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PHRAGMITES INVASION

Do ploidy level and nuclear genome size and latitude of origin modify the expression of *Phragmites australis* traits and interactions with herbivores?

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Abstract We studied the relationship between genome size and ploidy level variation and plant traits for the reed grass *Phragmites australis*. Using a common garden approach on a global collection of populations in Aarhus, Denmark, we investigated the influence of monoploid genome size and ploidy level on the expression of *P. australis* growth, nutrition and herbivore-defense traits and whether monoploid genome size and ploidy level play different roles in plant trait expression. We found that both monoploid genome size and latitude of origin contributed to variation in traits that we studied for *P. australis*, with latitude of origin being generally a better predictor of trait values and that ploidy level and its interaction with monoploid genome size and latitude of origin also contributed to trait variation. We also found that for four traits, tetraploids and octoploids had different relationships with the monoploid genome size. While for tetraploids stem height and leaf water content showed a positive relationship with monoploid genome size, octoploids had a negative relationship with monoploid genome size for stem height and no relationship for leaf water content. As genome size within octoploids increased, the number of aphids colonizing leaves decreased, whereas for tetraploids

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there was a quadratic, though non-significant, relationship. Generally we found that tetraploids were taller, chemically better defended, had a greater number of stems, higher leaf water content, and supported more aphids than octoploids. Our results suggest trade-offs among plant traits mediated by genome size and ploidy with respect to fitness and defense. We also found that the latitude of plant origin is a significant determinant of trait expression suggesting local adaptation. Global climate change may favor some genome size and ploidy variants that can tolerate stressful environments due to greater phenotypic plasticity and to fitness traits that vary with cytotype which may lead to changes in population genome sizes and/or ploidy structure, particularly at species' range limits.

Keywords Cytotype · Global climate change · Latitude of origin · Nuclear genome size · Plant defense · Plant invasion · Polyploidy

Introduction

Identifying traits that facilitate the success of invasive species and predicting outcomes of the interactions of such traits with the environment continues to be a priority for invasion ecologists (Küster et al. 2008; Pyšek et al. 2009, 2015). Over the last two decades it has become clear that no single trait can account for the invasion success of a species (Thébault et al. 2011) and invasion success can also vary over biogeographic space further complicating the identification of key invasive traits (Cronin et al. 2015).

Studies searching for determinants of invasiveness rarely consider underlying genetic factors such as genetic polymorphism, heterozygosity, and karyological factors, such as nuclear genome size, number of somatic chromosomes and ploidy level—all of which may differentially modify plant traits and interact with each other and environmental factors (e.g., climate) to affect trait expression (te Beest et al. 2011; Suda et al. 2015). Understanding how karyological variation (genome size and ploidy) influences plant traits across a range of environments is of particular interest for researchers seeking to improve predictive capacities in species invasions and range expansions (Suda et al. 2015). Moreover, studies have increasingly identified the role of intraspecific variation in contributing to the ecological breadth of a species and its ability to adapt to changing environments (Albert et al. 2010; Sides et al. 2014). Greater intraspecific functional variability is predicted to allow plant populations to adjust to a wider range of competitive and abiotic conditions thereby conferring a broader niche (Sides et al. 2014). Some evidence indicates that within-species genetic variation mirrors interspecific diversity (e.g. Hughes and Stachowicz 2004; Stachowicz et al. 2007; Cardinale et al. 2012) and that intraspecific variation, including karyological diversity, may drive community structure and ecosystem processes (Levin 2002; Reusch and Hughes 2006; Johnson et al. 2009; Crawford and Rudgers 2013) via plant functional traits (Lavorel et al. 2007; Hull-Sanders et al. 2009). Intraspecific functional variation may be important at small and intermediate scales due to environmental filtering and high interspecific variability at the global scale (Albert et al. 2010), and because adaptation to change plays out at the population and subpopulation scales (e.g., microevolutionary scale; Balao et al. 2011).

Both genome size and ploidy level have previously been implicated in invasion success (e.g. Kubešová et al. 2010; te Beest et al. 2011; Suda et al. 2015) but rarely considered simultaneously (but see Balao et al. 2011), even though including both in explanatory models may greatly increase their power to predict invasiveness (Pandit et al. 2014). For example, polyploidy can facilitate invasion success by "pre-adapting" species to conditions in the introduced range relative to diploids, and the associated higher genetic diversity in polyploids may enhance invasiveness (Henery et al. 2010; te Beest et al. 2011) whereas smaller holoploid (C-value) and monoploid (Cx-value) genome sizes (sensu Greilhuber et al. 2005) often contribute to faster growth rates (e.g. Lavergne et al. 2010; Fridley and Craddock 2015). Holoploid genome size is the amount of DNA in the whole chromosome complement of the nucleus with a chromosome number n, irrespective of ploidy number whereas monoploid genome size is the amount of DNA in one chromosome set of an organism (Suda et al. 2015).

Currently, little is also known about whether trade-offs exist between intraspecific cytotypes with respect to plant functional traits (but see Thébault et al. 2011; Hao et al. 2013), including plant defense against herbivores and pathogens, and most work in this direction has examined differences between diploids and higher ploidy levels. Data on enemy impact and

plant defenses in relation to ploidy level and genome size is sparse (Janz and Thompson 2002; Münzbergová 2006; Halverson et al. 2008; Hull-Sanders et al. 2009; Arvanitis et al. 2010; Hahn and Dornbush 2012) even though herbivores and pathogens play an important role in invasion success (e.g. Keane and Crawley 2002; Mitchell and Power 2003; Fagan et al. 2005; Mitchell et al. 2010). One example involves *Cardamine pratensis* where octoploids exhibited greater tolerance to herbivory than tetraploids (Boalt et al. 2010). Importantly, a gall-forming midge only attacked the octoploid suggesting that changes in ploidy can lead to host shifts in herbivores (Arvanitis et al. 2010). In other species, different cytotypes can suffer higher or lower levels of attack depending on both the plant and herbivore or pathogen (Halverson et al. 2008; Thompson and Merg 2008; Collins et al. 2013).

To gain greater insight into the roles of genome size and ploidy level variation in an invasive plant, we studied plant traits for the reed grass *Phragmites australis*—one of the best-studied plant species globally (Hulme et al. 2013; Meyerson et al. 2016). This topic is of particular interest because of the important habitat, including ecosystem services, reed grass provides across its native range in Europe and Asia, and its status as a highly invasive species in North America (Chambers et al. 1999; Kiviat 2013; Packer et al. 2016). *P. australis* is an ideal model species to investigate the interactions of genome size, ploidy level and plant traits because of its global distribution, high genetic and genomic diversity, and habitat breadth, ranging from brackish to freshwater systems, temperate to tropical, coastal to inland to high elevation (Meyerson et al. 2016). Using a common garden approach on a global collection of populations, we investigated (1) the influence of monoploid genome size and ploidy level on the expression of *P. australis* growth, nutrition herbivore-defense traits, and palatability to aphids, (2) and whether monoploid genome size and ploidy level play different roles in plant trait expression.

Methods

Plant material

We used the living collection of *P. australis* from a common garden at Aarhus University, Denmark (56°81′30″N; 10°80′70″E) during the summer of 2012 to examine the relationship between plant traits, genome size and ploidy (see "Appendix"), and herbivory. *P. australis* is a cosmopolitan species within Poaceae and is adapted to wide climatic and latitudinal ranges (±60°), including extreme environments. It exhibits a high genetic diversity (Saltonstall 2002), intra- and interspecific hybridization (Meyerson et al. 2010, 2012; Lambertini et al. 2012) and variation in ploidy (Clevering and Lissner 1999; Keller 2000; Lambertini et al. 2006). The species has globally distributed diverse cytotypes ($4x - 12x$, based on $x = 12$) and GS variability up to 22 % within cytotypes (Suda et al. 2015).

One hundred and sixty-six clones were planted in the garden in 2001. A decade passed prior to the start of this study, allowing plenty of time for the plants to acclimate to the Danish climate and for maternal environmental effects to have been virtually eliminated. All pots were maintained under identical watering (daily) and fertilization regimes (monthly) and all rhizomes were divided during spring 2012 and repotted in 60 L pots using commercial potting soil and sand mixture. Given the large genetic and genomic variation represented for *P. australis* in this common garden, the collection made it possible to address, at the intraspecific level, fundamental relationships among genetics and the environment (i.e. geography and herbivory).

