ECOLOGY LETTERS

Ecology Letters, (2012) **15**: 1016–1025 doi: 10.1111/j.1461-0248.2012.01824.x

LETTER

Invasion of diverse habitats by few Japanese knotweed genotypes is correlated with epigenetic differentiation

Christina L. Richards,[1]* Aaron W. Schrey[1] and Massimo Pigliucci[2]

[1]Department of Integrative Biology, University of South Florida, Tampa, FL, 33617, USA

[2]Philosophy Program, Graduate Center—City University of New York, NY, 10036, USA

*Correspondence: E-mail: clr@usf.edu

Abstract

The expansion of invasive species challenges our understanding of the process of adaptation. Given that the invasion process often entails population bottlenecks, it is surprising that many invasives appear to thrive even with low levels of sequence-based genetic variation. Using Amplified Fragment Length Polymorphism (AFLP) and methylation sensitive-AFLP (MS-AFLP) markers, we tested the hypothesis that differentiation of invasive Japanese knotweed in response to new habitats is more correlated with epigenetic variation than DNA sequence variation. We found that the relatively little genetic variation present was differentiated among species, with less differentiation among sites within species. In contrast, we found a great deal of epigenetic differentiation among sites within each species and evidence that some epigenetic loci may respond to local microhabitat conditions. Our findings indicate that epigenetic effects could contribute to phenotypic variation in genetically depauperate invasive populations. Deciphering whether differences in methylation patterns are the cause or effect of habitat differentiation will require manipulative studies.

Keywords

AFLP, DNA methylation, epigenetics, invasive species, Japanese knotweed.

Ecology Letters (2012) **15**: 1016–1025

INTRODUCTION

The expansion of invasive species not only provides an opportunity to investigate how organisms establish and adapt to new environments, but also challenges our understanding of the process of adaptation. As dramatically reduced genetic variation is expected to constrain the evolutionary potential of a given population or species, it is surprising that many invasive species appear to thrive even with low levels of sequence-based genetic variation (hereafter referred to simply as genetic variation). According to standard theory, the chances of a few individuals or a single genotype landing in a novel location and surviving are expected to be minimal. In fact, few cases of successful invasions have been reported to result from the introduction of specific genotypes that happen to be 'preadapted' to the invaded environment (Dlugosch & Parker 2007; Bossdorf *et al.* 2008a).

Although there are documented cases where invasive species appear to have acquired increased genetic variation through multiple introductions (Durka *et al.* 2005; Lavergne & Molofsky 2007; Rosenthal *et al.* 2008; Gammon & Kesseli 2010) or hybridisation (Mandák *et al.* 2005; Bailey *et al.* 2009), many invasives appear to do well even with low levels of genetic variation (Hollingsworth & Bailey 2000; Dlugosch & Parker 2008a,b; Richards *et al.* 2008; Loomis & Fishman 2009). Dlugosch & Parker (2008a) reported substantial losses of average molecular diversity compared to source populations across studies of 80 species of plants, animals and fungi. However, despite the observation of decreased genetic variance in these studies, only one reported a significant decline in phenotypic variance (Simberloff *et al.* 2000). The authors concluded that surprisingly large losses of up to 50% reduced heterozygosity compared to source populations may not necessarily translate into a loss of phenotypic variation.

Phenotypic plasticity has often been invoked in this context because it allows for phenotypic variation without variation at the genetic level (Baker 1965; Bossdorf *et al.* 2005). Studies have shown that plasticity in ecologically relevant traits may contribute to the success of invasive plant species by allowing increased fitness or fitness homeostasis across a range of habitats (Richards *et al.* 2006, 2008; Muth & Pigliucci 2007; Bossdorf *et al.* 2008a; Loomis & Fishman 2009; Walls 2010; but see Davidson *et al.* 2011). Recently, several authors have argued that this type of phenotypic plasticity can be mediated through epigenetic effects, and that these effects may lead to heritable phenotypic differences (Bossdorf *et al.* 2008b, 2010; Richards *et al.* 2008, 2010a; Jablonka & Raz 2009; Nicotra *et al.* 2010; Richards 2011; Scoville *et al.* 2011). The most studied epigenetic effect is DNA methylation, which has been shown to increase in variance in response to hybridisation (Salmon *et al.* 2005) and exposure to stress (Verhoeven *et al.* 2010), and has known effects on ecologically important phenotypes (Johannes *et al.* 2009; Bossdorf *et al.* 2010). Because epigenetic states can be altered in organisms facing novel or stressful environments, epigenetic effects could provide a rapid source of phenotypic variation without any change in genetic variation (Rapp & Wendel 2005; Bossdorf *et al.* 2008b), thereby affecting the evolutionary potential of colonising populations. This suggests that the chance sampling of genotypes, combined with epigenetic changes, could lead to divergence of invasive populations even in the absence of abundant genetic variation (Keller & Taylor 2008; Prentis *et al.* 2008).

