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Cultivar selection prior to introduction may increase invasiveness: evidence from *Ardisia crenata*

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Abstract

Ardisia crenata (Myrsinaceae), an evergreen shrub with attractive red fruits introduced from Japan to the USA for ornamental purpose, invades the understory of mesic hardwood forests, forming dense patches (up to 300 stems per m^2), and competitively displaces native understory plants by creating dense local shade. Comparison of the wild genotype that grows in mature evergreen broadleaf forests in central Kyushu, Japan, with the ecotype invading north central Florida revealed how selection for desirable cultivars might have inadvertently selected for traits that enhance the invasive potential of the species. In Japanese wild populations in deeply shaded evergreen forests, natural selection apparently maintained efficient architecture with a low degree of self-shading and large seed mass to enhance seedling shade tolerance. Cultivar selection for showy appearance can explain the greater fecundity but smaller seed size observed in the Florida populations compared to the Japanese population. Artificial selection for densely foliated appearance can also explain the greater degree of self-shading and less-efficient light use in the Florida genotype compared to the Japanese wild type grown under a common environment. Furthermore, the Florida ecotype allocated more biomass to root carbohydrate storage. These trait modifications resulted in slower growth rates, but greater competitive ability to cast shade upon neighbors and higher resprouting potential in the Florida populations. How traits are modified through the processes of artificial selection and cultivation must be taken into consideration in the evolutionary ecology of many other invasive plants introduced as ornamental plants.

Introduction

The majority of invasive non-indigenous plants in the USA have been deliberately introduced for agricultural and horticultural purposes (Mack and Erneberg 2002; Mack 2003). In Florida, the percentage of invasive species introduced as ornamental species is particularly high, due to active horticultural importation of both temperate and tropical species (Mack 2003). A total of 69% of the Category 1 Plant Pest species in Florida (species identified as altering natural communities, according to the Florida Plant Pest Council, FLEPPC, in 2001, updated from Gordon and Thomas 1998) were introduced as ornamental plants.

The fact that many invasive species are of ornamental origin may have a particular relevance in the ecological behavior of invading plant populations. The practice of cultivation may directly and indirectly affect the invasive potential of introduced plants. For example, the reproductive phenology of agricultural weeds can be highly selected by the cultivation regime of the crop with which they are associated (Barrett 1983). During the initial phase of invasion when environmental stochasticity is most likely to lead to the extirpation of small immigrant populations, cultivation by humans fosters the establishment of the minimal critical population size (Mack 2000). However, little is known about how artificial selection for desirable traits during cultivation may have modified the traits of wild genotypes prior to introduction. Artificial selection for greater reproductive output increases reproductive allocation and fecundity in cultivars (Harlan 1975). This change, in turn, may enhance colonization potential of introduced plants once they escape cultivation and become naturalized. Artificial selection for tolerance of greater geographical range may have increased the potential geographical area that escaped cultivars may invade. Selection for greater tissue concentration of medicinally useful secondary chemicals may increase allelopathy and pest resistance in cultivars that may act as 'novel weapons' (*sensu* Bais et al. 2003; Callaway et al. 2004) and, hence, increase their competitiveness.

Several common garden experiments have demonstrated significant genotype differences between the introduced and native range of invasive plant species (Blossey and Notzold 1995; Siemann and Rogers 2001; Wolfe 2002). It is often hypothesized that lack of natural enemies and changes in selective regimes result in the rapid evolution of reduced defensive traits and faster growth rates (Evolution of Increased Competitive Ability or EICA hypothesis, Blossey and Notzold 1995). Alternatively, difference in mean genotype between native and introduced range may be attributable to the founder effect, because an introduced population is usually derived from a small subset of genotypes in the native range (Muller-Scharer et al. 2004). Cultivar selection prior to introduction may contribute such that founding populations in the introduced range represent a non-random subset of genotypes in the native range. In the native country, wild populations consist of genotypes that tolerate an array of biotic and abiotic stresses, which may be achieved at the cost of less biomass allocation to reproduction. Since cultivated plants are protected by humans, it is possible to select for higher reproductive output even at the cost of lowered stress tolerance and defense. These potential biases in founding populations that descended from a cultivar have rarely been addressed.

The invading population of *Ardisia crenata* Sims. (Myrsinaceae) in the SE United States is clearly of cultivar origin. It is an evergreen shrub whose natural range extends from northern India (20°N) to 36°N along the coasts where the climate is mild in Japan (Ohwi 1984). In China, it is known as zhu-sha-gen (meaning 'red sand roots') and widely cultivated for medicinal purpose because of high concentrations of triterpenoid saponins in the roots (Jia et al. 1994). It is also cultivated for ornamental purpose, including *A. crenata* var. *hortensis* known as 'rich seed' variety that exhibits high fecundity (Shanghai Garden Online, http://www.lvhua.com/chinese/flora). In Japan, where it is known as manryo, as many as 80 cultivars were isolated from mutant seedlings during the horticultural boom of 1800′ (Souga 1989), of which 35 are still listed in horticultural literature (Tsukamoto 1980). But only three cultivars appear to be commercially available in the USA (Lee et al. 2003; also http://www.cultivar.org). The most commonly planted cultivar resembles the invasive ecotype in Florida in overall appearance with dense green foliage, crenate leaf margin and showy red fruits. The other two varieties (one with white fruits and another with variegated leaves) are extremely rare in the naturalized populations (KK and AMF, personal observations). According to published horticultural catalogues, *A. crenata* was imported to the USA by the early 1900s (Dozier 1999). It quickly became popular as Christmas berry or coral berry (Bailey 1922) and widely planted in shady yards (Conover and Poole 1989), but there was no evidence of horticultural selection within the USA. Due to ease of propagation from seeds and cuttings (Watkins and Sheehan 1975), it was perhaps not necessary to repeat importation after this initial introduction. Individuals that escaped cultivation have invaded the mesic forest understory in SE United States, often forming dense monodominant patches (Langeland and Burks 1998).