Genome size estimation

Holoploid genome sizes (i.e., the DNA content of the whole chromosome complement with chromosome number n irrespective of the degree of generative polyploidy; C-value) of analyzed plants were estimated using DNA flow cytometry following the simplified two step protocol using the Otto buffers as detailed in Doležel et al. (2007). *Bellis perennis* ($2C = 3.38$ pg; Schönswetter et al. 2007) was used as an internal reference standard and the nuclei isolated from intact young leaves of actively growing plants were stained by the intercalating fluorochrome propidium iodide. Karyologically confirmed tetraploid ($2n = 4x = 48$) and octoploid ($2n = 8x = 96$) *Phragmites* samples from the living collection of the Institute of Botany, The Czech Academy of Sciences in Průhonice were used as reference points to infer DNA ploidy levels of analyzed samples. Monoploid

genome sizes (i.e., the DNA content of the monoploid chromosome set, with chromosome number x; Cx-value) were calculated as 2C-value/ploidy level.

Growth, nutritional and defense traits

We measured 10 plant traits (Table 1) related to growth (stem height and number, the latter measured as the stem number per pot), nutritional quality (% carbon, % nitrogen, C:N), herbivore defense (total phenolics, leaf toughness), ambient aphid abundance per stem, and palatability to aphids, all in the same pots. Since water content of leaves can have a positive relationship with the population growth rate of aphids (e.g. Johnson 2009; Bhattarai et al. in review), we estimated this trait as the proportion of water per unit fresh biomass of three newly opened leaves collected from each pot. For nutrient analysis (% carbon, % nitrogen, C:N ratio), the top three leaves were collected during July 2012 from a single plant per pot. Leaves were oven-dried at 70 °C and ground to a fine powder. Carbon and nitrogen were analyzed using a CE Instruments Model NC2100 elemental analyzer at Brown University Environmental Chemistry Facilities (http://www.brown.edu/Research/Evchem/facilities).

Leaf toughness and total phenolics were our measures of plant defense against herbivores. In Poaceae, defenses are likely limited to structural defenses and phenolics. Using a penetrometer (Itin Scale Co., Inc., Brooklyn, NY), toughness was measured for the fully open uppermost leaf from a randomly selected stem (force in kg to push a 4.8 mm diameter blunt steel rod through the leaf). Total phenolics (nM/g of dried leaf tissue) were estimated using a microplate modified version of the Folin–Ciocalteu method (Waterman and Mole 1994; Cronin et al. 2015).

Aphid abundance and palatability to aphids

A wide diversity of herbivores are known to feed on *P. australis* (Tewksbury et al. 2002). Within Europe and North America, the mealy plum aphid *Hyalopterus pruni* (Homoptera: Aphididae) is the most common herbivore in terms of numbers and biomass (Cronin et al. 2015). The mealy plum aphid is a Eurasian species that was introduced to North America more than a century ago (Lozier et al. 2009). These aphids overwinter on their primary hosts, various *Prunus* species, but switch to grasses like *P. australis* during the spring and summer (Lozier et al. 2009). Mealy plum aphids often achieve densities >1000 per *P. australis* stem, and outbreaks can cause the die off of all aboveground vegetation (Cronin et al. 2015). We conducted two experiments investigating herbivory by the mealy plum aphid *Hyalopterus pruni* that has *P. australis* as its summer host.

Ambient aphid abundance per stem

Background densities of aphids per source population likely reflect a combination of plant defense, nutritional quality, and positional effects (i.e., location in the garden). Historical effects (e.g., temporal autocorrelation in abundance) are unimportant because the mealy plum aphid overwinters on other hosts and then returns to the garden. To quantify ambient aphid abundance, we first counted stems and the proportion of stems that were infested with aphids. Randomly selecting three aphid-infested stems in each pot, we counted the number of leaves with aphids. One infested leaf per stem was chosen at random and the aphids were counted. Mean aphids per stem were then estimated as the proportion of stems infested per pot × mean number of infested leaves per infested stem × mean number of aphids per infested leaf.

Palatability to aphids

We performed a caging experiment to access palatability of *P. australis* populations to mealy-plum aphid (*Hyalopterus pruni, Aphididae)*. We collected aphids from a single source in a naturally occurring stand of *P. australis* within 10 km of the common garden to minimize the genetic variation among aphids in the experiment. We caged adult aphids on live *P. australis* leaves to assess the palatability of the garden populations to aphids (see Bhattarai et al. in review for details). Aphid colonies were initiated with two adult aphids caged on the youngest fully open leaf on a randomly selected stem from each pot. Aphids reproduce parthenogenetically and produce a colony in a few days. After 10 days, leaves with aphid colonies were collected, transported on ice to the laboratory, and stored in a freezer at −20 °C. With a suitable host, aphid colonies can increase in size up to 100 times in

Table 1 Model selection results based on Akaike Information Criteria corrected for finite sample size (AICc)

Trait	Model	AICc	ΔAICc	Akaike Wt
% Carbon	Cx, L, L × L, H	287.4	0.0	0.13
	P, Cx, P × Cx, L, L × L, H	287.8	0.4	0.11
	L, L × L, H	288.0	0.6	0.10
	Cx, L, Cx × L, L × L, H	289.1	1.7	0.06
	P, Cx, L, L × L, H	289.2	1.8	0.05
% Nitrogen	Cx, L	105.2	0.0	0.20
	Cx, L, H	105.6	0.5	0.16
	Cx, L, Cx × L	106.5	1.3	0.10
	P, Cx, L, H	107.0	1.8	0.08
	Cx, L, Cx × L, H	107.1	1.9	0.08
	P, Cx, L	107.2	2.0	0.07
C:N ratio	Cx, L, H	569.8	0.0	0.35
	P, Cx, L, H	571.2	1.4	0.18
	Cx, L, Cx × L, H	571.7	1.9	0.14
Total phenolics	P, H	75.6	0.0	0.21
	P, L, H	75.9	0.2	0.18
	P, L, P × L, H	76.5	0.8	0.14
	P, Cx, H	77.5	1.8	0.08
Stem height	P, Cx, L, P × Cx, P × L	−38.0	0.0	0.11
	Cx, L, H	−37.7	0.3	0.10
	P, Cx, L, P × Cx	−37.4	0.7	0.08
	L, H	−36.7	1.4	0.06
	P, Cx, P × Cx	−36.3	1.7	0.05
	Cx, H	−36.2	1.9	0.05
Stem number	Cx, L, L × L, H	290.3	0.0	0.27
	P, Cx, L, P × Cx, L × L, H	290.7	0.5	0.21
	P, Cx, L, P × Cx, P × L, L × L, H	291.9	1.6	0.12
% Water content	P, Cx, L, P × Cx, P × L, Cx × L	−321.4	0.0	0.18
	P, Cx, L, P × L, Cx × L, Cx × Cx	−321.2	0.2	0.16
	P, Cx, L, P × L, Cx × L	−320.4	1.0	0.11
	P, Cx, L, P × L, Cx × Cx	−319.7	1.6	0.08
	P, Cx, L, P × L, Cx × L, Cx × Cx, H	−319.7	1.6	0.08
Leaf toughness	Cx, H	330.2	0.0	0.27
Aphids per stem	P, Cx, L, P × Cx, P × L, Cx × Cx	5378.0	0.0	0.60
Palatability to aphids (colony mass at 10 days)	P, Cx, L, P × L, Cx × Cx, L × L, H	1721.9	0.0	0.42

Candidate models with a Δ_i value (= $AICc_i - AICc_{min}$) of ≤2 and the AICc weights (w_i) are reported

P, ploidy (4*x* or 8*x*); Cx, monoploid genome size; L, latitude of origin; H, longitude of origin

10 days but still do not compromise colony growth because of intraspecific competition or leaf deterioration (Bhattarai et al. in review). Aphids per colony were counted, dried at 40 °C and weighed. We used colony mass at 10 days as an index of palatability.