Invasive populations of the *Fallopia* species complex (referred to as Japanese knotweed *sensu lato* or *s.l.*) are an excellent study system to investigate the role of epigenetic effects during invasion. Populations of the species complex are known to occupy a wide range of habitats in Europe (Mandák *et al.* 2005; Bailey *et al.* 2009) and have more recently colonised an even broader array of habitats in the

© 2012 Blackwell Publishing Ltd/CNRS

north-eastern USA, often growing next to or on the terrestrial margins of marshes that are inundated with *Phragmites australis* (Richards *et al.* 2008), and on beaches (Walls 2010). In previous work, we used cytology and Amplified Fragment Length Polymorphism (AFLP) markers to show that these populations have extremely low genetic diversity: some are made up of a single *Fallopia japonica* genotype found across Europe (Hollingsworth & Bailey 2000), whereas the majority consist of a few *F.* × *bohemica* hybrids (Richards *et al.* 2008; Walls 2010; Richards *et al.*, unpublished). Despite this low diversity, plants from the divergent habitats of roadside, beach and marsh demonstrated significant differences within and among sites for most traits and trait plasticities in response to controlled greenhouse studies and field reciprocal transplants.

In this study, we combined AFLP and methylation sensitive-AFLP (MS-AFLP) to compare genetic and epigenetic diversity across 16 populations from the three habitat types currently invaded by Japanese knotweed on and near Long Island, NY. With these data, we were able to test the hypothesis that rapid differentiation of invasive Japanese knotweed populations is not linked to genetic differentiation, but is correlated instead with epigenetic differentiation. Our study shows that epigenetic variation is much greater than genetic variation; because all materials were studied in a common garden setting, our data suggest that the epigenetic variation persists through clonal reproduction, potentially contributing to the colonisation of diverse habitats.

MATERIALS AND METHODS

Fallopia species complex and sampling sites

Historically, the taxonomy of the Japanese knotweeds has been complicated (see Richards *et al.* 2008). *F. japonica* ($2N = 44$ or 88) is considered a primary coloniser, important in the establishment of vegetation on newly formed, bare volcanic habitat. Japanese populations of *F. japonica* are extremely variable in morphology and at the molecular level (Bailey 2003). The distribution of the closely related species *F. sachalinensis* ($2N = 44$) is restricted to the Sakhalin Islands (Russia) and northern Japan, typically found along freshwater waterways (Bailey 2003). Hybrids (*F.* × *bohemica*; $2N = 44$, 66, or 88) are much more common in the invasive range of Europe, with greater genetic diversity and more rapid spread than either parent in the invasive range (Mandák *et al.* 2005). In the USA, recent studies suggest that spread of all three taxa takes place through both vegetative and sexual reproduction and that the morphological variation is much greater than that reported in Europe (Gammon *et al.* 2007; Grimsby *et al.* 2007; Gammon & Kesseli 2010).

We collected Japanese knotweed *s.l.* from five marsh sites, five beach sites and six roadside sites across eastern NY (Suffolk County, Long Island and Westchester County), Connecticut and Rhode Island (Table 1).

Taxonomy, AFLP genotyping and MS-AFLP epi-genotyping

Because methylation is environmentally labile, we collected rhizomes from the field and grew fresh leaf tissue in the greenhouse. At each site, we collected rhizomes *c.* 10 m apart to maximise the likelihood of collecting different genotypes (referred to throughout as 'genets'), and to represent the full area of each of the 16 sites. Each field-collected rhizome was treated as an individual in the subsequent analyses, although in many cases more than one 'genet' turned out to be the same genotype. In addition, for comparison, we grew fresh leaf tissue from rhizome pieces of the single *F. japonica* clone found across Europe (Hollingsworth & Bailey 2000). In a previous study, we also surveyed two to four individuals from each of the 16 populations to determine species or hybrid classes based on cytology and morphology (Richards *et al.* 2008; Richards *et al.* unpublished). Screening of these same individuals using AFLP identified a species-specific marker so that we could confirm the species identity for all individuals.

We screened a total of 215 individuals for genetic variation using AFLP (Table 1). We used the Qiagen DNeasy Plant Mini kit (Qiagen, Valencia, CA, USA) to perform duplicate DNA extractions from each of the samples at each site, which were subsequently run through the entire AFLP protocol to ensure reliable scoring of resultant AFLP fragments. We followed the standard protocol suggested by Qiagen, eluting the DNA with water instead of TE in the final step. We used an AFLP protocol based on standard methods with some modifications (see Richards *et al.* 2008). Briefly, we digested 200 ng of genomic DNA at 37 °C for 3 h with 10 units of *Eco*RI and 10 units of *Mse*I, immediately followed by ligation with 75 μM of *Eco*RI adaptors, 75 μM of *Mse*I adaptors and 20 units of T4 DNA ligase (NEB) overnight (16–20 h) at 4 °C. We used the same pre-selective amplification of the dilute ligation reaction as in Richards *et al.* (2008) with 40 pmol of *Eco*RI + A and *Mse*I + C primers and the following PCR conditions: 75 °C for 2 min; 20 cycles of 94 °C for 30 s, 56 °C for 30 s, 75 °C for 2 min; final extension at 60 °C for 30 min. We ran 5 μL of this PCR product on a 1% agarose gel to verify that the reactions had worked. Successful reactions produced a smear of DNA in the 100–1500 bp range.