The first objective of our study was to compare local density and seedling recruitment patterns between Florida populations that descended from a cultivar and the Japanese wild genotype of *A. crenata*. To explore the reasons for much lower seedling density in Japan, we also compared fecundity and seed size, examined seeds for possible presence of predispersal insect seed predators, and evaluated seed germination potential. Our second objective was to compare the functional traits of juveniles between the two ecotypes under a common environment, including germination characteristics, seedling morphology and architecture, and biomass allocation patterns including carbohydrate storage in roots. Examination of foliage display patterns may reveal how the invasive Florida ecotype differs from the wild genotype in patterns of light utilization and competitive ability for light as resource. Carbohydrate storage in the roots may have allowed this shrub species to survive stem loss from biotic (disease, stem aging, heavy shading) and abiotic stresses (drought, freezing) by enhancing resprouting potential from the base. Our main hypothesis was that cultivar selection for higher fruit production, dense foliage, and resprouting potential prior to introduction had enhanced the ability of *A. crenata* to invade forests in the SE United States.

Materials and methods

General growth habits of A. crenata across regions

Much of the following background information relevant for the study is based on observation of over 30,000 juveniles and adults by the authors in Florida and Japan. *A. crenata* does not exhibit any vegetative reproduction through rhizomatous or clonal growth, and all individuals establish from seeds in the wild. Plants become reproductive when they reach approximately 20 cm in height and start developing lateral branches. Shoot extension and leaf production are limited to July–September following flowering. Each lateral branch has a finite lifespan of 2 years; as a vegetative branch in the first year, and bearing flowers and fruits terminally in the second year. Evergreen leaves gradually senesce on fruit-bearing branches. Plants may become as tall as 1.5 m, although most are less than 1 m tall. Plants successfully acquire effective arbuscular mycorrhizal fungi in the soils of the mixed hardwoods in the introduced range (Bray et al. 2003). Each red drupe consists of a single seed inside a thin woody endocarp, which is embedded in a fleshy mesocarp and thin, waxy exocarp. In Japan, all mature fruits are dispersed by native birds such as *Ixos amaurotis* by June (personal communication with N. Noma, University of Shiga Prefecture, Japan). In Florida, fruits are dropped or removed at a slower rate by frugivorous birds including northern mocking bird, *Mimus polyglottus*, and a conspicuous number of ripe fruits remain even in October when the next cohort of fruits start to mature.

Sampling of wild genotype in Japan

A. crenata naturally grows in the evergreen broadleaf forests around Aya, Kyushu, Japan (32°03′ N, 131°12′ E, elevation 380–520 m, mean annual temperature 14.2 °C, precipitation 3070 mm; Sato et al. 1999). A total of 109 ha of steep terrain (average slope angle = 30°), dominated by various evergreen oaks and other hardwood canopy trees (Tanouchi and Yamamoto 1995), is protected as a Long-Term Ecological Research Site by the Forestry and Forest Product Research Institute of Japan (FFPRI) because of its value as a rare old growth forest in the warm-temperate region. FFPRI has set up a 4 ha permanent plot to study tree community dynamics, including 400, 2 m×2 m subplots laid every 10 m in a grid system for estimating percentage cover of each species and species richness in the understory (Sato et al. 1999). In 42 randomly selected 2 m×2 m plots, recruitment, survival, height, and status of canopy tree seedlings have been monitored every June–July since 1999. Starting in 2001, the survey also included enumeration of *A. crenata* seedlings in the same manner as for canopy tree seedlings. The understory (0–2 m from the ground) is less densely vegetated, compared to the understory of the mixed broadleaf forests in Florida.

In March 2001, we located a total of 18 reproductive individuals of *A. crenata* within the permanent plot area in Aya and another old-growth stand in Takaoka (17 km away from Aya). Two fruits were sampled from each plant. One fruit was cut open immediately and examined with a hand lens for any evidence of predispersal damage by insects or fungi. Within 5 days, the other fruit was transported to the University of Florida in a sealed plastic bag, the fresh mass of fruit and seed was measured, and the seed was placed in a Petri dish lined with moist filter paper to record the time-course of germination at constant 26 °C.

Sampling of Gainesville populations that descended from a cultivar

We selected five mixed hardwood stands that are dominated by deciduous and evergreen hardwood trees and pines and contain dense patches of *A. crenata* near Gainesville Florida, USA (29°44′ N 82°27′ W, elevation 51 m, annual mean temperature 20.6 °C, annual precipitation 1332 mm). The San Felasco population in a mature, mesic, mixed hardwood stand in the middle of a 28 km^2 State Preserve, at least 1.5 km away from any residential development, was likely established by a rare long-distance dispersal by a vertebrate. The remaining four populations in the mixed hardwood of unknown age probably originated from short-distance dispersal of fruits from *A. crenata* planted around nearby houses or abandoned farms within a few hundred meters. These populations were separated from each other at least by 5 km.

At each site, we set four 2 m wide transects radiating from the center of a dense *A. crenata* patch (one randomly oriented transect within each quadrant of compass direction), extending beyond the edge of the patch by at least 10 m after the last reproductive *A. crenata* individual was encountered on the transect. The length of each transect ranged from 14 to 61 m, averaging 36.2 m. Along each transect, we randomly set five 1 m×1 m plots inside, and three outside, where the last reproductive *A. crenata* was encountered. In each plot, we recorded the number of *A. crenata* stems greater than 20 cm in height, as well as percent vegetation cover by all understory species including *A. crenata.*

Fecundity and seedling recruitment were examined at two of these five sites in areas of intermediate *A. crenata* density. In each site, we selected 20 individuals >20 cm tall that were at least 1 m away from other reproductive individuals in early March 2001, by which time only a few fruits had been dispersed. Within a 1 m radius of the focal individual, we removed seeds, fruits and seedlings from the ground, as well as fruits borne on other reproductive plants within a 2 m radius. We counted all fruits on each focal plant and recorded seedlings emerging within the 1 m radius area every 2 months for one year starting March 2001. The study was repeated, starting in March 2002, with 20 different plants at one site and 10 at the other.

