ W124°24′	OR07-F	4*x* (2)	*a*	5
Conde B McCollough	OR	Coos	N43°26′ W124°12′	OR07-H	4*x* (1)	*a*	15
Coos Bay	OR	Coos	N43°22′ W124°13′	OR07-G	4*x* (7)	*a*	10
Prescott	OR	Cowlitz	N46°02′ W122°53′	OR06-C	4*x* (1)	*a*	10
Alfred A Loeb	OR	Curry	N42°04′ W124°12′	OR07-MM	2*x* (1), 8*x* (1)	–	15
Brookings	OR	Curry	N42°03′ W124°16′	OR07-A	2*x* (3), 4*x* (2)	*b*	65
Port Orford	OR	Curry	N42°44′ W124°29′	OR07-E	4*x* (2)	*a*	25
Canyonville	OR	Douglas	N42°55′ W123°16′	OR07-C	2*x* (1), 3*x* (1), 4*x* (1)	*a*	230
Riverfront	OR	Douglas	N43°13′ W123°22′	OR07-D	2*x* (2), 4*x* (3)	*a*	130
Grants Pass	OR	Josephine	N42°26′ W123°19′	OR07-B	2*x* (2)	–	295
Eugene	OR	Lane	N44°01′ W123°07′	OR07-R	2*x* (1)	–	240
Florence	OR	Lane	N43°58′ W124°07′	OR07-J	4*x* (1)	–	5
Woahink Lake	OR	Lane	N43°55′ W124°05′	OR07-I	4*x* (1)	–	15
Cape Perpetula	OR	Lincoln	N44°17′ W124°06′	OR07-K	4*x* (2)	*a*	45
Fogarty Creek	OR	Lincoln	N44°50′ W124°02′	OR07-N	4*x* (4)	*a*	5
Ona Beach	OR	Lincoln	N44°31′ W124°04′	OR07-M	2*x* (1)	–	5
Waldport	OR	Lincoln	N44°25′ W124°03′	OR07-L	2*x* (2)	–	5
Salem	OR	Marion	N44°55′ W123°02′	OR07-Q	2*x* (2), 3*x* (2)	*b*	50

Table 5 continued

Population	State	County	GPS coordinates	Accession no.	Ploidy (no. samples)	Haplotype	El. (m)
Woodburn	OR	Marion	N45°08′ W122°50′	OR07-S	4*x* (2)	–	60
Bridal Veil	OR	Multnomah	N45°33′ W122°10′	OR06-J	2*x* (1), 4*x* (10)	*a*	40
Guy Talbot	OR	Multnomah	N45°32′ W122°13′	OR06-K	4*x* (3)	–	30
Portland	OR	Multnomah	N45°37′ W122°48′	OR06-E	4*x* (3)	*a*	20
Saltzman	OR	Multnomah	N45°33′ W122°45′	OR06-F	4*x* (12)	*a*	95
Troutdale	OR	Multnomah	N45°30′ W122°21′	OR06-I	4*x* (1)	–	20
Tyron Creek	OR	Multnomah	N45°26′ W122°40′	OR06-G	4*x* (5)	*a*	80
Orangeburg	SC	Orangeburg	N33°29′ W080°52′	SC06-C	2*x* (3)	*a*	45
Columbia	SC	Richland	N34°01′ W081 02′	SC06-A	2*x* (9)	*c*	90
Dentsville	SC	Richland	N34°05′ W080°54′	SC06-B	2*x* (9)	*a*	75
Danville	VA	Danville	N36°33′ W079 27′	VA06-A	4*x* (6)	*a*	190
Richmond	VA	Henrico	N37°31′ W077°24′	VA06-B	2*x* (6), 4*x* (1)	*b*	30
Aberdeen	WA	Greys Harbor	N46°59′ W123°48′	WA06-T	4*x* (2)	*a*	10
Quinault Loop	WA	Greys Harbor	N47°27′ W123°51′	WA06-S	4*x* (1)	*a*	60
Carkeek	WA	King	N47°42′ W122°22′	WA06-CC	2*x* (8), 4*x* (2)	*a*	45
Discovery	WA	King	N47°39′ W122°24′	WA06-B	2*x* (2), 3*x* (2), 4*x* (3)	*a*	45
Lincoln	WA	King	N47°32′ W122°23′	WA06-A	2*x* (1), 3*x* (1), 4x (10)	*a*	50
Nolte	WA	King	N47°16′ W121°56′	WA06-I	2*x* (1), 4*x* (5)	*a*	240
Ravenna	WA	King	N47°40′ W122°18′	WA06-DD	4*x* (6)	*a*	40
St. Edward	WA	King	N47°43′ W122°15′	WA04-EE	2*x* (4), 3*x* (2), 4*x* (6)	*a*	100
Fay-Bainbridge	WA	Kitsap	N47°42′ W122°30′	WA06-R	4*x* (5)	*a*	20
Fort Ward	WA	Kitsap	N47°35′ W122°31′	WA06-Q	4*x* (9)	*a*	45

Table 5 continued

Population	State	County	GPS coordinates	Accession no.	Ploidy (no. samples)	Haplotype	El. (m)
Kitsap	WA	Kitsap	N47°49′ W122°39′	WA06-P	4*x* (6)	*a*	10
Beard's Hollow	WA	Pacific	N46°18′ W124°03′	WA06-W	4*x* (4)	*a*	15
Fort Columbia	WA	Pacific	N46°15′ W123°55′	WA06-X	4*x* (2)	–	35
Omerara	WA	Pacific	N46°20′ W123°57′	WA06-V	4*x* (4)	*a*	25
Raymond	WA	Pacific	N46°40′ W123°44′	WA06-U	4*x* (4)	*a*	5
Point Defiance	WA	Pierce	N47°18′ W122°31′	WA06-BB	4*x* (2)	*a*	30
Chuckanut	WA	Skagit	N48°37′ W122°26′	WA06-E	3*x* (2), 4*x* (4)	*a*	35
Hillcrest	WA	Skagit	N48°24′ W122°19′	WA06-L	2*x* (1), 4*x* (5)	*a*	50
Camano	WA	Snohomish	N48°07′ W122°29′	WA06-D	3*x* (1), 4*x* (3)	*a*	80
Forest Park	WA	Snohomish	N47°57′ W122°13′	WA06-M	4*x* (5)	*a*	100
Index	WA	Snohomish	N47°49′ W121°33′	WA06-H	4*x* (6)	*a*	160
Meadowdale	WA	Snohomish	N47°51′ W122°18′	WA06-N	4*x* (1)	–	125
Wallace Falls	WA	Snohomish	N47°52′ W121°40′	WA06-G	4*x* (2)	*a*	110
Wenberg	WA	Snohomish	N48°08′ W122°17′	WA06-C	2*x* (6), 3*x* (2), 4*x* (3)	*a*	125
Yost	WA	Snohomish	N47°48′ W122°21′	WA06-O	4*x* (4)	*a*	90
Olympia	WA	Thurston	N47°02′ W122°53′	WA06-Y	4*x* (1)	–	10
Priest Point	WA	Thurston	N47°04′ W122°53′	WA06-AA	4*x* (7)	*a*	0
Watershed	WA	Thurston	N47°02′ W122°53′	WA06-Z	4*x* (5)	*a*	20
Cornwall	WA	Whatcom	N48°46′ W122°28′	WA06-J	2*x* (1), 4*x* (4)	*a*	40
Lake Samish	WA	Whatcom	N48°40′ W122°24′	WA06-K	4*x* (1)	–	85
Larrabee	WA	Whatcom	N48°38′ W122°29′	WA06-F	4*x* (2)	*a*	60

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