In the selective amplification, we multiplexed 4 pmol of the 6-carboxy-fluorescein (6-FAM) fluorescently labelled *Eco*RI + AGC primers with 4 pmol of the 4,7,2′,4′,5′,7′-hexachloro-6-carboxy-fluorescein (HEX) fluorescently labelled *Eco*RI + ACG primers and 25 pmol of the *Mse*I + CAA in standard selective amplification reaction mixture and PCR conditions (Richards *et al.* 2008). We ran 5 μL of this PCR product on a 1% agarose gel to verify that the reactions had worked. Successful reactions produced a smear of DNA in the 50–500 bp range. We submitted the selective amplification products to the DNA Facility at Iowa State University, where they were electrophoretically separated on an ABI 3700. We visually inspected the AFLP fragments using the open source program Genographer (Benham *et al.* 1999) and manually categorised genotypes based on identical banding patterns across *c.* 200 total loci for the HEX and 6FAM primer sets combined. We assessed repeatability of banding patterns across duplicates for each sample by first comparing reactions to determine if banding patterns were consistent between duplicates. We then identified variable positions that could be reliably scored; any position that was not reliable was not included in the analysis. We scored all individuals at each variable position with a binary code, zero for band absent, one for band present. Throughout, we use 'locus' to indicate a specific fragment size in the AFLP and MS-AFLP results. We use 'haplotype' to indicate the collection of binary variable positions (dominant genotypes) for each individual at AFLP loci, and we use epi-genotype to indicate the collection of binary variable positions at MS-AFLP loci.

© 2012 Blackwell Publishing Ltd/CNRS

Table 1 Site locations and summary of species, AFLP genotypes and MS-AFLP epi-genotypes. Shared AFLP haplotypes and shared epi-genotypes are identified by a letter, whereas the epi-genotypes that are unique to one individual are numbered (1–101)

Site	Location	Latitude	Longitude	N	Species	AFLP Haplotypes (Frequency)	Epi-haplotype (Frequency)
Beach							
HBCT	Hammonasset Beach, State Park, Madison, CT	41 16.17	72 33.39	5	*F. japonica/ F.* × *bohemica*	F (4), G (1)	U (2), 83, 86, 87
HP	Hunt's Point, Southold, NY	41 05.17	72 26.68	10	*F. japonica*	F (10)	A, D (2), I (2), 6, 9, 19, 39, 41
MSH	Mount Sinai Harbour, Port Jefferson, NY	40 57.74	73 02.58	8	*F. japonica/ F.* × *bohemica*	C (2), D (2), E(1), F (1), G (2)	J (3), 34-38
PJB	Port Jefferson Beach, Port Jefferson, NY	40 57.88	73 03.17	12	*F. japonica/ F.* × *bohemica*	A (3), F (3), G (6)	E (2), K (2), 14, 15, 40, 42, 47, 68, 74, 75
RPB	Rocky Point beach, Rocky Point, NY	40 57.96	72 57.28	15	*F.* × *bohemica*	E (11), G(4)	T (2), X (2), 30–33, 67, 69, 73, 81, 82, 89, 90
Roadside							
CMR	Montauk Highway, Terrell River County Park, Center Moriches, NY	40 47.98	72 46.42	16	*F. japonica*	F (16)	AA (7), 43, 46, 50, 53, 54, 93, 97–99
HL	Hagerman, Landing Road, Rocky Point, NY	40 57.82	72 57.29	15	*F.* × *bohemica*	E (11), G (4)	Q (4), S (6), T, 22, 23, 72, 94
PWC	Purchase Street at Oak Valley Lane, Purchase, NY	41 03.04	73 43.09	7	*F.* × *bohemica*	B (5), E (2)	A (5), V (2)
RHC	Chauncey Road at Highway 24, Riverhead, NY	40 54.53	72 37.47	19	*F.* × *bohemica*	G (19)	W (5), Z (4), CC (3), EE (7)
RI	Johnson Lane at Old Post Road, Charlestown, RI	41 22.92	71 38.00	13	*F.* × *bohemica*	E (13)	3, 5, 8, 11-13, 44, 52, 57, 60, 76, 77, 95
ST	Route 25 in Given County Park, Smithtown, NY	40 51.473	73 12.60	14	*F.* × *bohemica*	G (14)	A (3), B (2), D, F (2), G (2), 1, 2, 4, 7
Marsh							
CBH	Crystal Brook 128 Hollow Road at Oakwood, Port Jefferson, NY	40 57.19	73 02.71	24	*F. japonica/ F.* × *bohemica*	D (1), E (2), F (5), H (1), G(15)	L (2), M (2), N (2), O (3), P (3), 16-18, 49, 58, 59, 61-64, 96, 101
CMM	Terrell River, County Park, Center Moriches, NY	40 47.96	72 46.41	13	*F. japonica*	F (13)	BB (3), 48, 51, 55, 56, 65, 70, 71, 88, 92, 100
OLCT	Route 156 at Ferry, Road, Old Lyme, CT	41 18.69	72 20.10	10	*F. japonica/ F.* × *bohemica*	F (1), G (9)	Q, R (2), Y (2), 78, 79, 84, 85, 91
RHBH	Privately owned boathouse, Peconic Bay Riverhead, NY	40 54.24	72 37.11	18	*F.* × *bohemica*	G (18)	DD (18)
WH	Wertheim National, Wildlife Refuge, Brookhaven, NY	40 46.23	72 53.86	10	*F.* × *bohemica*	G (10)	A (2), F (3), 10, 21, 45, 66, 80
European							
UK	*F. japonica* worldwide clone	-	-	12	*F. japonica*	F (6)	H (2), 20, 24-26