The density of juvenile stems (0–20 cm) was quantified in two of the five sites, and at two additional sites (0.5 ha of secondary forest, and 8 ha of mature forest stand within the Natural Area Teaching Lab, NATL) in the University of Florida campus, mapping all individuals along the permanent grid system (NATL) or using randomly placed plots (other sites).

Seedling morphology and resource allocation

Germinated seeds from the Japan and Gainesville populations were planted in plastic tubes (4.7 cm diameter at the top, 16 cm deep) filled with commercial potting soil (Proterra, Schultz, St. Louis, MO). These were grown in a growth chamber (Percival E36L, supplying PFD 300 μmol m^{-2} s^{-1}, set at 25 °C – 12 h day and 18 °C – 12 h night) until the initiation of the experiment (9 months for the Japanese population, 12 months for the Gainesville population that was planted 3 months earlier). This temperature regime was similar to the monthly mean temperature during growing season (May–September) both in Aya (20.1–27.7 °C) and Gainesville (23.7–27.2 °C). Five plants randomly chosen from each population were harvested for the determination of initial size and morphology. The remaining plants were transplanted to 3.5 l pots and assigned randomly to high and low light treatments in a glasshouse (eight plants per treatment per origin). The plants assigned to the low light treatment were covered with a combination of 30% shade cloth and a plastic film (PNTHR20 Dark, 3 M, St. Paul, MN) that reduced the red:far red ratio from 1.02 to 0.25 to simulate the light quality under a plant canopy.

According to spot measurements around noon on cloudy and sunny days, the high and low light treatments received on average 25 and 1.5% of full sun under open sky, respectively.

After 4 months, all seedlings were harvested and separated as leaves, stems (including petioles) and roots. Individual leaves were scanned with a flatbed scanner and analyzed for length, circumference, and area with NIH Image v. 1.62. Stem height, number of branches, internodal distance on the main stem, and petiole length were also recorded. Then, leaves, stem (including branches and petioles), and roots were dried at 65 °C for 5 days and weighed separately. Although Gainesville seedlings had greater initial total dry mass (mean $\pm$ SD $= 4.09 \pm 0.74$ g) than Japanese seedlings (1.29 ± 0.21 g), their mass was not significantly different at the end in either light treatment. Thus, the statistical comparison of size, morphology, and biomass allocation was done at a common final size, avoiding the bias due to size differences (Rice and Bazzaz 1989). Relative growth rates (RGR) for biomass were calculated for each plant harvested where RGR = (ln (final total biomass) −ln (mean initial biomass for each genotype))/30 weeks.

We used a three dimensional architecture model, YPLANT (Pearcy and Yang 1996), to simulate the effects of differences in internodal length, petiole length, and leaf morphology on light reception efficiency. We measured and described the architecture and leaf display for three seedlings of the Gainesville genotype per light treatment. From these measurements, the YPLANT program calculated the projected leaf area, with and without accounting leaf overlap, from 160 directions (8 azimuth and 20 elevation angle classes, equally dividing the hemisphere over the plant). Difference between these two values divided by the actual total leaf area is the relative measure of self-shading (SSP, self-shading proportion), and the mean for each elevation class was calculated, yielding 20 mean values for each plant. To simulate the effects of greater internode and petiole length in the Japanese genotype, each plant architecture data file of the Gainesville genotype was modified to create a 'hypothetical Japanese plant', by multiplying each internode and petiole value with the mean ratio of Japanese to Gainesville genotypes in the same light environment. To simulate the leaf shape effect, the leaf shape data file was replaced with a file created from digitized leaves of the Japanese genotype. Using these modified files, SSP values were calculated for each 'hypothetical Japanese plant' with the YPLANT program as for the Gainesville plants. The results from the six Gainesville plants and six 'hypothetical Japanese plants' were analyzed statistically with MANOVA, using 9 mean SSP values from the upper 9 elevation angle classes (from which most light would be received) as the response variables, to examine the effects of genotype, light environment, and genotype × light interactions. All statistical analyses were conducted with JMP-IN 4.0 software (SAS Institute, Cary, NC, USA).

Dried roots were ground with a Wiley mill to pass through a No. 40 mesh screen for determination of nonstructural carbohydrate (NSC) as described in Marquis et al. (1997). Reducing sugars were extracted with 80% ethanol, and starch in the remaining solid was then digested to glucose with amylogucosidase (Sigma A7255). The glucose concentration of sugar and starch in the final extract solution was determined with a phenol–sulfuric acid reaction and the results were expressed as mg glucose equivalent per gram dry mass.

Results

Spatial distribution in Florida vs Japan

The invading populations in Gainesville, Florida, exhibit much higher maximum densities of both adults and juveniles compared to the population in Aya, Japan (Table 1). While the wild genotype in Aya showed vegetation coverage less than 1%, Gainesville populations reach cover of 80–100%. In Japan, most adult plants had no seedlings within a 1 m radius, with the maximum number observed being less than five juveniles. In Gainesville, however, as many as 631 juveniles were encountered within the 1 m radius of an adult.

Comparison of reproductive traits

The seed size was significantly smaller for Gainesville populations compared to Aya, Japan (Table 1, *t*-test, $P < 0.0001$), weighing only 60%

Table 1. Comparisons of the local population density of juveniles (height ≤ 20 cm) and adults (height > 20 cm), as well as reproductive traits (seed size, fecundity, juvenile recruitments) of *A. crenata* between Gainesville, Florida (invading populations descended from a cultivar) and the Aya, Kyushu, Japan (native populations of the wild genotype).

Characteristics	Gainesville-FL	Aya-Jpn
Number of populations	6	2
Maximum cover (%)	50–100	< 1%
Maximum stem density (m^{-2})	14–96	2
Maximum juvenile density (m^{-2})*	184–286	5
Fresh fruit mass (g)[§]	0.253 ± 0.050 (40)	0.242 ± 0.024 (40)
Fresh seed mass (g)[§]	0.094 ± 0.014 (60)	0.159 ± 0.019 (17)
Number of fruits per reproductive branch**	13–18	< 12
Number of fruits per reproductive plant**	72–103	< 50
Seedling recruitment per plant per year**	15–74	0.2–1.8

For Gainesville, values show a range of means or maxima for the six populations, unless otherwise indicated.