Statistical analysis

We used generalized linear models to test whether the expression of *P. australis* growth, nutritional, defense and palatability to herbivore traits were influenced by

ploidy, monoploid genome size and the latitude and longitude of origin of the populations. Only the two most common ploidy levels of *P. australis* were considered in this study, 4*x* (n = 126 source populations) and 8*x* (n = 40). Because holoploid genome size (C-value) is closely correlated with ploidy level, we used monoploid genome size (Cx-value) as an explanatory variable. Ploidy was treated as a fixed effect in the model while Cx-value, latitude and longitude of origin were treated as covariates. Latitude of origin was included because the expression of plant traits, including those related to interactions with herbivores, often covary with latitude (e.g. Schemske et al. 2009) and this is particularly true for *P. australis* (Cronin et al. 2015). Longitude is closely correlated with previously described *P. australis* phylogeographic groups (see Lambertini et al. 2006). We also included a quadratic term for each covariate (e.g., latitude^2) to assess nonlinearity in the relationship between a trait and the covariate. We were particularly interested in testing for a ploidy × Cx-value (or ploidy × Cx-value^2) interaction that would indicate that the relationship between a trait and monoploid genome size differs fundamentally between ploidy levels. Finally, all first-order interactions among predictor variables were considered. Data were analyzed using SAS 9.3 Proc Glimmix with normally distributed errors and an identity link function (SAS Institute Inc., Cary, North Carolina).

Analysis of ambient aphid abundance per stem required a slightly different analytical approach. Aphid counts per stem was Poisson distributed. Therefore, for this plant metric, we used a generalized linear model with Laplace estimation method, Poisson distribution of errors and a log link function (SAS Proc Glimmix).

To help normalize data distributions and homogenize variances between ploidy levels, total phenolics, stem heights, number of stems per pot, and aphid mass were *ln* transformed. Quantile–quantile plots and studentized residuals were used to identify potential outliers in the distribution of trait estimates. However, in no case did the removal of these data points qualitatively change the conclusions of the model.

For each dependent variable, we used Akaike's Information Criteria corrected for finite sample size (AICc) to select the most informative model (Burnham and Anderson 2010). We began by assessing whether quadratic terms for the three covariates (Cx-value, latitude of origin, longitude of origin) should be included in the candidate models for each trait. The AICc score for the base model with the main predictor variables (ploidy, Cx-value, ploidy*Cx-value) and covariate in question was compared with the AICc score for the base model plus the quadratic term for the covariate in question. If the latter model did not reduce the AICc score by ≥2 relative to the base model, the quadratic term was excluded from the list of terms used for constructing candidate models (see Burnham and Anderson 2010).

Candidate models were constructed using all possible combinations of predictor variables. Restrictions to the possible combinations of variables included the requirement that interaction terms could only be present in the model if their main effects were also present in the model. Candidate models were ranked by AICc from lowest to highest value and AICs with a Δ_i value (= $AICc_i - AICc_{min}$) of ≤2 were deemed to have substantial support (Burnham and Anderson 2010). We also report the AICc weights (w_i) which indicate the weight of evidence (as a proportion) in favor of model *i* being the best model given the set of candidate models. As the Proc Glimmix procedure does not report goodness-of-fit for the models, we emphasize effect sizes of the factors in the model (i.e., proportional differences in least-squares means or slopes in relationships).

In order to visualize the relationship between a response variable (i.e., any of the plant traits) and a particular predictor variable (e.g., monoploid genome size), we used the following procedure. We repeated the generalized linear model analysis for the AICc-best model, with the exclusion of the predictor variable in question, and then obtained the residuals. A least-squares regression analysis or plot of the residuals against the predictor variable would reveal the effect of the predictor variable on the plant trait that is independent of the other model factors on that trait. For convenience, the R^2 and *P* values from the regression are provided for each case as a means to gauge the model fit.

To determine whether there were any significant relationships between climate and the latitudes and longitudes of origin of the *P. australis* populations included in our study, we also conducted a correlation analysis using the data base (http://www.worldclim.org/bioclim) at 30 Arc-seconds resolution for the following variables: annual mean temperature,

isothermality (mean diurnal range/temperature annual range), temperature seasonality (standard deviation of the temperature × 100), maximum temperature warmest month, minimum temperature coldest month, temperature annual range, annual precipitation, seasonal precipitation, precipitation wettest quarter and precipitation driest quarter, where a quarter = 3 months. Climate values were derived according to the methods of Hijmans et al. (2005).

Results

The monoploid genome size for 4*x* *P. australis* plants included in the experiment was 0.500 ± 0.002 pg (mean ± SE; $n = 126$) and ranged from 0.470 pg to 0.573 pg. For 8*x* plants, Cx-value was 0.501 ± 0.002 pg ($n = 40$) and ranged from 0.485 pg to 0.521 pg. The difference in Cx-values between tetra- and octoploids was not significant ($t_{164} = 0.25$, $P = 0.98$) suggesting that no genome downsizing occurred in the octoploids that we sampled.

For the 10 *P. australis* traits, the AICc best models are reported in Table 1. In general, the most likely models explaining variation in trait expression included Cx-value and latitude. Monoploid genome size was present in at least one of the candidate models with substantial support (ΔAICc ≤ 2) for all 10 traits. In fact, it was in the model with the highest likelihood (i.e., lowest AICc value) in 9 of 10 cases (the exception being total phenolics; Table 1). Among all supported models for the 10 traits ($n = 35$), Cx-value was a factor in 86 % of them. Latitude was in the AICc-best model for 8 of 10 traits and was a factor in 86 % of the models (Table 1). In descending order of importance, the percentage of models with longitude of origin, ploidy, ploidy × latitude of origin interaction, and ploidy × Cx-value interaction was 69 % (9 of 10 traits), 60 % (9 of 10 traits), 29 % (6 of 10 traits), and 23 % (5 of 10 traits), respectively (Table 1).

Although genome size was consistently included as a predictor variable of plant traits, the proportion of plant variation that was explained by Cx-value was generally low. Table 2 shows the least-squares regression model for the relationship between Cx-value and each plant trait after factoring out the effects of all other predictor variables from the AICc-best model. The coefficient of determination (R^2) was ≤0.10 in all cases and averaged 0.045 ± 0.01. One trait that was significantly related to Cx-value was leaf toughness—plants with larger monoploid genomes tended to have tougher leaves (Fig. 1).

For stem height, leaf water content, and ambient aphid abundance, the AICc-best model suggested that the relationship between monoploid genome size and each of these traits differed between tetra- and octoploids (i.e., a ploidy × Cx-value interaction; Table 1). Stem height increased significantly with increasing Cx-value for tetraploids ($R^2 = 0.067$, $P = 0.004$) but decreased with increasing Cx-value for octoploids ($R^2 = 0.054$, $P = 0.160$) (Table 2; Fig. 2a). Leaf water content (Table 2; Fig. 2b) was significant for increasing Cx-values in tetraploids but not octoploids. Finally, ambient aphid number per stem decreased significantly with Cx-value for octoploids whereas model selection favored a non-significant quadratic relationship between aphid number and Cx-value for the tetraploids (Table 2; Fig. 2c).

Latitude of origin was generally a better predictor of *P. australis* traits than genome size. Mean R^2 was 0.12 ± 0.03 for the 8 traits in which latitude of origin was in the AICc-best model (see Tables 1, 2); almost three times higher than the mean R^2 for Cx-value (see above). Regardless of ploidy level, % nitrogen decreased linearly with latitude of origin ($R^2 = 0.19$, $P < 0.001$) and the C:N ratio increased linearly with latitude of origin ($R^2 = 0.20$, $P < 0.001$). Interestingly, % carbon and stem number per pot peaked at intermediate latitudes of origin (the AICc-best model included a quadratic function; Table 2; Fig. 3).

For four traits, stem height, leaf water content, ambient aphid abundance and aphid palatability (colony mass at 10 days), the relationship between latitude of origin and trait expression differed between tetraploids and octoploids (Table 2). For example, stem number increased ($R^2 = 0.11$, $P = 0.041$) and leaf water content decreased ($R^2 = 0.32$, $P < 0.001$) with increasing latitude of origin for 8*x* plants (Fig. 4a) but no relationship was observed for 4*x* plants (Fig. 4b). A quadratic function best described the relationship between palatability to aphids and latitude of origin but 4*x* plants exhibited a trough while 8*x* plants exhibited a peak at intermediate latitudes of origin (Fig. 4c). The relationship between latitude of origin and ambient aphid abundance per stem was not statistically significant regardless of the ploidy level (Table 2).