© 2012 Blackwell Publishing Ltd/CNRS

We screened the 215 individuals for epigenetic variation with MS-AFLP using the same duplicate DNA extractions that were used in the AFLP (Table 1). For the MS-AFLP, we ran two separate protocols using essentially the same AFLP protocol, but replacing the *Mse*I enzyme with the same concentration of either *Msp*I or *Hpa*II, both of which are methylation sensitive, but vary in their sensitivity. Both enzymes recognise and cleave CCGG sequences, but cleaving by *Msp*I is blocked when the inner C is methylated whereas cleaving in *Hpa*II is blocked when either or both cytosines are fully or hemi-methylated. Together, four different types of variation can be scored (Salmon *et al.* 2008); Type I is when both enzymes cut at the restriction site and indicates no methylation; Type II is when *Msp*I does not cut and *Hpa*II does cut indicating the restriction site has a methylated internal C; Type III is when *Msp*I does cut and *Hpa*II does not cut indicating the restriction site has a methylated outer C; and Type IV is when neither enzyme cuts indicating that either both Cs are methylated or the restriction site has mutated. We pooled data into two categories, methylated (Type II, Type III) or not methylated (Type I, Type IV) restriction sites (Salmon *et al.* 2008; Schrey *et al.* 2012).

Genetic analysis: AFLP

We used GENALEX version 6.41 (Peakall & Smouse 2006) to identify shared haplotypes among individuals and to determine the haplotype diversity (*h*-AFLP) for each site. We also used GENALEX to calculate estimates of genetic differentiation among sites over all loci using an AMOVA framework. We conducted hierarchical AMOVA to compare variances among species (Φ_{RT}), among sites within species (*F. japonica* or *F.* × *bohemica*; Φ_{PR}) and among sites (Φ_{PT}). We also performed hierarchical AMOVA to compare variances among habitats (Φ_{RT}), among sites within habitats (Φ_{PR}) and among sites (Φ_{PT}). In both cases, we did not consider sites with less than five individuals. We then used GENALEX to perform locus-by-locus AMOVA to characterise genetic differentiation at each locus using both hierarchical data sets; and we performed AMOVA pairwise among all sites in the species hierarchy to determine which sites were significantly differentiated. For all AMOVA analyses, we used 9999 permutations to estimate statistical significance and the initial alpha = 0.05, and adjusted for multiple comparisons by the sequential Bonferroni method whenever multiple tests were conducted.

Epigenetic analysis: MS-AFLP

We followed the same framework for the epigenetic analysis using identical statistical methods as in the genetic analysis: identifying shared epi-genotypes, estimating the epi-genotype diversity (*h*-MS-AFLP), hierarchical AMOVA between species and among habitats, followed by locus-by-locus AMOVA and pairwise AMOVA comparisons among sites.

In addition, we analysed epigenetic variation among *F.* × *bohemica* individuals (n = 155), which occurred at 12 sites: 3 beach (MSH n = 7, PJB n = 9, RPB n = 15), 5 roadside (HL n = 15, PWC n = 7, RHC n = 19, RI n = 13, ST n = 14) and 4 saltmarsh (CBH n = 19, OLCT n = 9, RHBH n = 18, WH n = 10). We calculated diversity estimates and conducted AMOVA to compare variances among habitats (Φ_{RT}), among sites within habitats (Φ_{PR}) and among sites (Φ_{PT}). We then performed locus-by-locus AMOVA.

