Contrasting growth phenology of native and invasive forest shrubs mediated by genome size

Jason D. Fridley and Alaïa Craddock

Department of Biology, Syracuse University, Syracuse, NY 13244, USA

Author for correspondence:
Jason D. Fridley
Tel: +1 315 443 3098
Email: fridley@syr.edu

Received: 14 November 2014
Accepted: 23 February 2015

doi: 10.1111/nph.13384

Key words: biological invasions, budbreak, deciduous forests, flow cytometry, nuclear DNA content (2C DNA).

Summary

- Examination of the significance of genome size to plant invasions has been largely restricted to its association with growth rate. We investigated the novel hypothesis that genome size is related to forest invasions through its association with growth phenology, as a result of the ability of large-genome species to grow more effectively through cell expansion at cool temperatures.
- We monitored the spring leaf phenology of 54 species of eastern USA deciduous forests, including native and invasive shrubs of six common genera. We used new measurements of genome size to evaluate its association with spring budbreak, cell size, summer leaf production rate, and photosynthetic capacity.
- In a phylogenetic hierarchical model that differentiated native and invasive species as a function of summer growth rate and spring budbreak timing, species with smaller genomes exhibited both faster growth and delayed budbreak compared with those with larger nuclear DNA content. Growth rate, but not budbreak timing, was associated with whether a species was native or invasive.
- Our results support genome size as a broad indicator of the growth behavior of woody species. Surprisingly, invaders of deciduous forests show the same small-genome tendencies of invaders of open habitats, supporting genome size as a robust indicator of invasiveness.

Introduction

The size of a plant’s genome has both genetic and ecological consequences (Lynch, 2007), and ecologists have been quick to integrate genome size into the panoply of key traits defining plant strategy theory (Grime & Mowforth, 1982; Grime et al., 1985; Thompson, 1990; Grotkopp et al., 2004; Morgan & Westoby, 2005; Beaulieu et al., 2007b; Knight & Beaulieu, 2008; Hodgson et al., 2010). Ecological associations of genome size and species behavior stem from two primary mechanisms. First, because cells of large-genome species divide more slowly (Darlington, 1965; Cavalier-Smith, 2005; Gregory, 2005), species of rapid life history characteristics are biased toward small genomes (Bennett et al., 1998). In this case, the use of ‘genome size’ refers to the total holoploid nuclear DNA content in a cell (Greilhuber et al., 2005) – the unreplicated genome – and its association with the duration of the cell cycle (Grotkopp et al., 2004; Francis et al., 2008). Secondly, genome size, and more particularly the total amount of DNA in the nucleus (often called the ‘2C value’), is associated with larger cell volume (Beaulieu et al., 2007a, 2008; Hodgson et al., 2010). Cell volume, in turn, has important ecological consequences, including those involving seed mass (Thompson, 1990; Knight et al., 2005; Beaulieu et al., 2007b), guard cell size (Beaulieu et al., 2008), and whole-plant water balance as a function of stomatal control (Hodgson et al., 2010). To the extent that differences in genome size within major plant groups are independent of phylogeny (Bennett & Leitch, 2005; Beaulieu et al., 2008; Chen et al., 2010; Pandit et al., 2014), this relatively simple plant trait is thus a promising indicator of plant strategies associated with life history and growth economics (Morgan & Westoby, 2005).

Genome size has attracted particular attention as an indicator of species invasiveness (te Beest et al., 2012; Pandit et al., 2014). To the extent that growth is limited by the rate of cell division, large-genome species are expected to have slower relative growth rates and may be at a competitive disadvantage in habitats that favor a strategy of resource pre-emption through fast acquisition (Grime, 2006). Several lines of evidence support this hypothesis. For example, species designated as ‘weeds’ have smaller genomes than nonweeds, assessed both regionally (e.g. Bennett et al., 1998; for the UK; Kubešová et al., 2010; for the Czech Republic) and globally (Chen et al., 2010; Pandit et al., 2014). Such differences have also been found within specific taxonomic groups. Grotkopp et al. (2004) showed that genome size differences across 85 species of Pinus were associated with a broad suite of ecological characters relating to invasiveness, including both growth and reproductive attributes. Similarly, invasive species in the genus Artemisia have smaller genomes than nonintruders (Garcia et al., 2008), and populations of the North American wetland invader Phalaris arundinacea have smaller genomes than those of its native European range (Lavergne et al., 2010). It is noteworthy that all of these comparisons have involved species in...
relatively high light habitats, where fast growth is an important contributor to competitive ability. It remains unclear whether genome size should be a general indicator of species invasiveness outside of a ‘weediness’ context, particularly for less disturbed or more stressful habitats where growth rate per se is less relevant to fitness (Grime, 1977).

We suggest an alternative strategy of invader success relating to genome size in temperate ecosystems, involving links between cell volume, growth through cell expansion, and growth phenology—that is, that large-genome species are able to leaf out earlier in the spring and thus gain competitive advantage over small-genome species. The relationship of growth phenology and genome size was first reported by Grime & Mowforth (1982) for grassland species in northern England, where early-season, large-genome grasses and geophytes containing larger cells are able to achieve fast rates of tissue growth via expansion of cells divided during the previous warm season (Grime et al., 1985). Because growth by means of cell division is more limited by low temperatures than growth via cell expansion, particularly below 10°C (Francis & Barlow, 1988; Körner, 1991, 2003), the correlation of genome size and cell size may allow larger genome species to better exploit cell expansion-based growth and thus be more advantaged during early spring growth periods (Veselý et al., 2012). Although this has never been addressed in the context of species invasions, the widely reported phenomenon of early leaf phenology in many invasive woody species in temperate ecosystems (Harrington et al., 1989; Schierenbeck & Marshall, 1993; Xu et al., 2007) suggests that cell expansion-based growth differences may be more relevant than those based on cell division, particularly in forested habitats where spring leaf phenology is strongly tied to the carbon economy of understory species (Augsperger et al., 2005; Fridley, 2012). To our knowledge, no study has yet addressed linkages of genome size and growth phenology in woody species, despite the demonstrated importance of cell expansion to leaf emergence in trees (Dengler et al., 1975; Kozlowski & Pallardy, 1997).