Table 2 Effect of the monoploid genome size (Cx-value) and latitude of origin on each *Phragmites australis* trait studied

Factor	Trait	Model	R^2	*P*
Monoploid genome size	% C	−0.43(Cx) + 0.23	0.001	0.884
	% N	−1.71(Cx) + 0.85	0.098	0.263
	CN ratio	7.22(Cx) − 3.59	0.005	0.395
	ln Stem height			
	4*x*	2.51(Cx) − 1.26	0.067	**0.004**
	8*x*	−5.08(Cx) + 2.54	0.054	0.160
	ln Stem number	−3.68(Cx) + 1.86	0.015	0.116
	% water			
	4*x*	0.84(Cx) − 0.42	0.067	**0.009**
	8*x*	−1.18(Cx) + 0.59	0.063	0.187
	Leaf toughness	5.87(Cx) − 2.95	0.030	**0.029**
	Aphid number			
	4*x*	167.44(Cx) − 163.46(Cx^2) − 42.79	0.006	0.699
	8*x*	−24.59(Cx) + 12.00	0.107	**0.048**
	ln Aphid colony mass	−627.0(Cx) + 614.7(Cx^2) + 159.6	0.035	0.057
Latitude of origin	% C	0.11(L) − 0.001(L^2) − 2.62	0.061	**0.018**
	% N	−0.02(L) + 0.80	0.193	**<0.001**
	CN ratio	0.11(L) − 4.68	0.203	**<0.001**
	ln Stem height			
	4*x*	0.002(L) − 0.085	0.007	0.362
	8*x*	0.011(L) − 0.424	0.111	**0.041**
	ln Stem number	0.190(L) − 0.002(L^2) − 4.72	0.293	**<0.001**
	% water			
	4*x*	−0.0004(L) + 0.022	0.005	0.492
	8*x*	−0.005(L) + 0.194	0.324	**0.001**
	Aphid number			
	4*x*	0.017(L) − 0.949	0.026	0.082
	8*x*	−0.004(L) − 0.220	0.001	0.817
	ln Aphid colony mass			
	4*x*	−0.550(L) + 0.006(L^2) + 12.05	0.064	**0.019**
	8*x*	0.504(L) − 0.005(L^2) − 12.08	0.173	**0.030**

If the AICc-best model contained a ploidy × Cx-value interaction, separate models were reported for each ploidy level. *P* values in bold are significant ($P \leq 0.05$). Values for each trait were obtained as the residuals from separate generalized linear model analyses that included all variables from the AICc-best model except the trait in question. Least-squares regressions were performed on the residuals to obtain the model and associated statistics

Tests of the relationship between Cx-value and plant-trait value were performed using residuals from the AICc-best model, minus Cx-value and all interactions involving Cx-value. The same approach was used for assessing the relationship between latitude of origin and each plant trait

In addition to ploidy-level effects on how trait expression varies with latitude of origin, ploidy *per se* was a modestly important predictor for one half of the plant traits considered in this study (Table 3). The most pronounced differences were that tetraploids had 30 % higher total phenolics, 14 % fewer stems per pot, and 7 % fewer aphids per stem than octoploids. There were other significant differences between ploidy levels, but those differences were associated with very small effect sizes—tetraploids had 3 %

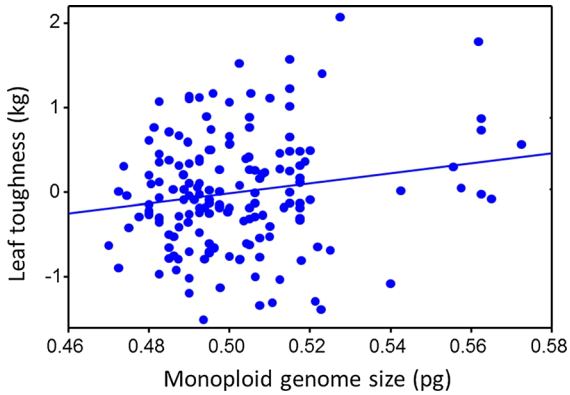

Fig. 1 The relationship between monoploid genome size (Cx-value) and *P. australis* leaf toughness (kg). Leaf toughness values were obtained as the residuals from a generalized linear model analysis that included all variables from the AICc-best model, excluding monoploid genome size. *Line* is fit by least-squares regression

taller stems and 2 % less % water content than octoploids. All other traits were indistinguishable between ploidy levels (Table 3).

As indicated by the frequent occurrence of longitude of origin in the AICc-best models (see Table 1), there are differences among continents/phylogeographic groups. Interestingly, there were no interactions between longitude of origin and ploidy number or genome size. As this study is about karyological diversity and its effects on plant trait expression, no further discussion of longitude of origin is warranted.

Finally, using the variables listed above from the Bioclim database (see "Statistical analysis" section), we found a strong negative relationship ($r = -0.795$) between latitude of origin and annual mean temperature and moderately strong relationships between latitude of origin and minimum temperature in the coldest month ($r = -0.595$) and isothermality ($r = -0.508$). The correlation coefficient was weak ($r \leq 3$) for all other variables except temperature seasonality, which was also weak at 0.306. All correlation coefficients relating longitude of origin to the 10 variables were weak ($r = <3$) except precipitation, which showed a moderate negative relationship with longitude of origin ($r = -0.435$) and annual precipitation, which showed a weak negative linear relationship ($r = -0.361$).

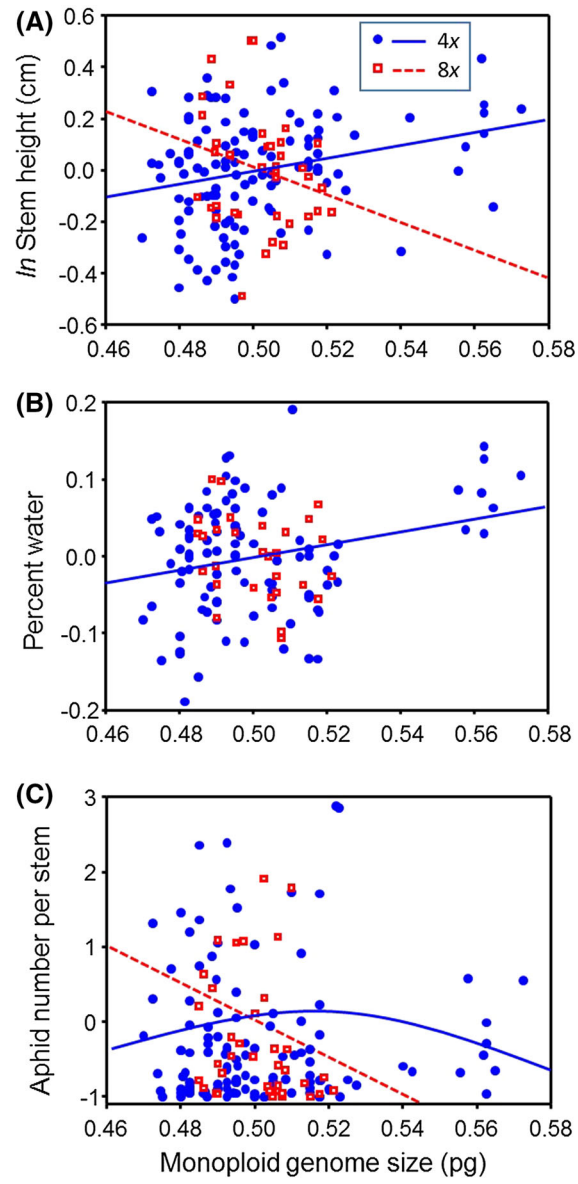

Fig. 2 The relationship between monoploid genome size (Cx-value) and *P. australis*. **a** ln stem height (cm), (**b**) percent water content, and **c** ambient aphid abundance per stem. Values for stem height, percent water and aphid abundance were obtained as the residuals from separate generalized linear model analyses that included all variables from the AICc-best model, excluding monoploid genome size. Because the AICc-best model (Table 1) included a ploidy*Cx-value interaction, separate least-squares regression lines were fit to tetraploids and octoploids (see Table 2). Quadratic regression was used for tetraploids in (**c**). *Note*: only a single, pooled regression lines is provided in (**b**) because separate lines almost completely overlapped