To specifically control for genotype and look at the behaviour of epigenetic variation across sites, we investigated epigenetic variation among *F. japonica* individuals and among *F.* × *bohemica* individuals with the two most common AFLP haplotypes. The *F. japonica* dataset had a relatively low sample size and two of the three habitats were represented by a single site (HP n = 10 Beach; CMR n = 16 Roadside; CBH n = 5, CMM n = 13 Saltmarsh). For *F. japonica*, we calculated diversity estimates and conducted AMOVA to compare variances among habitats (Φ_{RT}), among sites within habitats (Φ_{PR}) and among sites (Φ_{PT}). We then performed locus-by-locus AMOVA.

To control for genotype in *F.* × *bohemica* individuals, we identified the two most common AFLP haplotypes, haplotypes E and G. Haplotype E (n = 38) occurred in two beach (MSH n = 1, RPB n = 11) and three roadside (HL n = 11, PWC n = 2, RI n = 13) populations, whereas haplotype G (n = 102) occurred in four beach (HBCT n = 1, MSH n = 2, PJB n = 6 and RPB n = 4), three roadside (HL n = 4, RHC n = 19 and ST n = 14) and four saltmarsh (CBH n = 15, OLCT n = 9, RHBH n = 18 and WH n = 10) populations. Again, we limited our analyses to sites with at least five individuals. We used a hierarchical AMOVA to compare the amount of epi-genotype differentiation attributed to AFLP-haplotype with that associated with different sites. We then calculated diversity estimates for haplotype G (higher sample size) and performed an AMOVA to compare variances among habitats (Φ_{RT}), among sites within habitats (Φ_{PR}) and among sites (Φ_{PT}). We also performed locus-by-locus AMOVA. We estimated statistical significance following 9999 permutations for all analyses and adjusted for multiple comparisons by the sequential Bonferroni method whenever multiple tests were conducted (alpha = 0.05).

RESULTS

Genetic diversity and structure

Although we detected little sequence-based variation using AFLP, we did find significant differentiation among species. We identified only four variable positions among individuals from *c.* 200 loci across the *Mse*I AFLP selective PCR products, which formed eight haplotypes (Table 1). Haplotype diversity (*h*-AFLP) ranged from 0 to 0.38 among sites (Table 2). We observed no genetic variation within *F. japonica* (including the specimens from Europe); all were haplotype F (Table 1), which had a fixed difference from *F.* × *bohemica* at Locus 4.

Hierarchical AMOVA revealed that a greater portion of the genetic differentiation was attributed to differences between species ($\Phi_{RT} = 0.644$, $P < 0.001$), than among sites within species ($\Phi_{PR} = 0.532$, $P < 0.001$), and significant differentiation was present among sites ($\Phi_{PT} = 0.833$, $P < 0.001$). Locus-by-locus AMOVA showed that two loci were significantly differentiated between species, three loci were significantly differentiated among sites within species and all four polymorphic loci were significantly differentiated among sites (Table 3). However, hierarchical AMOVA revealed that genetic differentiation was not attributable to differences among habitats ($\Phi_{RT} = -0.062$, $P = 1.0$).

Pairwise AMOVA also identified genetic differentiation between *F. japonica* and *F.* × *bohemica*. Of 136 pairwise comparisons, 44 (32%) were significant after sequential Bonferroni correction at alpha = 0.05 (Table 4). The majority of the significant tests, 24

© 2012 Blackwell Publishing Ltd/CNRS

Table 2 Diversity compared between genetic (*h*-AFLP) and epigenetic (*h*-MS-AFLP) loci for all individuals at each site, *F. japonica* individuals, *F.* × *bohemica* individuals and only *F.* × *bohemica* with the most common haplotype (G)

	All samples		*F. japonica*		*F.* × *bohemica*		*F.* × *bohemica* Hap G	
	h-AFLP	*h*-MS-AFLP	*h*-AFLP	*h*-MS-AFLP	*h*-AFLP	*h*-MS-AFLP	*h*-AFLP	*h*-MS-AFLP
Site Beach								
HBCT	0.080 0	0.093	–	–	–	–	–	–
HP	0	0.181	0	0.181	–	–	–	–
MSH	0.297	0.120	–	–	0.245	0.120	–	–
PJB	0.375	0.307	–	–	0.333	0.260	0	0.222
RPB	0.098	0.167	–	–	0.098	0.167	–	–
Roadside								
CMR	0	0.352	0	0.215	–	–	–	–
HL	0.098	0.387	–	–	0.098	0.165	–	–
PWC	0.306	0.204	–	–	0.306	0.322	–	–
RHC	0	0.354	–	–	0	0.052	0	0.052
RI	0	0.355	–	–	0	0.29	–	–
ST	0	0.477	–	–	0	0.088	0	0.088
Marsh								
CBH	0.190	0.232	0	0.194	0.144	0.243	0	0.259
CMM	0	0.258	0	0.177	–	–	–	–
OLCT	0.045	0.303	–	–	0	0.159	0	0.159
RHBH	0	0	–	–	0	0	0	0
WH	0	0.355	–	–	0	0.144	0	0.144
European	0	0.193	0	0.193	–	–	–	–
All	0.090	0.255	0	0.192	0.102	0.167	0	0.132