[§] Mean ± SD (total number of fruits or seeds) for pooled samples across populations for each genotype.

*Sampled in four sites in Gainesville (see Materials and methods).

**Sampled in two sites in Gainesville.

as much, even though fruit size was similar between the two populations (*t*-test, $P > 0.05$). However, the number of fruits was much higher in Gainesville than in Aya, both per branch and per plant (Table 1). In Aya, 19 of 37 adult-size plants had fruits. No predispersal insect or fungal damage was observed in any fruits cut open, and all seeds planted in a Petri dish germinated within 2 weeks for both Gainesville and Florida populations.

Seedling recruitment per plant per year was much higher in Gainesville than in Aya (Table 1). For each year between 2001 and 2004, only 9, 2, 2, and 1 new seedlings, respectively, were found in the 42 census plots in Aya. Assuming that vegetation cover of 0.5 and 1% corresponds to 1 and 2 reproductive plants per understory census plot, 0.2–1.8 seedlings were recruited each year per adult in Aya. In contrast, in two Gainesville populations, the mean number of seedlings recruited per adult per year was 48 (between sites and years this ranged from 15 to 74).

Seedling growth, morphology and allocation

Under a common environment, the Gainesville genotype grew much more slowly than the Aya genotype (Figure 1, Table 2). The relative growth rate of biomass was significantly slower for the Gainesville genotype than the Aya genotype in both high and low light treatments (Figure 1b). Both genotypes exhibited better growth under higher light availability. Although Gainesville seedlings were older and significantly larger at the beginning, after 4 months under the high light treatment, the faster RGR of Aya genotype resulted in similar total biomass and taller stature (25.1 cm compared to 18.2 cm for Gainesville genotype: Table 2).

These differences in growth rates can be explained by differences in morphology and biomass allocation patterns between the Gainesville and Aya genotypes. The Aya genotype had significantly longer internodes (Figure 2a), which partly explains its faster growth in height. The Aya genotype also had significantly longer petioles (Figure 2b). While total leaf area did not differ between genotypes, plants from Aya had more elongated leaves in terms of the ratio of leaf length to width (3.91 vs 3.09) and ratio of leaf length to leaf area (0.54 vs 0.41) than the Gainesville ecotype (Table 2, Figure 3a).

According to the simulation of leaf display patterns using YPLANT, the greater internodal length, petiole length and more elongated leaf shape in the Aya genotype resulted in a significantly reduced degree of self-shading compared to the Gainesville genotype (Figure 3b, showing significant effects of the genotype in MANOVA, df = 1/8, $F = 833.6$, $P = 0.0001$, without significant effects of light, genotype × light interaction, and within-subject effects, $P > 0.1$). The Aya genotype exhibited biomass allocation patterns that enhance biomass RGR, including a higher LAR (Figure 2c) and lower Root/Shoot biomass ratio (Figure 2d).

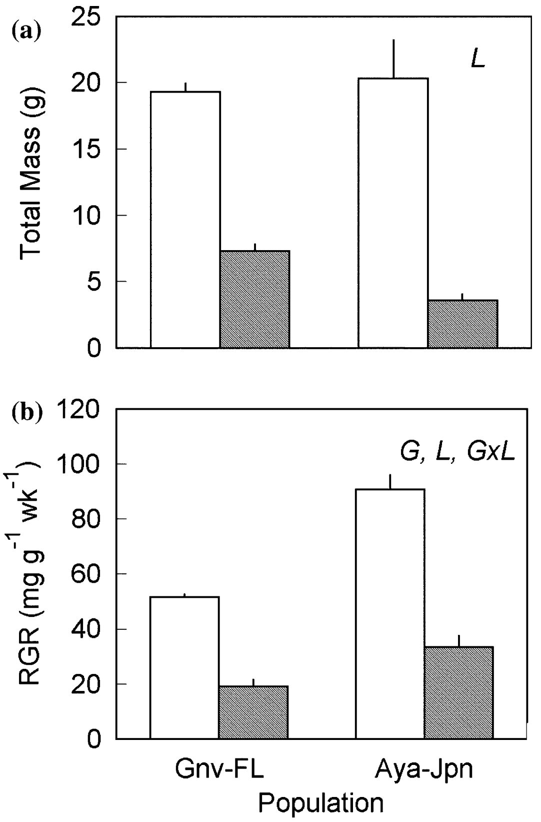

Figure 1. Genotype differences in size and growth of *A. crenata* seedlings between the invading populations in Gainesville, Florida (Gnv-FL) vs wildtype collected in Aya, Japan (Aya-Jpn) examined under light treatments (open: 30%, shaded: 1.5% of full sun). Mean (+SE) for (a) total dry mass and (b) relative growth rates of biomass (RGR). Italic letters at the upper right corner indicate significant genotype (*G*), light (*L*) and genotype×light interactions (*G*×*L*) in 2-way ANOVA ($P < 0.05$, see Table 2 for details).

There was no genotype×light interaction for either variable (Table 2). Higher light availability significantly decreased LAR in both genotypes, but had no effect on Root/Shoot ratio. Higher Root/Shoot ratios in the Gainesville genotype compared to the Aya genotype reflected greater total root biomass in the former. But this difference was solely due to high carbohydrate storage in the Gainesville genotype, which had significantly higher NSC in roots, both in terms of tissue concentration (Figure 2e) and total amount (Figure 2f) compared to the Aya genotype. Indeed, in terms of structural root biomass (= total root biomass–total NSC pool in roots), the two genotypes were not significantly different (Table 2).

Discussion

The populations of *A. crenata* that descended from a cultivar and invading north central Florida exhibit large phenotypic differences from the wild populations in Japan. A greater number of smaller seeds were produced per adult in the Florida populations compared to the wild populations. The glasshouse experiment demonstrated that the phenotypic differences in vegetative traits reflected genetic differences between the two ecotypes. These differences may have enhanced resource competition ability, resprouting ability, seedling recruitment success, and the establishment of high local densities in the Florida ecotype compared to the Japanese wild type.