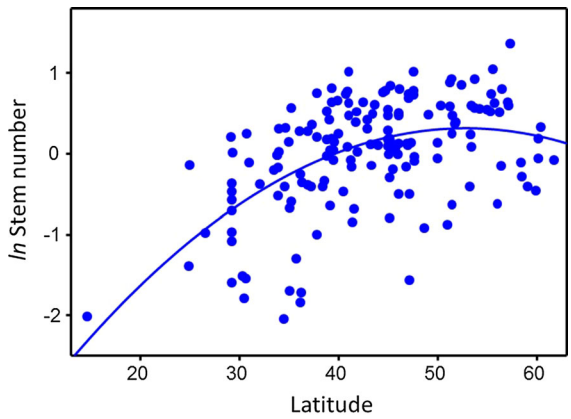

Fig. 3 The relationship between latitude of origin and number of *P. australis* stems per pot. Number of stems are the residuals from a generalized linear model analysis that included all variables from the AICc-best model, excluding latitude of origin. Line is fit by least-squares regression, band represents the 95 % confidence limits and dashed lines present the 95 % prediction limits

Discussion

Recent studies indicated a relationship between genome size and plant traits associated with invasiveness such as growth rate and phenology (e.g. Kubešová et al. 2010), while others showed differences between plant defense traits across ploidy levels (e.g. te Beest et al. 2011); however, the effects of genome size and ploidy level have rarely been addressed simultaneously (Pandit et al. 2014). In our common garden study we examined 126 populations of tetraploid *P. australis* spanning from 14.6°N to 61.8°N and 40 octoploid populations spanning from 39.5°S to 51.2°N. We have documented significant intraspecific variation in genome size across globally distributed populations of *P. australis*. Furthermore, we examined the roles monoploid genome size, ploidy and latitude of origin (and their interactions) play in trait expression in a common garden setting that allowed us to explore the relative contributions of those factors on particular plant traits.

We found that both monoploid genome size and latitude of origin contributed to variation in nine out of the 10 traits that we studied for *P. australis*, with latitude of origin being generally a better predictor of trait values. Moreover, we found that ploidy level and its interaction with monoploid genome size and latitude of origin also contributed to trait variation.

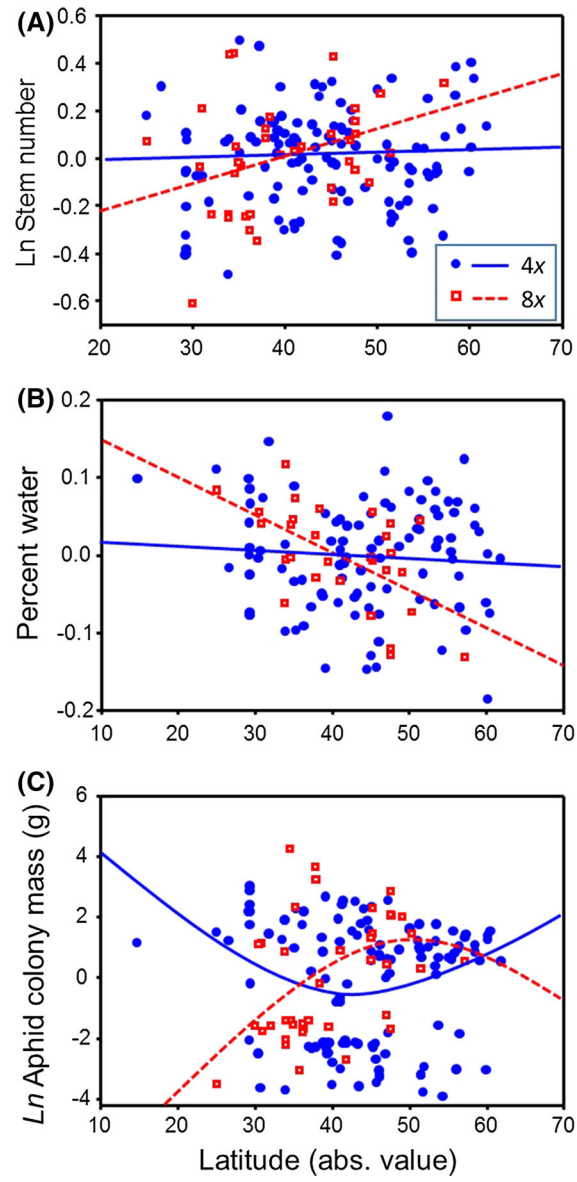

Fig. 4 The relationship between latitude of origin and *P. australis*. **a** stem number, **b** percent water content, and **c** palatability to aphids (colony mass at 10 days). As with the previous figures, values for each variable are the residuals from the generalized linear model analyses from the AICc-best model excluding latitude of origin

This suggests that while genome size and ploidy are each important factors that help determine plant traits, each is only a contributing factor that interacts with the other, as well as the environment, and reinforces the idea that no single trait can account for the invasion success of a species (Thébault et al. 2011). There was a

Table 3 Least-squares mean ± SE for 4*x* and 8*x* *Phragmites australis*

Trait	Tetraploid (4*x*)	Octoploid (8*x*)	F	*P*
% C	46.71 ± 0.08	46.50 ± 0.19	3.66	0.058
% N	3.01 ± 0.043	2.91 ± 0.08	0.86	0.350
CN ratio	15.69 ± 0.21	16.21 ± 0.47	0.82	0.368
Total phenolics	1902.1 ± 1.03	1462.9 ± 1.07	10.22	**0.002**
Stem height	117.33 ± 1.02	113.72 ± 1.04	4.13	**0.044**
Stem number	44.53 ± 1.06	52.02 ± 1.14	4.07	**0.045**
% water	0.49 ± 0.01	0.50 ± 0.01	4.92	**0.028**
Leaf toughness	2.16 ± 0.07	2.14 ± 0.14	0.01	0.910
Aphid number	18.76 ± 1.02	20.01 ± 1.06	67.58	**<0.001**
Aphid colony mass	0.0022 ± 0.001	0.0004 ± 0.002	0.78	0.378

The model used to compute the least-squares means was the AICc-best model that included ploidy (see Table 1). If ploidy was not in any of the candidate models, then it was added to the model with the lowest AICc value

significant positive relationship between monoploid genome size and leaf toughness (Fig. 1), a plant defense trait, suggesting that tougher leaves are associated with larger genomes, at least in *P. australis*. In a previous field study, we found that for European populations of *P. australis*, herbivory by leaf chewers and stem gallers was negatively correlated with leaf toughness; suggesting leaf toughness is an herbivore-defense trait (Cronin et al. 2015). While small genome size is associated with faster growth (Küster et al. 2008; Fridley and Craddock 2015), larger monoploid genome size in *P. australis* resulted in better-defended leaves potentially suggesting a trade-off between defense and growth rate.

We also found that for four traits, tetraploids and octoploids had different relationships with the monoploid genome size. While for tetraploids stem height and leaf water content showed a positive relationship with monoploid genome size, octoploids had a negative relationship with monoploid genome size for stem height and no relationship for leaf water content. As genome size within octoploids increased, the number of aphids colonizing leaves decreased whereas for tetraploids there was a quadratic, though non-significant, relationship. In general, we found that tetraploids were taller, chemically better defended (as suggested by the content of total phenolics), had a greater number of stems, higher leaf water content, and supported more aphids (likely due to higher leaf water content) than octoploids. However, these differences need to be interpreted with caution since the variation in genome size in tetraploids (n = 126) was much greater than in octoploids (n = 40). Earlier work by Clevering et al. (2001), Hansen et al. (2007) and Achenbach et al. (2012) reported variability in the significance of plant size and physiological responses between octoploids and tetraploids depending on origin making it difficult to conclusively determine the relationship between ploidy level and *P. australis* stature. In addition, while it was more or less continuous in the latter, there was some gap in tetraploids (note that small- and large-genome tetraploids could be distinguished as distinct groups, Fig. 2). Therefore, at least some relationships may be affected by a few tetraploids with large genomes. Nevertheless, our results provide robust evidence that a wide spectrum of traits with a range of functional roles are modified in their expression by the interaction with genome size; we are not aware of any former study pointing to this phenomenon.