(55%) occurred in comparisons between *F. japonica* and *F.* × *bohemica*, whereas 20 (45%) tests among *F.* × *bohemica* sites were significant. No pattern was detected among the significant tests within *F.* × *bohemica*. All pairwise comparisons among *F. japonica* were not significant because *F. japonica* had no genetic variation.

Epigenetic diversity and structure

Nearly five times as many variable positions were detected among individuals in the epigenetic analysis compared to the genetic analysis. Nineteen variable positions were detected among 180 loci observed, which formed 128 epi-genotypes (Table 1). There were significantly more variable positions in the epigenetic analysis than the genetic analysis (*t*-test, $P = 0.0004$). Most epi-genotypes (98 of 128) were unique to one individual; yet, some epi-genotypes occurred multiple times and some were shared among sites (Table 1). Haplotype diversity for epi-genotypes (*h*-MS-AFLP) among sites ranged from 0 to 0.477 and was greater than *h*-AFLP at 13 sites (Table 2).

Hierarchical AMOVA revealed that a greater portion of the epigenetic differentiation was attributed to differences among sites within species ($\Phi_{PR} = 0.610$, $P < 0.001$), rather than between species ($\Phi_{RT} = 0.070$, $P < 0.001$), which is opposite to the genetic results. Significant epigenetic differentiation occurred among sites ($\Phi_{ST} = 0.637$, $P < 0.001$). Locus-by-locus AMOVA identified that five loci were significantly differentiated between species, whereas all loci were significantly differentiated among sites within species and among sites (Table 3). Hierarchical AMOVA detected greater differences among sites within habitats ($\Phi_{PR} = 0.639$, $P < 0.001$), than among habitats ($\Phi_{RT} = -0.087$, $P = 1$). Also, locus-by-locus AMOVA found no significant differences among habitats.

Pairwise AMOVA of sites identified much greater differentiation among epi-genotypes than observed among genetic markers. Here, 127 of 136 comparisons (93%) were significant after sequential Bonferroni correction at alpha = 0.05 (Table 4). In direct contrast to the genetic results, all pairwise comparisons among *F. japonica* were significant at epigenetic markers. All but four comparisons among *F.* × *bohemica* were significant, and all but five comparisons between *F. japonica* and *F.* × *bohemica* were significant. The majority (6 of 9; 67%) of non-significant results occurred in comparisons that included *F.* × *bohemica* from site PWC.

In addition to greater epigenetic than genetic differentiation, 67% of pairwise comparisons between sites disagreed between epigenetic and genetic markers. Thus, these results showed that epigenetic differentiation was not dependent on genetic differentiation: 87 (64%) tests were significant at epigenetic, but not genetic markers and 4 (3%) were non-significant at epigenetic, but significant at genetic markers.

Analysis of *F.* × *bohemica* found epigenetic variation to be more than 10 times greater than genetic variation. There were only seven AFLP-haplotypes but 85 epi-genotypes among the 155 individuals. Epigenetic diversity (*h*-MS-AFLP) ranged from 0 to 0.322 and was higher than *h*-AFLP at nine sites (Table 2). Over all loci, AMOVA identified significant epigenetic differentiation among sites ($\Phi_{PT} = 0.634$, $P < 0.001$) and sites within habitats ($\Phi_{PR} = 0.670$, $P < 0.001$), but not among habitats ($\Phi_{RT} = -0.108$, $P = 1$). Locus-by-locus AMOVA identified significant differentiation among sites and among sites within habitats at all loci, but no loci were significant among habitats (Table 3).

The analysis of individuals with no genetic variation indicates more clearly the potential of epigenetic variation. For *F. japonica*, the 19 polymorphic loci formed 34 epi-genotypes, 30 of which were unique to one individual; *h*-MS-AFLP ranged from 0.177 to 0.215 among sites (Table 2). Over all loci, AMOVA identified significant epigenetic differentiation among sites ($\Phi_{PT} = 0.447$, $P < 0.001$) and sites within habitats ($\Phi_{PR} = 0.487$, $P < 0.001$), but not among habi-