In this study, we addressed two alternative links between genome size and invasiveness for native and nonnative, invasive woody understory species common to deciduous forests of the eastern USA (EUS; Table 1). First, we hypothesize that woody forest understory invaders are successful in part as a result of unusually fast summer growth rates compared with native species, and that fast growth is enabled by both a high capacity for carbon gain (photosynthetic rate) and a faster rate of cell division allowed by a smaller genome (Fig. 1, left). For this hypothesis, our mechanism of interest is related to unreplicated genome size, which we measured as nuclear DNA content (2C DNA) divided by ploidy level (Grotkopp et al., 2004; Greilhuber et al., 2005). All species in the present study are diploids except for two species of Lonicera (Table 1). Many, but not all, woody invaders in EUS deciduous forests have been shown to have high rates of carbon assimilation compared with congeneric native species (Heberling & Fridley, 2013), so the novelty of the present approach is an assessment of whether such differences in assimilation capacity are linked to differences in canopy growth rate, and whether this general ‘fast return’ strategy necessarily includes small genome size.

Our second hypothesis is that understory species gain advantage from early foliar budbreak phenology, and that such species have relatively large cells that facilitate growth via cell expansion at low spring temperatures (Fig. 1, right). Cell size, in turn, should be a positive function of both genome size and ploidy level, which together determine the total amount of nuclear DNA per cell (2C value) and differentiate ‘genotype size’ from ‘nuclear DNA content’ in hypotheses 1 and 2 (Fig. 1) with respect to two polyploids in the present study. Although early foliar phenology as a general mechanism of EUS deciduous forest invasions has been shown to be less relevant than late-season foliar retention (Fridley, 2012), there remains a core group of forest invaders that do leaf out before most native species (e.g. Elaeagnus umbellata, Lonicera japonica and Rosa multiflora), and, conversely, a core group of native species with exceptionally late leaf-out times in relation to the forest canopy (e.g. Eunonymus americanus, Hamamelis virginiana and Lindera benzoin). It is an open question as to whether nuclear DNA content plays a role in phenological growth differences between woody understory species in a seasonal environment, and whether other genome-based associations—such as mid-season growth rate—may limit the ability of some invaders to exploit early-season cell expansion-based growth.

We evaluated these hypotheses using new measurements of nuclear DNA content for 54 woody shrubs and lianas via flow cytometry, combined with laboratory assays of plant guard cell volume, growth through cell expansion, and growth phenology, summer growth rate, and leaf gas exchange. Our primary objective in the analysis was to compare growth strategies of invaders with those of related native species, thus controlling for broad phylogenetic differences in genome evolution and growth behaviors. Our secondary objective was to better understand relationships between genome size and growth behavior in woody angiosperms, regardless of invasion status. We achieved both objectives by selecting species on the basis of replicated taxonomic groups containing both native and nonnative, invasive species (Table 1), and estimating bivariate relationships jointly in a hierarchical Bayesian model that allowed for phylogenetic autocorrelation in dependent variables (de Villemereuil et al., 2012).

Materials and Methods

Study species

Focal species (Table 1) are part of a long-term common garden study of the growth phenology of native and nonnative shrubs and lianas present in the understory of deciduous EUS forests (Fridley, 2012). DNA extractions and field measurement data come from individuals of 54 species (29 native and 25 nonnative), each represented in three replicate blocks of a shade garden in Syracuse, NY, USA (43°09′ N, 76°09′ W). Species were chosen based on phylogenetically controlled contrasts of EUS native and naturalized nonnative species within several genera, and contrasts of nonnative species with widespread but unrelated EUS forest shrubs (e.g. Lindera benzoin and Hamamelis virginiana). All species are present in EUS forests or woodlands, and all but...
Table 1  Focal species of the present study, with nonnative species in bold