Latitude is often used as a proxy to investigate how species undergoing range expansions or introduction to novel environments will respond to global climate change (e.g., De Frenne et al. 2013; Kambo and Kotanen 2014) and it is expected that as the global climate warms, populations will expand their ranges poleward. The latitudes of origin for the populations used in our study showed a strong negative linear relationship with annual temperatures. Our results also showed that latitude of origin, rather than genome size or ploidy, was a better predictor of plant trait expression. Leaf nitrogen content declined at higher latitudes of origin for both ploidy levels while percent carbon increased. As with our results for monoploid genome size discussed above, we found different relationships between trait expression and latitude of origin depending on the ploidy level of the population, but these relationships were only significant for octoploid stem height (positive) and leaf water content (negative), and aphid abundance (quadratic) showed significant but opposite relationships by ploidy level.

While many of the relationships between plant traits and genome size, ploidy and latitude of origin were weakly significant, it is worth noting that the populations included in this study represented 47° of latitudinal span for tetraploids and 90° of latitude for octoploids, and we therefore suggest that the results are biologically meaningful. As noted above, we analyzed three times as many tetraploids than octoploids in our sample set, which may have influenced the significance of our results. Investigating the effects of greater latitudinal range in octoploids versus tetraploids with a much larger data set could yield insights into the factors driving latitudinal differences between them.

Our results suggest that there are potential trade-offs among plant traits mediated by monoploid genome size and ploidy with respect to fitness and defense and that the latitude of plant origin is a significant determinant of trait expression even after a decade or more of growing in a common garden setting. Under climate change, some genome size and ploidy variants (both within and among plant species) may more successfully cope with changing external filters (e.g., temperature, salinity, drought), owing to greater phenotypic plasticity and to fitness traits that vary with cytotype (e.g. Knight and Ackerly 2002; Bennett and Leitch 2005; Knight et al. 2005; Suda et al. 2015). As such, some cytotype and genome size variants may be favored by natural selection leading to changes in population genome size and/or ploidy structure, particularly at species range limits. Such changes could foster "bottom up" effects and further interact with climate change and distribution of natural enemies that are, and will continue to be, important drivers of range expansions and species invasions.

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Appendix

Name	Country	Continent	Latitude of origin	Longitude of origin	Ploidy	Genome size (pg)
145 8× (Pa 8× AU) Australia N.S.W. Telowie, Coorong	AU	AU	−32.05	138.07	8×	4.05
148 8× (Pa 8× AU) Australia N.S.W. Brewarrina	AU	AU	−29.95	146.87	8×	3.98
149 8× (Pa 8× AU) Australia N.S.W Bora Channel	AU	AU	−33.88	151.22	8×	4.08
150 8× (Pa 8× AU) Australia N.S.W. Old Willbriggie Road	AU	AU	−34.47	146.02	8×	4.00
156 8× (Pa 8× AU) Australia N.S.W. Monkeygar Creek	AU	AU	−30.75	147.73	8×	4.11
157 8× (Pa 8× AU) Australia N.S.W. Edward River	AU	AU	−35.74	145.27	8×	3.91
158 8× (Pa 8× AU) Australia N.S.W. Victoria Leneva Creek	AU	AU	−36.22	146.90	8×	3.97
162 8× (Pa 8× AU) Australia N.S.W. Victoria Bonegilla	AU	AU	−36.15	147.00	8×	4.04
167 8× (Pa 8× AU) Australia N.S.W. Victoria Huges Creek	AU	AU	−36.90	145.23	8×	4.03
173 8× (Pa 8× AU) Australia N.S.W. Victoria Tullaroop Creek	AU	AU	−37.82	144.97	8×	4.02
176 8× (Pa 8× AU) Australia N.S.W. Victoria Bethanga Creek	AU	AU	−36.12	147.10	8×	4.07
96 8× (Pa Core Group) Australia N.S.W. Botany Wetlands	AU	AU	−33.97	151.20	8×	4.00

Name	Country	Continent	Latitude of origin	Longitude of origin	Ploidy	Genome size (pg)
76 8× (Pa 8× AU) Australia A.C.T. Ginninderra Creek	AU	AU	−35.20	149.08	8×	4.02
136 M 8× Australia Cortina Lake	AU	AU	−34.93	138.60	8×	4.05
218 8×? Australia Victoria Melbourne	AU	AU	−37.83	144.90	8×	4.04
146 4× (Pa Core Group) Belgium Scheldt, Konkelschoor, Berlare	BE	EU	51.22	4.42	4×	1.98
67 m 4×? (Pa core group) Belgium Scheldt-estuarie Burcht Antwerp	BE	EU	51.22	4.42	4×	1.96
131 M 4×? (Pa Core Group) Canada Quebec Huntingdon	CA	NA	45.08	−74.18	4×	2.05
151 4× (NJ Pa Alt. Coast) Canada Quebec Chemin de la Butte	CA	NA	45.50	−73.58	4×	2.07
152 4× (NJ Pa Alt. Coast) Canada Quebec Duvernay-est, Montreal	CA	NA	45.57	−73.85	4×	2.16
153 4× (NJ Pa Alt. Coast) Canada Quebec Ormstown	CA	NA	45.13	−74.00	4×	2.10
154 4× (Pa Core Group) Canada Quebec Saint de Joliette	CA	NA	46.03	−73.43	4×	1.98
155 4× (Pa Core Group) Canada Quebec Ste-Martine	CA	NA	45.23	−73.80	4×	2.00
204 Canada MW Manitoba Lake Manitoba III, Inkster Farm	CA	NA	49.97	−98.30	4×	2.25
129 M 4×? (Pa Core Group) Canada Ontario Cootes Paradise	CA	NA	43.67	−79.42	4×	2.09
132 4× (Pa Core group) Canada Quebec I'lslet sur Mer	CA	NA	46.80	−71.17	4×	1.97
130 M 4× (Pa MW) Canada Manitoba Lake Manitoba Blind Channel	CA	NA	49.97	−98.30	4×	2.25
801 Switzerland Zurich Lake	CH	EU	47.33	8.53	4×	1.94
122 M 8× (Pa 8× AU) China Mai Po, Hong Kong	CN	AS	34.53	118.86	8×	4.05
680 China Living garden 21.04.07	CN	AS	30.66	104.06	4×	2.09
123 M 4× (Pa Core Group) China Lanzhou	CN	NA	36.06	103.79	4×	2.02
620 M 4× (Pa core group) CZ-3 (CZR-L10)	CR	EU	48.65	14.37	4×	1.96
671 4×? (Pa Africa Basel gr.) Cyprus Coral Beack, Pafos	CY	EU	35.04	32.43	4×	1.95
672 4× (Pa Africa basel gr.) Cyprus Afrodites Bath, Polis	CY	EU	35.04	32.43	4×	2.03
641 M 4× ØT 107 Germany	DE	EU	51.43	13.62	4×	1.97
639 M 4× ØT A Germany	DE	EU	51.82	13.82	4×	2.01
640 M 4× ØT 76 Germany	DE	EU	51.43	13.62	4×	1.97
665 4×? Germany D-W6	DE	EU	51.00	9.90	4×	1.96
609 M 4× (Pa core Group) Vejlerne DK-4 (DK-W1)	DK	EU	57.09	9.05	4×	1.97
49 M 4× (Pa Core Group) Denmark Norsminde Fjord	DK	EU	56.02	10.27	4×	1.99
20 Denmark Fano	DK	EU	55.40	8.45	4×	1.93
689 Denmark Laeso Haltermmen	DK	EU	57.29	10.96	4×	1.92
21 Denmark Endelave Lynger	DK	SA	55.76	10.24	4×	1.96
68 (Pa Africa Basel grade) Algeria Guebbour, south af Hassi Messaoud	DZ	AF	31.70	6.05	4×	1.97
159 4× (Pa Core Group) Estonia Lake Peipsi	EE	EU	59.02	27.73	4×	1.93