Recent studies suggest that invading populations may rapidly evolve in response to changes in selection regimes (Maron et al. 2004), often in response to a lack of natural enemies, such as specialist herbivores, in the introduced range (Blossey and Notzold 1995; Siemann and Rogers 2001; Wolfe 2002). However, many other invasive plants fail to show such ecotypic differences between the native and introduced ranges (Willis et al. 2000; Thebaud and Simberloff 2001; van Kleunen and Schmid 2003; Vila et al. 2003; Bossdorf et al. 2004). Genetic diversity is often lower in the introduced range than in the native range because of founder effects (Bartlett et al. 2002), which may even enhance invasiveness (Tsutsui et al. 2000). More generally, genetic bottlenecks bias mean trait values for quantitative traits, such as size and fecundity, in either direction (Simons 2003). Horticultural selection prior to introduction of cultivars may further exacerbate the bias in genetic make up in the introduced populations compared to the native range.

Trait modification by cultivar selection prior to introduction is strongly suspected in the case of *A. crenata*. Popular ornamental cultivars in China and Japan bear many large fruits (KK personal observations). Plants sampled from six residential backyards in Japan, near Tokyo, had significantly larger fruits (mean fresh mass = 0.300 g) than both the Aya wild genotype and the Gainesville populations (Table 1). However, the mean seed fresh mass was intermediate (0.133 g) and significantly smaller than for the Aya wild type (0.159 g). Although seed

Table 2. The results of two-way ANOVA for the effects of genotype (the site of seed collection, GNV-Florida vs Aya-Japan) and light treatment (high vs low) on size, morphology, and biomass allocation characteristics in glasshouse-grown seedlings of *Ardisia crenata.*

Variable	Genotype		Light		Interaction	
	F	P	F	P	F	P
Height	5.2	0.041	20.1	< 0.001	4.3	0.06
Total dry mass	0.8	NS	92.6	< 0.0001	2.5	NS
Total leaf area	0.5	NS	31.6	< 0.0001	7.4	0.018
Biomass RGR	58.6	< 0.0001	165.1	< 0.0001	12.4	0.004
Internodal length	7.3	0.02	8.1	0.01	0.3	NS
Petiole length	59.6	< 0.0001	< 0.1	NS	1.7	NS
Leaf length/width	25.8	0.0003	0.5	NS	< 0.1	NS
Leaf length/area	53.8	< 0.0001	9.3	0.003	3.2	NS
Leaf area ratio	10.4	0.007	36.4	0.0001	1.2	NS
Root/Shoot	14.0	0.003	< 0.1	NS	< 0.1	NS
Total root mass	9.7	0.009	64.6	< 0.0001	< 0.1	NS
Root NSC conc.	12.8	0.004	11.4	0.007	0.05	NS
Root NSC pool	10.2	0.0085	30.8	0.0002	1.4	NS
Structural root mass	4.4	NS	68.6	< 0.0001	2.2	NS

NS for $P > 0.1$.

size exhibits considerable variation within a population, there is a significant genetic component leading to variation among populations (Winn and Gross 1993; Susko and Lovett-Doust 2000; Buckley et al. 2003). Both fresh and dry seed mass differed significantly among four Gainesville populations, two of which were not significantly different from the Japanese cultivar (mean fresh mass ranging from 0.089 to 0.120 g, all significantly smaller than the Aya wild type, KK unpublished data). This may reflect a founder effect as each Gainesville population were probably established from a few escaped individuals. More generally, because of seed size – number trade-offs, artificial selection for greater fecundity, as well as natural selection for enhanced dispersal, may decrease seed size (Geritz 1995). In contrast, large seed size is likely to be selected for in the deeply shaded understory of evergreen broadleaf forests in Aya, because large seed size is important for seedling establishment in light-limited environments (Poorter and Rose 2005). Semi-deciduous forest canopy, combined with milder winter temperature (mean January temperature is 13.6 °C in Gainesville, compared to 7.6 °C in Aya), probably benefit net carbon balance and aid the establishment of seedlings from smaller seeds in Gainesville.

Few other studies have compared seed size between native and introduced ranges. Buckley et al. (2003) found significantly larger seeds for invading population of *Cytisus scoparius* compared to populations in the native range, and attributed this difference to a rapid evolutionary change to enhance competitiveness in the invading population. However, the same study did not find a significant difference in seed size of another invasive species, *Ulex europaeus*, between native and introduced ranges, and our study found smaller seeds in the Japanese cultivar as well as *A. crenata* naturalized in Florida. Thus, no generalization is possible as to how seed size may evolve in invading populations. The period of 100 years since introduction may be sufficient for natural selection for smaller seed size and lower seed to pulp ratio in *A. crenata* to enhance dispersal, but the role of artificial selection prior to introduction was probably more important. Seed size exhibits obvious variation among populations across SW China (observation of *A. crenata* specimens at the Chinese National Herbarium in Beijing, China, by KK). A survey of the genetic variation of wild and cultivated populations across their geographical ranges is necessary to test unambiguously whether genetic differences between the native and introduced range reflect