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>2C DNA</th>
<th>Cell L</th>
<th>Cell W</th>
<th>Leaf rate</th>
<th>$A_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caprifoliaceae</td>
<td>Lonicera canadensis</td>
<td>2.42</td>
<td>29.46</td>
<td>22.98</td>
<td>0.52</td>
<td>9.76</td>
</tr>
<tr>
<td></td>
<td>Lonicera fragrantissima</td>
<td>1.83</td>
<td>22.92</td>
<td>20.78</td>
<td>3.25</td>
<td>17.17</td>
</tr>
<tr>
<td></td>
<td>Lonicera hirsuta</td>
<td>2.32</td>
<td>22.64</td>
<td>15.42</td>
<td>0.89</td>
<td>14.38</td>
</tr>
<tr>
<td></td>
<td>Lonicera involucrata</td>
<td>2.48</td>
<td>29.61</td>
<td>21.69</td>
<td>0.75</td>
<td>14.36</td>
</tr>
<tr>
<td></td>
<td>Lonicera japonica</td>
<td>1.82</td>
<td>23.41</td>
<td>20.25</td>
<td>3.25</td>
<td>15.46</td>
</tr>
<tr>
<td></td>
<td>Lonicera korolkowii</td>
<td>1.50</td>
<td>21.52</td>
<td>19.55</td>
<td>4.80</td>
<td>17.90</td>
</tr>
<tr>
<td></td>
<td>Lonicera maackii</td>
<td>1.57</td>
<td>26.61</td>
<td>18.90</td>
<td>3.56</td>
<td>15.53</td>
</tr>
<tr>
<td></td>
<td>Lonicera morrowii</td>
<td>1.72</td>
<td>18.62</td>
<td>14.04</td>
<td>2.31</td>
<td>14.63</td>
</tr>
<tr>
<td></td>
<td>Lonicera oblongifolia</td>
<td>2.58</td>
<td>23.63</td>
<td>19.92</td>
<td>0.52</td>
<td>12.97</td>
</tr>
<tr>
<td></td>
<td>Lonicera periclymenum</td>
<td>5.94†</td>
<td>35.25</td>
<td>26.53</td>
<td>2.83</td>
<td>19.42</td>
</tr>
<tr>
<td></td>
<td>Lonicera ruprechtiana</td>
<td>1.67</td>
<td>21.17</td>
<td>15.64</td>
<td>1.72</td>
<td>17.76</td>
</tr>
<tr>
<td></td>
<td>Lonicera sempervirens</td>
<td>4.12‡</td>
<td>31.52</td>
<td>26.41</td>
<td>1.89</td>
<td>16.68</td>
</tr>
<tr>
<td></td>
<td>Lonicera standishii</td>
<td>1.95</td>
<td>22.43</td>
<td>17.06</td>
<td>0.90</td>
<td>15.65</td>
</tr>
<tr>
<td></td>
<td>Lonicera tatarica</td>
<td>1.67</td>
<td>21.87</td>
<td>14.25</td>
<td>0.58</td>
<td>10.94</td>
</tr>
<tr>
<td></td>
<td>Lonicera × bella</td>
<td>1.77</td>
<td>21.63</td>
<td>17.25</td>
<td>0.46</td>
<td>12.76</td>
</tr>
<tr>
<td></td>
<td>Lonicera xylsteuim</td>
<td>1.90</td>
<td>25.53</td>
<td>19.32</td>
<td>1.18</td>
<td>14.65</td>
</tr>
<tr>
<td>Celastraceae</td>
<td>Celastrus orbiculatus</td>
<td>0.77</td>
<td>29.58</td>
<td>23.31</td>
<td>1.22</td>
<td>15.70</td>
</tr>
<tr>
<td></td>
<td>Celastrus scandens</td>
<td>0.85</td>
<td>30.57</td>
<td>24.18</td>
<td>1.23</td>
<td>15.41</td>
</tr>
<tr>
<td></td>
<td>Euonymus alatus</td>
<td>1.35</td>
<td>25.83</td>
<td>24.91</td>
<td>0.75</td>
<td>15.47</td>
</tr>
<tr>
<td></td>
<td>Euonymus americanus</td>
<td>1.39</td>
<td>28.63</td>
<td>26.49</td>
<td>0.31</td>
<td>11.45</td>
</tr>
<tr>
<td></td>
<td>Euonymus atropurpureus</td>
<td>0.83</td>
<td>29.49</td>
<td>24.04</td>
<td>0.48</td>
<td>10.11</td>
</tr>
<tr>
<td></td>
<td>Euonymus bungeanus</td>
<td>0.88</td>
<td>28.76</td>
<td>22.08</td>
<td>0.58</td>
<td>14.00</td>
</tr>
<tr>
<td></td>
<td>Euonymus europaeus</td>
<td>1.77</td>
<td>32.04</td>
<td>24.84</td>
<td>0.31</td>
<td>13.86</td>
</tr>
<tr>
<td></td>
<td>Euonymus hamiltonianus</td>
<td>0.89</td>
<td>33.95</td>
<td>25.70</td>
<td>0.48</td>
<td>13.66</td>
</tr>
<tr>
<td></td>
<td>Euonymus obovatus</td>
<td>0.98</td>
<td>27.61</td>
<td>20.38</td>
<td>0.64</td>
<td>10.20</td>
</tr>
<tr>
<td></td>