Name	Country	Continent	Latitude of origin	Longitude of origin	Ploidy	Genome size (pg)
83 M 4×? (Pa Core Group) Estonia Lake Vortsjarv	EE	EU	58.43	25.41	4×	1.93
72 M 4× (Pa Core Group) Spain Gallocanta N	ES	EU	41.00	−1.50	4×	1.98
300 4×? (Pa Core Group) Spain Mallorca Alcudia	ES	EU	39.87	3.12	4×	1.93
74 M 4× (Pa Core Group) Spain I' encanyissada (ebro)	ES	EU	40.72	0.58	4×	2.06
160 4× (Pa Core Group) Finland Mariehamn, Aland	FI	EU	60.10	19.95	4×	1.93
217 4×? (Pa Core Group) Finland Rsisionlahti, Turku	FI	EU	60.40	22.10	4×	1.95
53 4× (Pa Africa Basel gr) Tunisia Ras Taguermes Djerba (fine)	FI	EU	33.82	11.03	4×	1.98
70 M 4× (Pa c. gr. basel 8× AU) France Campignol, Narboone	FR	EU	43.18	3.00	4×	1.96
663 8× (Pa core group) H-L3 8×	HU	EU	47.60	17.03	8×	3.95
664 4× (Pa Core Group) Hungary H-L1 4×	HU	EU	47.60	17.03	4×	1.94
58 M 4×? (Pa Core Group) Ireland Kilcock	IE	EU	53.40	−6.67	4×	1.99
164 4× (Pa Core Group) Ireland Lake Roe	IE	EU	53.33	−6.25	4×	1.98
165 4× (Pa Core Group) Ireland Hazelhatch	IE	EU	53.25	−7.12	4×	1.88
166 4 ×Ireland Lowtown	IE	EU	53.43	−7.95	4×	1.93
90 m 4× (Pa c. gr. Basel 8× AU) Israel Yerokham, Negev Highland	IL	ME	30.99	34.93	4×	1.94
91 M 4× (Pa Core Group) Israel Dead Sea South-West Coast	IL	ME	30.99	34.93	4×	1.96
8 Italy Sardinia S. Antioco (Saline)	IT	EU	39.09	8.36	4×	1.93
12 Italy Sardinia Isola Rossa	IT	EU	41.00	8.87	8×	3.92
10 (Short) Italy Sardinia Valledoria	IT	EU	40.93	8.80	4×	1.98
11(Tall) Italy Sardinia Valledoria	IT	EU	40.93	8.80	4×	1.98
207 4×? (Pa Core Group) Italy Albano S. Alessandro Bergamo	IT	EU	45.68	9.77	4×	1.94
75 4× (Pa Core Group) Italy Gorgona	IT	EU	43.44	9.92	4×	1.93
684 Italy 4×? (Pa Core Group) Valle Bentivoglia Malalbergo Bolonga	IT	EU	44.47	11.63	4×	2.06
685 Italy 4×? (Pa Core Group) Valle Le Tombe Malalbergo Bologna	IT	EU	44.72	11.53	4×	2.06
120 M 8× (Pa 8× AU) Japan Okoyama	JP	AS	34.65	133.92	8×	4.03
686 Kuwait	KW	ME	29.32	47.48	4×	1.90
85 M 4× Lithuania Silute	LT	EU	55.35	21.48	4×	1.95
14 Libya Nemes 9	LY	AF	24.91	17.76	4×	1.95
15 Libya Mafu	LY	EU	26.56	13.12	4×	1.95
602 M 4× (Pa core group) NL-3 (NL-L10)	NL	EU	52.38	4.82	4×	1.98
163 4× (Pa Core Group) Holland Verdroken Land Van Saeftnghe	NL	EU	51.33	4.15	4×	1.96
142 M 8× (Pa 8× AU) New Zealand Tutaekuri River Napier	NZ	AU	−39.48	176.92	8×	4.05
78 M 4× (Pa Core Group) Poland Krakow	PL	EU	51.73	18.52	4×	1.92
624 M 8× (Pa core group) RO-1 (RO-01)	RO	EU	45.17	29.33	8×	3.91
643 M 4× (Pa core group) RO-L4	RO	EU	45.00	29.22	4×	2.01
652 8× (Pa core group) RO-L 3B 8×	RO	EU	45.00	29.22	6×	2.91

Name	Country	Continent	Latitude of origin	Longitude of origin	Ploidy	Genome size (pg)
661 RO9-8 8× (Pa Core Group)	RO	EU	45.17	29.33	8×	3.92
657 L 5 B 4× (Pa Core Group)	RO	EU	45.00	29.22	4×	2.02
654 8× (Pa Core Group) RO-L6A 8×	RO	EU	45.00	29.22	8×	3.92
655 4× (Pa Core Group) RO-L6A 4×	RO	EU	45.00	29.22	4×	2.03
659 8× (Pa Core Group) RO-L8-13 8×	RO	EU	45.00	29.22	8×	3.88
662 4× RO-09-8 4×	RO	EU	46.17	30.33	4×	1.93
84 M 8× (Pa Core Group) Romania L. Oborny	RO	EU	45.70	25.80	6×	2.97
107 4× (Pa basel MW) Russia Moscow B, G (RAS)	RU	EU	56.40	38.65	4×	2.23
687 Russia Novosibrisk	RU	EU	55.02	82.88	4×	1.97
169 4× (Pa Core Group) Russia St. Petersburg	RU	EU	59.89	30.26	4×	2.02
215 8–10×? (Pa 8× AU) Russia Sakhalin Pokrovka Nayba	RU	EU	47.55	143.32	8×	4.06
201 8× (Pa 8× AU) Russia Sakhaln Pugachevo (makaraovsky distr)	RU	EU	49.07	143.28	8×	3.96
212 8–10× (Pa 8× AU) Russia Sakhaln Laguna Busse (Korsakovsky Distr)	RU	EU	47.03	143.30	8×	4.15
138 4× (Pa Core Group) Russia Nazyvaevsk, Omsk	RU	EU	55.57	71.35	4×	1.98
216 6–8× (Pa Core Group) Russia Sakhaln Zaozemaya	RU	EU	50.30	156.40	8×	4.14
178 8× (Pa 8× AU) Russia Sakhalin Yuzhno-Sakhalinski B,G	RU	EU	46.95	142.74	8×	4.17
213 6–8× (Pa 8× AU) Russia Sakhalin Voskhod (Tymovsky Distr)	RU	EU	51.42	143.08	8×	4.14
205 4× (Pa Core Group) Russia Sakhalin Novikovo (Korsakovsky Distr)	RU	EU	47.03	143.30	4×	1.89
214 8–10× (Pa 8× AU) Russia Sakhalin River Manuy (Dolynsky Distr)	RU	EU	47.55	143.32	8×	4.12
306 8×? Russia Rostov	RU	EU	57.18	39.45	8×	3.89
110 M 8× (Pa 8× AU) Russia Sakhalin Okhotsk (Dolynsky Distr)'	RU	EU	47.55	143.32	8×	4.06
615 M 4× (Pa core group) SE 4 A (S-W3)	SE	EU	58.45	14.90	4×	1.89
1 4×? Sweden Hornslandet Rogsta Halsingland	SE	EU	61.76	17.21	4×	1.91
79 M 4×? (Pa Core Group) Silvenia Zadnij kraj Lake cerknisko	SI	EU	46.06	14.51	4×	1.92
170 4× (Pa Core Group) Slovenia Gornje jezero Lake Cerknisko	SI	EU	45.97	14.43	4×	1.99
171 4× (Pa Core Group) Solvenia Pond Dress Ljubljana	SI	EU	46.06	14.51	4×	1.97
172 4× (Pa Core Group) Slovenia Veena pot Ljubljana	SI	EU	46.06	14.51	4×	1.96
800 Slovenia Graga Pri Igu	SI	EU	45.95	14.54		NA
102 M 4× Senegal Potte'd Oie Dakar	SN	AF	14.67	−17.44	4×	1.90
50 M 4× (Pa Core Group) Denmark Knebel Vig	TN	AF	56.22	10.50	4×	1.93
97 4× (Pa African grade) Tunisia Ras Tagermes Djerba (giant)	TN	AF	33.82	11.03	8×	3.92
174 4× (Pa Core Group) Tunisia Chenini (Gabes)	TN	AF	33.88	10.12	4×	1.92
89 M 8× Turkey Aksehir	TR	EU	38.36	31.42	8×	4.07
682 4× GB-L8	UK	EU	53.70	−1.70	4×	1.92
208 4× England River Severen	UK	EU	53.70	−1.70	4×	1.92
209 4× (Pa Core Group) England Thamesmead	UK	EU	51.50	−0.12	4×	1.93