© 2012 Blackwell Publishing Ltd/CNRS

Table 3 Summary of significant locus-by-locus tests of differentiation (Φ_{ST}) for A) AFLP among sites (Φ_{PT}), among sites within species (Φ_{PR}) and between species (Φ_{RT}); B) MS-AFLP for all individuals between species (Φ_{RT}), among sites within species (Φ_{PR}) and among sites (Φ_{PT}). For only *F. japonica* among habitats (Φ_{RT}), and among sites (Φ_{PT}). Only *F.* × *bohemica* among sites within habitats (Φ_{PR}), and among sites (Φ_{PT}), only *F.* × *bohemica* with haplotype G among sites within habitats (Φ_{PR}), and among sites (Φ_{PT}). An asterisk denotes significant locus-by-locus tests after sequential Bonferroni correction

A. AFLP analysis

	All individuals		
Locus	Φ_{RT}	Φ_{PR}	Φ_{PT}
1	–	0.474*	0.448*
2	–	0.443*	0.433*
3	0.127*	0.598*	0.649*
4	0.990*	–	0.990*

B. MS-AFLP analysis

						F. × *bohemica*			
	All individuals			*F. japonica*		All		Haplotype G	
Locus	Φ_{RT}	Φ_{PR}	Φ_{PT}	Φ_{RT}	Φ_{PT}	Φ_{PR}	Φ_{PT}	Φ_{PR}	Φ_{PT}
1		0.603*	0.559*	0.836*	0.809*	0.546*	0.509*	0.768*	0.719*
2		0.537*	0.499*			0.710*	0.647*	0.716*	0.649*
3		0.598*	0.560*		0.524*	0.612*	0.608*	0.659*	0.619*
4		0.548*	0.562*	0.437*	0.424*	0.572*	0.592*	0.816*	0.805*
5		0.441*	0.400*			0.568*	0.496*	0.657*	0.583*
6		0.677*	0.642*		0.661*	0.733*	0.669*	0.871*	0.845*
7		0.553*	0.530*			0.683*	0.635*	0.763*	0.717*
8	0.294*	0.808*	0.864*		0.455*	0.893*	0.874*	0.933*	0.914*
9	0.341*	0.628*	0.755*		0.671*	0.620*	0.636*	0.908*	0.883*
10	0.357*	0.632*	0.763*		0.535*	0.662*	0.584*	0.825*	0.770*
11		0.657*	0.625*	0.688*	0.638*	0.686*	0.664*	0.875*	0.841*
12	0.315*	0.593*	0.721*			0.656*	0.606*	0.837*	0.793*
13		0.625*	0.587*	0.522*	0.526*	0.688*	0.659*	0.814*	0.769*
14		0.538*	0.575*			0.606*	0.555*	0.437*	0.447*
15		0.715*	0.677*	0.777*	0.742*	0.788*	0.735*	0.943*	0.927*
16		0.620*	0.609*	0.623*	0.606*	0.683*	0.653*	0.729*	0.660*
17		0.568*	0.592*			0.583*	0.552*	0.696*	0.662*
18		0.723*	0.772*		0.302*	0.851*	0.850*	0.874*	0.845*
19		0.561*	0.568*		0.286*	0.622*	0.593*	0.825*	0.805*

tats ($\Phi_{RT} = -0.079$, $P = 1$). However, locus-by-locus AMOVA identified significant differentiation among habitats at 6 loci and among sites at 13 loci (Table 3; Fig. 1), which suggests that some epigenetic loci may be associated with different habitats.

For *F.* × *bohemica*, AFLP haplotype did not account for a significant portion of the variation in MS-AFLP epi-genotype. There was no significant differentiation in epi-genotypes between AFLP-haplotypes ($\Phi_{RT} = -0.102$, $P = 1$), yet there was significant differentiation among sites within AFLP-haplotypes ($\Phi_{PR} = 0.689$, $P < 0.001$), and among sites ($\Phi_{ST} = 0.657$, $P < 0.001$).

Individuals of *F.* × *bohemica* with the most common AFLP-haplotype (haplotype G) were highly variable at epigenetic markers. There were 53 epi-genotypes among the 102 individuals. Of the seven sites with more than five individuals, *h*-MS-AFLP ranged from 0 to 0.259 (Table 2); over all loci, AMOVA identified significant epigenetic differentiation among sites ($\Phi_{PT} = 0.720$, $P < 0.001$) and sites within habitats ($\Phi_{PR} = 0.794$, $P < 0.001$), but not among habitats ($\Phi_{RT} = -0.361$, $P = 1$). Locus-by-locus AMOVA identified significant differentiation among sites and among sites within habitats at all loci, but not among habitats (Table 3). The proportion of