<td>Euonymus phellomanus</td>
<td>1.31</td>
<td>31.80</td>
<td>26.79</td>
<td>1.01</td>
<td>13.68</td>
</tr>
<tr>
<td>Elaeagnaceae</td>
<td>Elaeagnus angustifolia</td>
<td>1.27</td>
<td>23.94</td>
<td>16.76</td>
<td>2.29</td>
<td>14.19</td>
</tr>
<tr>
<td></td>
<td>Elaeagnus commutata</td>
<td>1.33</td>
<td>22.28</td>
<td>17.86</td>
<td>0.92</td>
<td>18.15</td>
</tr>
<tr>
<td></td>
<td>Elaeagnus multiflora</td>
<td>1.60</td>
<td>19.59</td>
<td>16.29</td>
<td>3.91</td>
<td>15.91</td>
</tr>
<tr>
<td></td>
<td>Elaeagnus umbellata</td>
<td>1.61</td>
<td>20.08</td>
<td>18.09</td>
<td>3.28</td>
<td>17.31</td>
</tr>
<tr>
<td></td>
<td>Shepherdia argentea</td>
<td>3.08</td>
<td>28.89</td>
<td>21.33</td>
<td>2.54</td>
<td>19.40</td>
</tr>
<tr>
<td>Rhamnaceae</td>
<td>Frangula alnus</td>
<td>0.71</td>
<td>23.51</td>
<td>15.85</td>
<td>1.43</td>
<td>12.85</td>
</tr>
<tr>
<td></td>
<td>Frangula caroliniana</td>
<td>0.87</td>
<td>22.39</td>
<td>18.18</td>
<td>1.44</td>
<td>10.98</td>
</tr>
<tr>
<td></td>
<td>Rhamnus alnifolia</td>
<td>0.58</td>
<td>23.56</td>
<td>15.25</td>
<td>0.67</td>
<td>13.49</td>
</tr>
<tr>
<td></td>
<td>Rhamnus cathartica</td>
<td>1.08</td>
<td>23.92</td>
<td>15.23</td>
<td>0.36</td>
<td>15.08</td>
</tr>
<tr>
<td></td>
<td>Rhamnus davurica</td>
<td>1.06</td>
<td>28.29</td>
<td>19.46</td>
<td>0.77</td>
<td>16.13</td>
</tr>
<tr>
<td></td>
<td>Berberis vulgaris</td>
<td>3.55</td>
<td>29.58</td>
<td>23.10</td>
<td>1.21</td>
<td>16.12</td>
</tr>
<tr>
<td>Sapindaceae</td>
<td>Acer pensylvanicum</td>
<td>2.24</td>
<td>21.57</td>
<td>14.80</td>
<td>0.24</td>
<td>12.03</td>
</tr>
<tr>
<td></td>
<td>Acer spicatum</td>
<td>1.69</td>
<td>22.01</td>
<td>14.49</td>
<td>0.88</td>
<td>14.37</td>
</tr>
<tr>
<td>Calycanthaceae</td>
<td>Calycanthus floridus</td>
<td>1.96</td>
<td>26.84</td>
<td>16.78</td>
<td>0.38</td>
<td>14.06</td>
</tr>
<tr>
<td>Clethraceae</td>
<td>Clethra acuminata</td>
<td>2.28</td>
<td>19.64</td>
<td>13.77</td>
<td>0.67</td>
<td>12.78</td>
</tr>
<tr>
<td>Cornaceae</td>
<td>Cornus alternifolia</td>
<td>1.93</td>
<td>23.37</td>
<td>16.94</td>
<td>1.19</td>
<td>14.13</td>
</tr>
<tr>
<td></td>
<td>Cornus amomum</td>
<td>1.91</td>
<td>23.24</td>
<td>16.69</td>
<td>1.88</td>
<td>14.03</td>
</tr>
<tr>
<td></td>
<td>Cornus florida</td>
<td>2.94</td>
<td>25.61</td>
<td>19.58</td>
<td>0.67</td>
<td>12.31</td>
</tr>
<tr>
<td>Diervillaceae</td>
<td>Diervilla lonicera</td>
<td>2.28</td>
<td>30.28</td>
<td>22.46</td>
<td>1.08</td>
<td>14.63</td>
</tr>
<tr>
<td></td>
<td>Diervilla rivilaris</td>
<td>2.27</td>
<td>28.70</td>
<td>20.89</td>
<td>0.57</td>
<td>13.29</td>
</tr>
<tr>
<td>Thymelaeaceae</td>
<td>Dicta palustris</td>
<td>3.29</td>
<td>37.81</td>
<td>30.18</td>
<td>0.29</td>
<td>8.36</td>
</tr>
<tr>
<td>Hamamelidaceae</td>
<td>Hamamelis virginiana</td>
<td>1.97</td>
<td>31.69</td>
<td>21.77</td>
<td>0.25</td>
<td>11.76</td>
</tr>
<tr>
<td>Hydrangeaceae</td>
<td>Hydrangea arborescens</td>
<td>2.46</td>
<td>22.42</td>
<td>15.94</td>
<td>0.56</td>
<td>16.54</td>
</tr>
<tr>
<td>Laureaceae</td>
<td>Lindera benzoin</td>
<td>3.03</td>
<td>25.43</td>
<td>15.95</td>
<td>0.34</td>
<td>14.71</td>
</tr>
<tr>
<td>Rutaceae</td>
<td>Ptelea trifoliata</td>
<td>1.64</td>
<td>24.57</td>
<td>15.32</td>
<td>0.43</td>
<td>13.81</td>
</tr>
</tbody>
</table>