Name	Country	Continent	Latitude of origin	Longitude of origin	Ploidy	Genome size (pg)
60 M 4× Scotland Tay estuary	UK	EU	56.46	−3.05	4×	1.94
63 M 4× England River Humber	UK	EU	54.20	−0.31	4×	1.90
117 6× (Pa Gulf Coast) United States Florida SFWCA2A	US	NA	25.79	−80.13	6×	3.16
125 6–8× (Pa Gulf Coast) United States Louisiana Weeks Island	US	NA	29.81	−91.81	6×	3.16
101 6×? (Pa Gulf Coast) United States Alabama Dauphin island	US	NA	30.26	−88.11	6×	3.09
111 M 4× (Pa MW) United States Utah Green River	US	NA	40.46	−109.53	4×	2.29
190 4× (NJ Pa Alt. Coast) United States New York, Buffalo, Orchard Park	US	NA	42.89	−78.88	4×	NA
193 4× (Pa Core Group) United States Virginia Upshur Creek	US	NA	39.30	−75.18	4×	2.03
116 M 4× (NJ Pa Atl. Coast) United States Washington Moses Lake	US	NA	47.13	−119.28	4×	2.04
126 4×? United States Louisiana Madisonville	US	NA	30.38	−90.16	6×	3.09
109 Santa Rosa Island Florida USA	US	NA	30.40	−86.23	6×	3.12
69 4×? (Pa Core Group) United States Delaware Burtons Island	US	NA	38.58	−75.26	4×	2.09
86 4× (Pa Core Group) United States Michigan Ives Road Adrian	US	NA	41.90	−84.04	4×	2.22
54 M 4×? (Pa Core Group) Finland Husoviken Aland	US	NA	60.10	19.95	4×	1.95
99 4× (Pa Core Group) United States North Carolina Avon, Pea island	US	NA	36.27	−77.59	4×	2.04
55 4× (Pa MW) United States Minnesota Bluestern prairie	US	NA	46.87	−96.77	4×	2.25
113 Rhode Island Galilee	US	NA	47.13	−119.28	4×	2.04
115 M 4× United States Maryland Easton Talbot	US	NA	38.77	−76.08	4×	2.02
114 M 4×? (NJ Pa Atl. Coast) United States Ohio Maumee Bay	US	NA	41.56	−83.65	4×	2.06
113 M 4×? Unites States Rhode Island Galilee	US	NA	41.38	−71.51	4×	2.05
210 4×? United States Virginia James Town island	US	NA	37.21	−76.77	4×	2.02
128 4×? United States Massachusetts Buzzards Bay	US	NA	41.75	−70.62	4×	2.06
61 4× United states Illinois La salle-Peru	US	NA	41.33	−89.11	4×	2.00
121 4× (Pa Core Group) United States Florida Crayton	US	NA	30.33	−86.17	4×	1.96
OCT 1 Greeny1-214 Mississippi River Delta Louisiana USA	US	NA	29.21	−89.22	4×	1.99
ROMS7 Delta-210 Mississippi River Delta Louisiana USA	US	NA	29.25	−89.24	4×	1.92
WHS2 EU-211 Mississippi River Delta Louisiana USA	US	NA	29.21	−89.21	4×	2.03
202 4×? United States New Mexico Bitter River	US	NA	33.47	−104.42	4×	2.25
WHS3 Land-212 Mississippi River Delta Louisiana USA	US	NA	29.21	−89.21	6×	3.17
203 4× United States New Jersey Stone Harbor	US	NA	39.06	−74.77	4×	2.07
65 4×? (Pa MW) United States Michigan Daytin West Prairie	US	NA	41.00	83.00	4×	2.26
119 6× (Pa Core Group) United States Louisiana Cocodrie	US	NA	29.25	−90.66	4×	1.95

Name	Country	Continent	Latitude of origin	Longitude of origin	Ploidy	Genome size (pg)
71 4×? (Pa Core Group) United States New York Buffaki, Great Baehre	US	NA	42.89	−78.88	4×	2.08
ROM2 EU-209 Mississippi River Delta Louisiana USA	US	NA	29.26	−89.24	4×	2.08
ROM4 Delta-215 Mississippi River Delta Louisiana USA	US	NA	29.26	−89.24	4×	2.07
112 M 4×? (NJ Pa Alt. Coast) United States Delaware Roosevelt Inlet	US	NA	39.94	−74.39	4×	2.17
ROM16 Land-207 Mississippi River Delta Louisiana USA	US	NA	29.20	−89.25	6×	3.17
SEP107 Greeny3-107 Mississippi River Delta Louisiana USA	US	NA	29.14	−89.14	4×	1.98
179 4× (NJ Pa Atl. Coast) United States Delaware Dover	US	NA	39.16	−75.52	4×	2.00
180 4× (NJ Pa Atl. Coast) United States Loms Pond	US	NA	39.58	−75.71	4×	2.07
181 4× (NJ Pa Atl. Coast) United States Delaware Odessa	US	NA	39.46	−75.66	4×	1.97
182 4× United States Delaware Willow Creek	US	NA	38.78	−75.11	4×	2.07
185 4× United States Maryland Webster Field	US	NA	38.79	−77.29	4×	2.05
186 4× (Pa Core Group) United States Virginia Oyster Delmarva	US	NA	37.29	−75.92	4×	1.99
187 4×? (NJ Pa Atl. Coast) United States Virginia Parramore Island	US	NA	39.30	−75.18	4×	2.06
189 4× (NJ Pa Atl. Coast) United States Virginia Mutton Hunk	US	NA	37.78	−75.60	4×	2.00
191 4× (Atl. Coast) United States New York N. Wheatfield Bear Ridge	US	NA	43.28	−77.28	4×	2.02
192 4× (NJ Pa Atl. Coast) United States Virginia Swash Bay	US	NA	39.30	−75.18	4×	1.97
194 4× (NJ Pa Atl. Coast) United States Virginia Virginia Beach	US	NA	36.85	−75.98	4×	2.11
197 4× (NJ Pa Alt. Coast) United States New York Buffalo, Depew	US	NA	42.90	−78.69	4×	2.00
199 4× (NJ Pa Atl. Coast) United States Massachusetts Bedford Boston	US	NA	42.49	−71.28	4×	2.07
200 4× (NJ Pa Atl. Coast) United States Rhode Island Silver Spring Lake	US	NA	41.79	−71.37	8×	4.12
206 4× (NJ Pa Atl. Coast) United States Connecticut Milford	US	NA	41.22	−73.06	4×	1.99
211 4×? (Pa MW) United States Minnesota Pipestone	US	NA	44.00	−96.32	4×	2.02
224 4×? (Pa Gulf Coast) United States Mississippi Christian	US	NA	35.22	−88.04	4×	1.97
144 Rockefeller Louisiana USA	US	NA	29.74	−92.82	6×	3.09
ROMS4 Delta-208 Mississippi River Delta Louisiana USA	US	NA	29.26	−89.24	4×	2.07
188 8× (NJ Pa 8× ZA) Rep. South Africa Loeriesfontein	ZA	AF	−30.97	19.45	8×	3.95
195 8× (NJ Pa 8× ZA) Rep. South Africa Brandvlei	ZA	AF	−30.45	20.48	8×	3.88
105 M 8× (Pa Core Group) Rep. South Africa Keurboom Estuary	ZA	AF	−33.95	18.46	8×	3.93
311 8× (NJ Pa 8× ZA) Rep. South Africa Kalkgat	ZA	AF	−24.98	28.63	8×	3.89

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