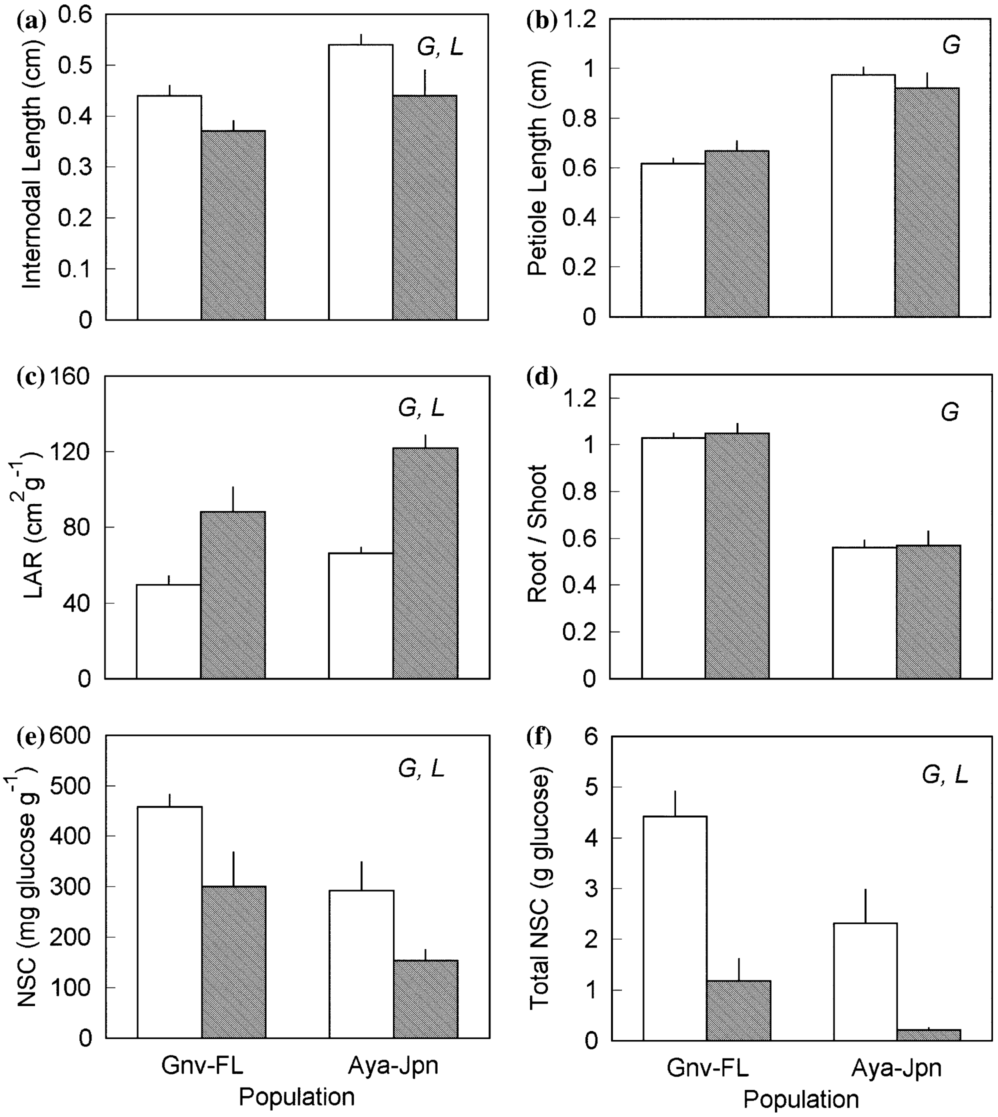

Figure 2. Genotype differences in morphological and biomass allocation patterns of *A. crenata* seedlings established from seeds collected in Gainesville, Florida (Gnv-FL) and Aya, Japan (Aya-Jpn) after 4 months of growth in high (open bars) and low (shaded bars) light availability in a glasshouse. Means (+SE) for (a) average internodal length, (b) petiole length, (c) leaf area ratio (leaf area divided by total plant mass), (d) Root/Shoot biomass ratio, (e) root NSC concentration, (f) total pool size of root NSC. Italic letters at the upper right corner indicate significant genotype (*G*), light (*L*) and genotype×light interactions (*G*×*L*) in 2-way ANOVA (see Table 2 for details).

pre-adaptation *via* cultivar selection, or evolutionary change post-introduction.

The invasive ecotype of *A. crenata* in Florida grows more slowly than the wild genotype in Japan. This is the opposite of the EICA hypothesis that predicts faster growth rates and larger size in invading populations compared to those in the native range (Blossey and Notzold 1995). The wild type of *A. crenata* growing in the understory of evergreen broadleaf forests allocates more biomass to shoots and avoids self-shading *via* longer petioles and internodes. The resulting high light-use efficiency is probably important for maintenance of positive carbon balance in Aya's deeply

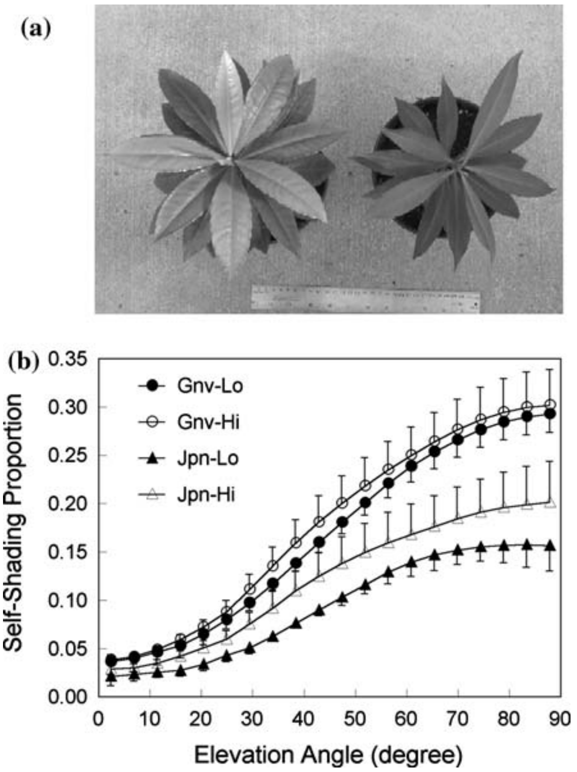

Figure 3. (a) Typical leaf display patterns viewed from the top of 1.5 year old seedlings grown from seeds collected in Gainesville, Florida (left) and Aya, Japan (right) under shade in a glasshouse. (b) The mean self-shading proportion for each elevation angle using a three-dimensional architecture simulation with the YPLANT program, for seedlings of the Gainesville genotype grown in high and low light treatments (Gnv-Hi and Gnv-Lo, respectively; means of three plants each, with error bars indicating SD). The effects of elongated leaf, longer petiole and internode of the Aya, Japan, genotype were simulated by multiplying the actual values for these variables by the average ratios between the Aya genotype and the Gainesville genotype under each light environment (Jpn-Hi and Jpn-Lo, respectively).

shaded understory. In contrast, the Florida ecotype descended from a cultivar that exhibits dense foliage and consequently lower light-use efficiency and slower biomass growth rate. The low light-use efficiency was probably less of a challenge for the maintenance of carbon balance in Florida mixed hardwoods, where the partially deciduous canopy provides an opportunity for carbon gain during winter.