*Lianas.
†Hexaploid.
‡Tetraploid.
*For authority, see USDA, NRCS (2015).

Summary data include nuclear DNA content (2C; pg), mean length (L) and width (W) of guard cells (μm), maximum leaf production rate in summer (new leaves d$^{-1}$), and maximum area-based photosynthetic rate ($A_{\text{max}}$; μmol CO$_2$ m$^{-2}$ s$^{-1}$).
six of the 25 naturalized species are managed as invaders in the EUS (for details on invader and habitat designations, see Fridley, 2008). *Berberis canadensis* was initially present in the garden as the native congener of *Berberis thunbergii* and *Berberis vulgaris*, but has subsequently been removed from analyses because of suspected hybrid origin. In addition, 13 species of *Viburnum* in the garden were not used for DNA analysis as a consequence of the presence of DNA-staining inhibitors that preclude easy estimates of nuclear DNA content from flow cytometry analysis (Jedrzejczyk & Sliwinska, 2010).

Individuals were established in the common garden from transplants in 2006–2007, collected locally from wild populations when possible and where necessary purchased commercially from growers at similar latitude (including Forestfarm Nursery, Williams, OR and Musser Forests, Indiana, PA, USA; see supplementary data in Fridley, 2012). Individuals were spaced 1 m apart under 80% neutral shade cloth (black knitted polypropylene; DeWitt Co., Sikeston, MO, USA), deployed seasonally to mimic local canopy closure and leaf fall phenology (c. 20 May and 24 October, respectively).

Spring leaf phenology

We monitored spring bud and leaf development of each plant in 2008–2010 by taking photographs of selected leaf nodes at 2–5 d intervals from early March to mid-May. Buds were classified into development stages of: (1) dormant; (2) active (apparent bud swelling, scale development, visibility of inner scales, or scales changing in color); (3) exposed (inner bud tissue apparent, including secondary cataphylls, transitional leaves, or tips of first leaves; first leaf reflexion in species lacking bud scales); (4) budbreak (‘burst’; general loosening of all bud structures including inner leaves, and some exposure of leaf lamina; second leaf reflexion in species lacking bud scales); and (5) flushed/true leaf (full laminar surface of true leaf visible).

Photosynthetic capacity and growth rate

Photosynthetic rates of selected leaves of each individual were measured monthly (2008–2010) using photosynthetic light curves (Li-Cor 6400 with red-blue LED light source; 400 μmol CO₂ mol⁻¹, 700 μmol s⁻¹ flow rate, 20°C; Li-Cor Biosciences, Lincoln, NE, USA) with equilibration at 800 μmol photon m⁻² s⁻¹ and descending to 300, 100, and 50 μmol photon m⁻² s⁻¹ for 2 min each. Photosynthetic capacity of each species was estimated with the procedure described in Fridley (2012), involving fitting of light response data to the four-parameter nonrectangular hyperbolic function (Lambers et al., 1998) for each measurement period. Light-asymptotic ($A_{max}$) values used in the present study are means of an average of 24 light curves for each species (2008–2010).

The maximum growth rate of each species was estimated as leaf production rate from leaf counts on growing branches monitored at 2-wk intervals over each growing season (2008–2010). Five healthy terminal branches were monitored on each plant (max. 15 per species yr⁻¹), and total extant leaves on existing buds and new shoots on each branch were counted at each interval. A new leaf was counted once it had reflexed by 20°. The maximum rate of leaf production for each species was estimated as the annual maximum number of new leaves produced by any branch over any 2-wk period after initial leaf flush, standardized to leaves d⁻¹, and averaged over the 3-yr period.

Cell size

We used guard cell length, width, and area as indices of cell size for each species because of the consistency of stomatal size among epidermal cell types and their presumed low rate of endopolyploidy (Melaragno et al., 1993). We prepared slides of the abaxial surfaces of one to two mature leaves of individuals of each species using epidermal peels. In several instances where peels yielded insufficient epidermal area for analysis, we created slides of epidermal impressions with clear ethyl acetate applied to the abaxial leaf surface. Mounted epidermal peels or epidermal impressions were photographed at ×40 with a Moticam 2000 digital camera (Motic, Richmond, British Columbia, Canada) mounted on a Zeiss Axiosar Plus microscope (Bio-Rad, Cambridge, MA, USA). For random fields of view in each image, we measured the length and width of closed guard cells of 40 stomata (maximum 5 per field of view) using Motic Images Plus 2.0 software (Motic, Richmond, BC, Canada). Reported measurements are species means across individuals.

Nuclear DNA content

We measured nuclear 2C DNA content via flow cytometry following the protocol of Doležel et al. (2007). Leaf samples were collected from experimental individuals immediately before
sample preparation and kept on ice. Leaf material (c. 30 mm²) was chopped according to Galbraith et al. (1983), using the co-chopping technique for internal standardization. Leaves were stacked in a Petri dish placed on ice, and covered with 1–2 ml of one of several buffers, including those of Galbraith et al. (1983), Arumuganathan & Earle (1991), and Loureiro et al. (2007). Leaves were minced with razor blades for 1 min and allowed to sit briefly in buffer. Extract was removed and filtered into a cap tube through a 30-µm mesh nylon filter (Spectrum Laboratories, Rancho Dominguez, CA, USA). The extract was centrifuged at 16 000 g for 30 min and the supernatant was discarded; 400 µl of 50 µg RNA digestion was allowed to take place overnight at 4°C vortexed. Cap tubes were placed in a slurry of ice and water, and RNA digestion was allowed to take place overnight at −1.0°C. Cap tubes were centrifuged at 16 000 g for 30 s and the supernatant was discarded; 500 µl of 100 µg propidium iodide ml⁻¹ buffer solution was added and tubes were vortexed. Cap tubes were placed in a slurry of ice and water, and RNA digestion was allowed to take place overnight at −1.0°C. Cap tubes were centrifuged at 16 000 g for 30 s and the supernatant was discarded; 500 µl of 100 µg propidium iodide ml⁻¹ buffer solution was added, and tubes were vortexed. Time from staining to sample analysis ranged from 45 min to 2.5 h, during which time samples were kept in a slurry of ice and water. Samples from recalcitrant species were prepared and run with alternate buffers, standards, amounts of material and additives for controlling the effects of cytosolic compounds.

Samples were analyzed with a Becton Dickinson LSR II flow cytometer (BD Biosciences, San Jose, CA, USA). The instrument was set to record 10 000 events with forward scatter (FSC) of PI-stained particles thresholded at 5000, fluorescence intensity (FI) thresholded at 2000, and side scatter (SSC) thresholded at 2000. We collected FSC, SSC, and FI parameters using FACSDIVA software (BD Biosciences). At each session, the voltage of the FI parameter was set according to an initial run of the standard nuclei alone and kept the same for each sample that included that parameter. Channels encompassing the G1 and G2 peaks of test and standard species were gated to eliminate the sometimes large quantity of debris at very low fluorescence. Standard species included *Lyopersicon esculentum* ‘Stupke’ (1.96 pg), *Glycine max* ‘Polanka’ (2.5 pg), and *Pisum sativum* ‘Ctirad’ (8.75 pg).

FCS output files were converted to text files in WinMDI 2.8 (J. Trotter, The Scripps Institute, La Jolla, CA, USA) and imported into R 2.03 (R Core Team, 2014) to calculate 2C values from FI parameter distributions. The mean FI values of the test and standard G1 peaks were determined using a bootstrapping technique (1000 random samples with replacement) and converted to nuclear content as:

\[ 2C \text{DNA(pg)} = \frac{\text{sample G1 mean FI}}{\text{standard G1 mean FI}} \times \text{standard 2C value} \]

The percentage coefficient of variation (%CV) for each test species distribution was calculated at half height of the peak, following Marie & Brown (1993). Runs were considered successful if they met the following criteria: > 1000 events; low debris; < 50 channel shift of internal standard when compared with a standard run in isolation; and CV < 5%. Final estimation of genome size for each species was the average of four runs. A list of all 2C DNA estimates for each species, along with standards and buffers used for each run, is presented in Supporting Information Table S1.

**Statistical analysis**

Genome size was measured as the 2C value divided by ploidy, accounting for two polyploids in our study: *Lonicera periclymenum* (hexaploid) and *Lonicera sempervirens* (tetraploid). Because our five stages of the timing of spring bud and leaf development were highly correlated, we present results only for stage 4 (budbreak), the most commonly used measure of spring foliar phenology. Similarly, among cell size metrics we present results only for guard cell length; use of cell width or area gave qualitatively similar results (see Fig. S1). All traits except budbreak were log-transformed for analysis.

**Phylogenetic relationships**

We created a phylogenetic tree of our 54 target species using Phylocomatic (Webb & Donoghue, 2005). Intragenic assignments of *Lonicera* species are based on Smith (2009) and Smith & Donoghue (2010). *Lonicera canadensis* is not yet available in a molecular phylogeny; rather than exclude this species, it was placed according to the original morphological treatment of Rehder (1903), which overall remains consistent with recent molecular phylogenies (Theis et al., 2005). Intragenic molecular phylogenies are not yet available for the majority of our species of *Rhamnus, Elaeagnus*, or *Euonymus* (cf Simmons et al., 2012), which we treat here as polytomies. Branch lengths were estimated using the Phylocomatic BLADJ algorithm (Webb et al., 2008) based on the node ages ofWikström et al. (2001). The phylogeny used for analysis is presented as Fig. S2.

**Hypothesis testing**

We used a hierarchical Bayesian (HB) approach for testing relationships between traits as described in Fig.1 in a single modeling framework. This approach offers several advantages over other bivariate or multivariate methods. First, it allows us to estimate species-level differences in budbreak phenology despite the occurrence of missing values in a given year, without the need for applying year-based corrections (cf Panchen et al., 2014). Secondly, it allows us to fit slope parameters across traits simultaneously without concerns of multiple testing or P-value correction. Thirdly, it allows us to take advantage of simultaneous estimation of phylogenetic signal (λ; Pagel, 1999) across dependent variables in a single analysis, and incorporate phylogenetic autocorrelation across all relationships using a common correlation matrix (Σ) based on shared branch lengths in the phylogeny (de Villemereuil et al., 2012). Finally, posterior distributions of our hypothesized relationships in Fig.1 (bf s) are a direct statement of the probability of our hypotheses.

Our model includes four dependent variables, of which three are continuous and one is a binary variable of whether the species is native or invasive:
Continuous variables are normally distributed with mean $\mu$, variance $\sigma^2$, and correlation structure $\Sigma$. Note that we treat ‘native’ or ‘invasive’ as a dependent variable in this framework, consistent with our hypothesis structure in Fig. 1, but one could instead compare the distribution of covariates (e.g. growth rate and budbreak) between natives and invaders with equivalent results. Regression components of the model follow Fig. 1 and are of the form:

$$growth\ rate_i \sim N(\mu_{growth\ rate\ i}; \sigma^2_{growth\ rate}; \Sigma)$$

$$cell\ size_i \sim N(\mu_{cell\ size\ i}; \sigma^2_{cell\ size}; \Sigma)$$

$$budbreak_i \sim N(\mu_{budbreak\ i}; \sigma^2_{budbreak}; \Sigma)$$

$$invasive_i \sim Bernoulli(p_i)$$

where the terms are $\lambda$ for the intercept and $\beta$ for the slope refer to relationships in Fig. 1, and growth rate, cell size, and invasive vectors of elements refer to species values ($i$) in Table 1. Budbreak values were standardized between years by modeling them in a random effects-only model with year, species, and the overall mean as predictor variables, and extracting posterior means for fixed effect coefficients, and gamma priors (rate = 1; shape = 1) for the precision of random effects of phylogenetic autocorrelation, based on de Villemereuil et al. (2012). Regression goodness-of-fit statistics (pseudo-$R^2$) were calculated as the coefficient of determination of fitted versus observed values of dependent variables. R code implementing the model is available in Notes S1.