Unlike disturbance dependent weeds for which competitiveness is a function of the growth rates in height and above-ground biomass (Blossey and Notzold 1995), climax trees and understory shrubs out-compete neighbors by extracting light as the limiting resource more completely (i.e., lower R* *sensu* Tilman 1988; Canham et al. 1994; Kitajima et al. 2005). Light transmitted below the crowns of isolated, adult individuals of *A. crenata* was indeed less than below the crowns of *Prunus caroliana* saplings, an abundant component of the native woody vegetation in north central Florida (KK and M Dooley, University of Florida, unpublished data). Rounder leaves, shorter petioles, and reduced internodal length all contribute to lower light-use efficiency, but allow greater light competitiveness in invading populations of *A. crenata.* However, this enhanced competitiveness is unlikely to be the result of rapid evolution after introduction. Extremely high monospecific densities of invading populations mean that intraspecific competition is more important than interspecific competition. An adaptive evolutionary strategy in such a setting would be to allocate more to shoots, produce longer internodes, and become taller more rapidly than conspecific neighbors (Anten 2002). Instead, the Florida ecotype exhibits architectural patterns similar to cultivars, and consequently, has inefficient light-use and slow growth rates, but high tolerance of abiotic stress.

Greater allocation to root carbohydrate storage in the Florida ecotype also contributes to its slower relative growth rate compared to the Japanese wild type. The Florida ecotype that descended from a cultivar exhibits one of the highest carbohydrate concentrations in roots, compared to other temperate and tropical woody species (Canham et al. 1999; KK unpublished data for seedlings of 50 neotropical woody species). This high root carbohydrate storage in the Florida ecotype was unlikely due to post-introduction enemy release or selection for increased competitiveness, and cultivar selection is again a likely suspect. In both Japan and Florida, leaf herbivory of *A. crenata* appears extremely rare and minor. However, stems become increasingly vulnerable to drought stress as they grow taller, and they die back to the base in response to drought and freezing events (KK and AMF unpublished data). According to herbarium specimens, wild plants in subtropical SW China frequently reach more than 2 m tall, which is much taller than both Japanese and Florida populations. Common cultivation practice in Japan includes repeated stem-trimming and air-layering for short and densely foliated appearance and protection from freezing to allow cultivation in a colder climate than that of wild populations (Souga 1989). In response to this practice, greater root carbohydrate storage may have been artificially selected for. Medicinal use of roots in China may also have selected for greater biomass allocation to roots (Jia et al. 1994). An unfortunate and unintended consequence of the high resprouting ability of the Florida ecotype is the difficulty of eradication; *A. crenata* recovers easily from repeated mowing and above-ground removal by resprouting from the stem base within a year (KK unpublished data).

In conclusion, the role of cultivar selection prior to introduction is strongly suspected as an explanation for the differences between the wild and introduced populations of *A. crenata*. Overall, the wild type in Japan and the invading Florida ecotype exhibit similar degrees of phenotypic plasticity in vegetative traits and inhabit shaded and mesic understory as preferred habitat. However, cultivar selection may have favored a particular genotype that was pre-adapted as an invader in its new range where it can rapidly expand population size *via* many small seeds and monopolize light in the less shaded understory. Many other invasive plants were also introduced as ornamentals or for production purposes. How cultivar selection prior to introduction may enhance or decrease naturalization into a new range is difficult to generalize across species. However, artificial selection for higher growth rates and fecundity may enhance invasiveness, resulting in a similar pattern as predicted by the EICA hypothesis. For this reason as well, the process of cultivation is a step that deserves more attention when studying the evolutionary ecology of invasive species (Mack 2000). We propose that for effective comparisons of ecological habits between the native and introduced ranges, how traits are modified through the processes of artificial selection must be considered. In relation to common garden experiments, future studies should employ a molecular genetics approach to decipher the evolutionary ecology of species invasions (Lee 2002; Maron et al. 2004).

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References

Anten NPR (2002) Evolutionarily stable leaf area production in plant populations. Journal of Theoretical Biology 217: 15–32

Bailey LH (1922) The Standard Encyclopedia of Horticulture. Vol. 1. McMillan, New York

Bais HP, Vepachedu R, Gilroy S, Callaway RM and Vivanco JM (2003) Allelopathy and exotic plant invasion: from molecules and genes to species interactions. Science 301: 1377–1380

Barrett SCH (1983) Crop mimicry of weeds. Annual Review of Ecology and Systematics 5: 1–25

Bartlett E, Novak SJ and Mack RN (2002) Genetic variation in *Bromus tectorum* (Poaceae): differentiation in the eastern United States. American Journal of Botany 89: 602–612

Blossey B and Notzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants – a hypothesis. Journal of Ecology 83: 887–889

Bossdorf O, Prati D, Auge H and Schmid B (2004) Reduced competitive ability in an invasive plant. Ecology Letters 7: 346–353

Bray SR, Kitajima K and Sylvia DM (2003) Differential response of an exotic invasive shrub to mycorrhizal fungi: growth, physiology and competitive interaction. Ecological Applications 13: 565–574

Buckley YM, Downey P, Fowler SV, Hill R, Memmot J, Norambuena H, Pitcairn M, Shaw R, Sheppard AW, Winks C, Wittenberg R and Rees M (2003) Are invasives bigger? A global study of seed size variation in two invasive shrubs. Ecology 84: 1434–1440

Callaway RM, Thelen GC, Rodriguez A and Holben WE (2004) Soil biota and exotic plant invasion. Nature 427: 731–733

Canham CD, Finzi AD, Pacala SW and Burbank DH (1994) Causes and consequences of resource heterogeneity in forests: interspecific variation in light transmission by canopy trees. Canadian Journal of Forest Research 24: 337–349