**Results**

Consistent with hypothesis 1, summer growth rate was lower in large-genome species (Fig. 2a; $\beta_1$, Fig. 3) and higher in species with high photosynthetic capacity ($A_{max}$, Fig. 2b; $\beta_2$, Fig. 3). The mean effect size of standardized $A_{max}$ (0.64) was more than double that of genome size ($-0.24$), suggesting that assimilation rate is a larger driver of maximum shrub growth rate than genome size constraints. Although the 95% credible interval (2.5–97.5 percentiles) for the genome size coefficient posterior ($\beta_1$) overlapped zero (Fig. 3), there was strong evidence that genome size contributes to growth rate ($R(\beta_1 < 0) = 0.96$). Together, these variables explained about a third of the variation in summer whole-plant growth rate among species (pseudo-$R^2 = 0.34$). Growth rate in turn was a strong predictor of whether 54 shrub and liana species were native or invasive ($\beta_3$, Fig. 3). Using an invasiveness probability of 50% from the fitted model, growth rate correctly distinguished between native and invader identity in all but 16 species (70% accuracy; nine false positives and seven false negatives).

Consistent with hypothesis 2, species of high nuclear DNA content had larger cell sizes (Fig. 2c; $\beta_4$, Fig. 3), and species of large cell sizes exhibited earlier budbreak (Fig. 2d; $\beta_5$, Fig. 3). However, early-budbreak species were not more likely to be invasive than late-budbreak species ($\beta_6$, Fig. 3), suggesting that, although nuclear DNA content is linked to spring leaf phenology in understory shrub species, consistent with a cell expansion mechanism, spring phenology per se is not linked to invasiveness.

Estimated values of phylogenetic signal (Pagel’s $\lambda$) for dependent variables in the model suggest that growth rate and budbreak phenology behaviors are more conserved across the phylogeny than cell size, although all exhibit significant phylogenetic autocorrelation (inset, Fig. 3).

**Discussion**

**Genome size and temperate forest invasions**

Our analysis of spring and summer growth behaviors of native and invasive woody understory species supports small genome size as a driver of forest invasions through the mechanism of fast summer growth (hypothesis 1; Fig. 1), and rejects hypothesis 2, whereby invasive species of larger genomes are able to maintain earlier spring leaf phenology. Invaders of EUS forests do not as a group leaf out earlier than related or unrelated native forest species, but rather have significantly smaller genomes, have higher capacities for photosynthesis, and grow new leaves and shoots at a faster rate during peak summer growth periods. This supports the prevailing idea that plant invaders generally have small genomes (Bennett et al., 1998; Chen et al., 2010; Kubečková et al., 2015 New Phytologist Trust).
2010; Pandit et al., 2014), as a result of selection on fast-growing species to maintain small genomes that can be replicated quickly during mitosis (Grotkopp et al., 2004; Cavalier-Smith, 2005). It is noteworthy that this remains true of invaders common to relatively stressful ecosystems such as forest understories, where shade tolerance rather than fast growth is presumably an important component of fitness (Martin et al., 2008). We further note, however, that some of the invaders in our study are also abundant in more open habitats (particularly Lonicera spp.), which may be partly responsible for the support of hypothesis 1 in our data set.

Exceptions to the ‘small genome invader’ rule in our focal species are notable. Invasive barberries (B. thunbergii and B. vulgaris) have relatively large genomes, and B. thunbergii has nonetheless a high potential growth rate. Conversely, several invaders have relatively high photosynthetic capacities but low rates of leaf growth, including Euonymus spp., Lonicera × bella, and Rhamnus cathartica; this is particularly surprising for the latter two as members of genera that are otherwise represented by fast-growing species (including Lonicera morrowii and Lonicera tatarica, the parents of L. × bella). Given that most of these invaders and nearly all of the others in our study also exhibit significantly delayed autumnal leaf senescence, which has been shown to drive greater annual carbon gain in these species (Fridley, 2012), it is likely that summer growth rate per se decreases in importance for invaders as the density of the canopy increases. Berberis thunbergii, for example, has a relatively large genome but is exceptionally shade tolerant and able to rapidly adjust leaf physiology to changing seasonal light conditions (Xu et al., 2007).

We were also surprised to discover that our core group of EUS native understory species, and particularly those unrelated to EUS invaders, couple relatively large genomes with both slow growth and late growth phenology. This includes some of the most abundant and widespread natives in EUS forests, such as L. benzoin, Cornus florida, and Hydrangea arborescens, as well as more geographically restricted and taxonomically unusual species such as Calycanthus floridus and Dirca palustris. What is the advantage of having both a slow rate of canopy expansion and late growth phenology that takes little advantage of early spring high light intensities? One hypothesis is that the leaf physiology of these species is highly specialized for a low light environment; such species are expected to have low growth rates but high photosynthetic capacities in the shade.
metabolic and assimilation rates, and as a consequence would be damaged by high light intensities before canopy closure (Demmig-Adams & Adams, 2006). Consistent with this hypothesis are the overall low levels of $A_{\text{max}}$ of these species in our study, their low-statured growth form (i.e. multi-branched (e.g. Lindera and Hydrangea) or layered canopy (e.g. Cornus)), and their strong association with late successional forests of low light availability (Gleason & Cronquist, 1991). The larger genome of these species may have arisen as a result of weak selection on maintaining a small genome for fast growth. Given that this strategy is common to many EUS forest understory natives, it is curious that there is not an obvious forest invader with these traits, although the small-genomed Euonymus alatus also has a low growth rate and late spring budbreak phenology. We suspect that there are more such species present in EUS as ornamentals, but they are not yet invasive because of the low propagule pressure of introduced species into the darker interiors of less disturbed forest stands.

Genome size as an indicator of growth phenology in woody angiosperms

The relationship we illustrate between nuclear DNA content and spring leaf emergence is, to our knowledge, the first such demonstration for woody species, and suggests that growth at low temperatures through cell expansion may be an important feature of both herbaceous and woody communities in temperate regions (Grime & Mowforth, 1982; Grime et al., 1985). This is surprising given the relatively small range of 2C values for herbaceous species (Hodgson et al., 2010), and woody angiosperms in particular (only c. 7% of the total range of 2C values for herbaceous angiosperms; Ohri, 2005). In this study, 2C values were highest for the two polyploid Lonicera lianas (up to 5.94 pg for L. periclymenum), and between 0.5 and 4 pg for diploids. By contrast, species in European grasslands characteristic of early spring versus mid-summer peak growth span a range larger by nearly an order of magnitude; compare for example the early spring-active Helictotrichon pratense (36 pg) with the summer-growing Brachypodium pinnatum (2.5 pg), both calcareous grasses (Grime et al., 1988). Our results suggest that even small differences in nuclear DNA content between closely related species can have implications for growth phenology.

If one mechanism of leafing early in a deciduous forest understory is through a selective increase in cell size promoting cell expansion at low temperatures, and cell size increases can occur from one generation to the next through the accumulation of nongenic nuclear DNA (Lynch, 2007), a potential consequence is a reduction in maximum growth rate from increased mitotic duration (Gregory, 2005). Whether this is an important constraint on fitness depends on whether growing season growth rate contributes to a species’ long-term survival or reproductive capacity, and how this varies with respect to environmental properties. In cool temperate ecosystems, high seasonal light availability and the relatively fertile substrate of young soils should be ideal conditions for generating distinct functional groups of ‘small-genome fast growers’ and ‘large-genome early growers’, often present as spring geophytes in woodlands (Veselý et al., 2012) and spring-flowering C3 grasses in grasslands (Grime et al., 1985). In more resource-limited systems, growth rate per se may be less relevant to fitness, with a shift in the importance of early season growth, particularly in a highly seasonal light environment (Augspurger et al., 2005).

There was no evidence of a trade-off between summer growth rate and the capacity for early spring leaf growth across the 54 woody species in our study. To the contrary, species of higher summer growth rate exhibited earlier leaf emergence, whether or not phylogenetic autocorrelation was included in the regression (Fig. 4). Thus, despite significant associations between genome size and growth rate ($\beta_1$) on the one hand, and significant associations between nuclear DNA content and spring budbreak on the other ($\beta_4$ and $\beta_5$), such relationships are not strong enough to generate a trade-off between spring and summer growth potentials in woody plants, at least for our focal understory species. We suggest that the stronger trade-off at play here concerns adaptations for sun versus shaded conditions, in that species that emerge early in high spring light conditions should exhibit high photosynthetic capacity (high leaf nitrogen concentration and specific leaf area), the same leaf investment strategy adopted by species that are able to grow quickly through cell division in summer. Consistent with this, species in the upper left corner of Fig. 4 are members of shade-tolerant genera (e.g. Euonymus) or are natives associated with shaded habitats as described above. This is in contrast to temperate herbaceous species in less light-limited habitats, where we expect the spring–summer growth trade-off, mediated by genome size, to be more apparent (Grime et al., 1985).
Conclusions

For native and invasive shrubs and lianas of EUS deciduous forests, we demonstrate that species of larger genomes exhibit earlier spring budbreak phenology, a behavior that is better represented by native species. Invaders, by contrast, exhibit fast canopy growth during the growing season, and, as is typical of invaders across many other habitat types, have smaller genomes that allow faster rates of growth via cell division. Establishing whether genome size in woody plants is related to leaf development via cell expansion during cool spring temperatures, as has been demonstrated for herbaceous species, requires additional measurements of the development of leaf primordia in large- and small-genome species. Although there does not appear to be a fundamental trade-off in spring phenology and summer growth rate due to their opposing correlation with genome size, genome size nonetheless appears to be an important indicator of growth behavior and the native-invader contrast in woody, shade-tolerant species.

Acknowledgements

We gratefully acknowledge garden assistance from S. Anderson, E. Fridley, S. Kelley, and A. Surace. We thank R. Duncan for modeling advice, and the many who provided insights into the ecological role of genome size in plants, including J. P. Grime, J. Hodgson, and members of the Fridley Lab at Syracuse. We also thank two anonymous referees, D. Ackerly, and J. Friedman for providing valuable comments on the manuscript. The Syracuse Shrub Garden was established with funds from Syracuse University.

References


Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Relationship of mean stomatal length and width, along with SD, for 40 measurements per species.

Fig. S2 Phylogenetic tree of 54 native and nonnative species used in this study.

Table S1 2C DNA values for individual flow cytometry measurements for each species, along with species standards and chemical buffers used for each run.

Notes S1 R code for hierarchical Bayesian model estimating relationships described in Fig. 1, fitted via JAGS using the R2JAGS library.

Please note: Wiley Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the New Phytologist Central Office.