Canham CD, Kobe RK, Latty EF and Chazdon RL (1999) Interspecific and intraspecific variation in tree seedling survival: effects of allocation to roots versus carbohydrate reserves. Oecologia 121: 1–11

Conover CA and Poole RT (1989) Production and use of *Ardisia crenata* as a potted foliage plant. Foliage Digest 12: 1–3

Dozier H (1999) Plant introductions and invasion: history, public awareness, and the case of *Ardisia crenata*. Ph.D. Thesis, University of Florida, 113 pp

Geritz SA (1995) Evolutionarily stable seed polymorphism and small-scale spatial variation in seedling density. American Naturalist 146: 685–707

Gordon DR and Thomas KP (1998) Florida's invasion by nonindigenous plants: history, screening and regulation. In: Simberloff D, Schmitz DC and Brown TC (eds) Strangers in Paradise: Impact and Management of Nonindigenous Species in Florida, Island Press, Washington, DC

Harlan JR (1975) Crops and Man. American Society of Agronomy and Crop Science Society of America, Madison, p. 295

Jia ZH, Koike K, Nikaido T, Ohmoto T and Ni MY (1994) Triterpenoid saponins from ardisia crenata and their inhibitory activity on camp phosphodiesterase. Chemical and Pharmaceutical Bulletin 42: 2309–2314

Kitajima K, Mulkey SS and Wright SJ (2005) Variation in crown light utilization characteristics among tropical canopy trees. Annals of Botany 95: 535–547

Langeland KA and Burks KC (1998) Identification and Biology of Non-native Plants in Florida's Natural Areas. University of Florida, Gainesville, p. 165

Lee A, Suh J, Roh MS and Slovin JP (2003) Analysis of genetic relationships of *Ardisia* spp. using RAPD markers. Journal of Horticultural Science and Biotechnology 78: 24–28

Lee CE (2002) Evolutionary genetics of invasive species. Trends in Ecology and Evolution 17: 386–391

Mack RN (2000) Cultivation fosters plant naturalization by reducing environmental stochasticity. Biological Invasions 2: 111–122

Mack RN (2003) Plant naturalizations and invasions in the eastern United States: 1634–1860. Annals of the Missouri Botanical Garden 90: 77–90

Mack RN and Erneberg M (2002) The United States naturalized flora: largely the product of deliberate introductions. Annals of the Missouri Botanical Garden 89: 176–189

Maron JL, Vila M, Bommarco R, Elmendorf S and Beardsley P (2004) Rapid evolution of an invasive plant. Ecological Monographs 74: 261–280

Marquis RJ, Newell EA and Villegas AC (1997) Non-structural carbohydrate accumulation and use in an understory rainforest shrub and relevance for the impact of leaf herbivory. Functional Ecology 11: 636–643

Muller-Scharer H, Schaffner U and Steinger T (2004) Evolution in invasive plants: implications for biological control. Trends in Ecology and Evolution 19: 417–422

Ohwi J (1984) The Flora of Japan. Smithsonian Institution, Washington, DC, p. 1067

Pearcy RW and Yang WM (1996) A three-dimensional crown architecture model for assessment of light capture and carbon gain by understory plants. Oecologia 108: 1–12

Poorter L and Rose SA (2005) Light dependent changes in the relationship between seed mass and seedling traits: a meta-analysis for rain forest tree species. Oecologia 142: 378–387

Rice SA and Bazzaz FA (1989) Quantification of plasticity of plant traits in response to light intensity: comparing phenotypes at a common weight. Oecologia 78: 502–507

Sato T, Kominami Y, Saito S, Niiyama K, Manabe T, Tanouchi H, Noma N and Yamamoto S (1999) An introduction to the Aya Research Site, a long-term ecological research site, in a warm temperate evergreen broadleaved forest ecosystem in southwestern Japan: Research topics and design. Bulletin of Kitakyushu Museum of Natural History 18: 157–180

Siemann E and Rogers WE (2001) Genetic differences in growth of an invasive tree species. Ecology Letters 4: 514–518

Simons AM (2003) Invasive aliens and sampling bias. Ecology Letters 6: 278–280

Souga T (1989) Engei Shokubutsu Daijiten (The Encyclopedia of Horticultural Plants). Vol. 5, Shougakkan, Tokyo, Japan,

Susko DJ and Lovett-Doust L (2000) Patterns of seed mass variation and their effects on seedling traits in *Alliaria petiolata* (Brassicaceae). American Journal of Botany 87: 56–66

Tanouchi H and Yamamoto S (1995) Structure and regeneration of canopy species in an old-growth evergreen broadleaved forest in Aya district, soutwestern Japan. Vegetatio 117: 51–60

Thebaud C and Simberloff D (2001) Are plants really larger in their introduced ranges? American Naturalist 157: 231–236

Tilman D (1988) Dynamics and Structure of Plant Communities. Princeton University Press, Princeton, p. 360

Tsukamoto Y. (1980) Garden Plants of the World in Color, Vol. 15, Ornamental Trees and Shrubs. Hoikusha, Osaka, Japan

Tsutsui ND, Suarez AV, Holway DA and Case TJ (2000) Reduced genetic variation and the success of an invasive species. Proceedings of the National Academy of Sciences of the United States of America 97: 5948–5953

van Kleunen M and Schmid B (2003) No evidence for an evolutionary increased competitive ability in an invasive plant. Ecology 84: 2816–2823

Vila M, Gomez A and Maron JL (2003) Are alien plants more competitive than their native conspecifics? A test using *Hypericum perforatum* L. Oecologia 137: 211–215

Watkins JV and Sheehan TJ (1975) Florida Landscape Plants. University of Florida, Gainesville

Willis AJ, Memmott J and Forrester RI (2000) Is there evidence for the post-invasion evolution of increased size among invasive plant species? Ecology Letters 3: 275–283

Winn AA and Gross KL (1993) Latitudinal variation in seed weight and flower number in *Prunella Vulgaris*. Oecologia 93: 55–62

Wolfe LM (2002) Why alien invaders succeed: Support for the escape-from-enemy hypothesis. American Naturalist 160: 705–711