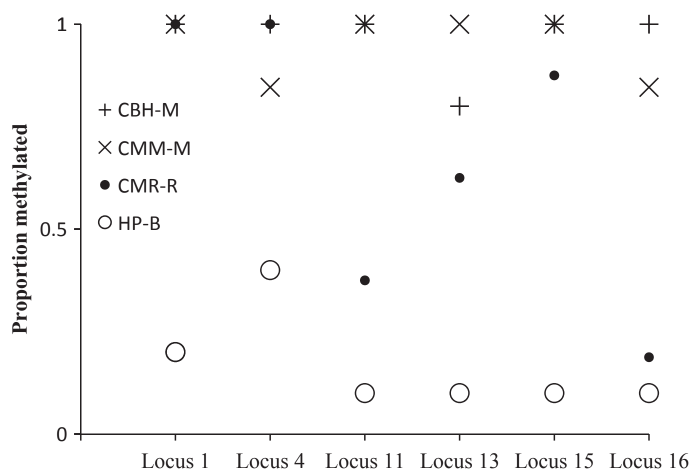

Figure 1 The proportion of *F. japonica* individuals with methylation at MS-AFLP loci that showed significant epigenetic differentiation among habitats. These individuals had no genetic variation. Site abbreviations are defined in Table 1.

© 2012 Blackwell Publishing Ltd/CNRS

Table 4 Pairwise AMOVA among sites identified by species *F. japonica* (Fj) and *F.* × *bohemica* (F × b). Results from genetic markers are presented below the diagonal and those from epigenetic markers are presented above the diagonal. Site abbreviations are defined in Table 1. An asterisk denotes statistical significance after sequential Bonferroni correction (alpha = 0.05)

	CBH-F × b	HL-F × b	MSH-F × b	OLCT-F × b	PJB -F × b	PWC-F × b	RHBH-F × b	RHC-F × b	RI-F × b	RPB-F × b	ST-F × b	WH-F × b	B-Fj	CBH-Fj	CMM-Fj	CMR-Fj	HP-Fj
CBH-F × b		0.492*	0.513*	0.421*	0.246*	0.531*	0.655*	0.418*	0.396*	0.512*	0.741*	0.690*	0.504*	0	0.503*	0.515*	0.659*
HL-F × b	0.420*		0.632*	0.338*	0.383*	0.568*	0.787*	0.662*	0.546*	0.309*	0.796*	0.737*	0.575*	0.550*	0.604*	0.620*	0.708*
MSH-F × b	0.256	0.357		0.618*	0.480*	0.521*	0.866*	0.770*	0.413*	0.660*	0.816*	0.743*	0.607*	0.541*	0.654*	0.607*	0.719*
OLCT-F × b	0	0.658	0.478*		0.268*	0.522*	0.739*	0.648*	0.462*	0.366*	0.813*	0.737*	0.538*	0.505*	0.605*	0.570*	0.707*
PJB-F × b	0.114	0.276	0.004	0.25		0.393*	0.711*	0.610*	0.310*	0.478*	0.704*	0.633*	0.417*	0.280*	0.500*	0.531*	0.600*
PWC-F × b	0.485	0.613	0.222	0.626*	0.067		0.824*	0.789*	0.268	0.534*	0.199	0.06	0.344	0.493	0.466*	0.336*	0.051
RHBH-F × b	0.033	0.735*	0.617*	0	0.379	0.741*		0.891*	0.694*	0.805*	0.942*	0.917*	0.885*	0.852*	0.801*	0.747*	0.894*
RHC-F × b	0.037	0.742*	0.628*	0	0.39	0.749*	0		0.657*	0.669*	0.916*	0.886*	0.759*	0.598*	0.763*	0.758*	0.864*
RI-F × b	0.685*	0.196	0.616*	0	0.504*	0.756	0	0		0.581*	0.541*	0.469*	0.273*	0.347*	0.316*	0.355*	0.422*
RPB-F × b	0.420*	0	0.357	0.658	0.276	0.613	0.735*	0.742*	0.196		0.798*	0.719*	0.536*	0.555*	0.624*	0.578*	0.683*
ST-F × b	0.016	0.706*	0.566	0	0.33	0.700*	0	0	0	0.706*		0.144	0.692*	0.807*	0.694*	0.599*	0.170*
WH-F × b	0	0.669	0.499	0	0.268	0.644*	0	0	0	0.669*	0		0.591*	0.726*	0.600*	0.434*	0.025
B-Fj	0.646	0.829*	0.694	0	0.566*	0.718	0	0	0	0.829*	0	0		0.486*	0.466*	0.507*	0.528*
CBH-Fj	0.633*	0.822*	0.67	0	0.541	0.695	0	0	0	0.821*	0	0	0		0.499*	0.495*	0.684*
CMM-Fj	0.711*	0.871*	0.794*	0	0.680*	0.811*	0	0	0	0.871*	0	0	0	0		0.245*	0.529*
CMR-Fj	0.731*	0.883*	0.819*	0	0.712*	0.835*	0	0	0	0.883*	0	0	0	0	0		0.402*
HP-Fj	0.687*	0.856*	0.761*	0	0.641*	0.781*	0	0	0	0.856*	0	0	0	0	0	0	

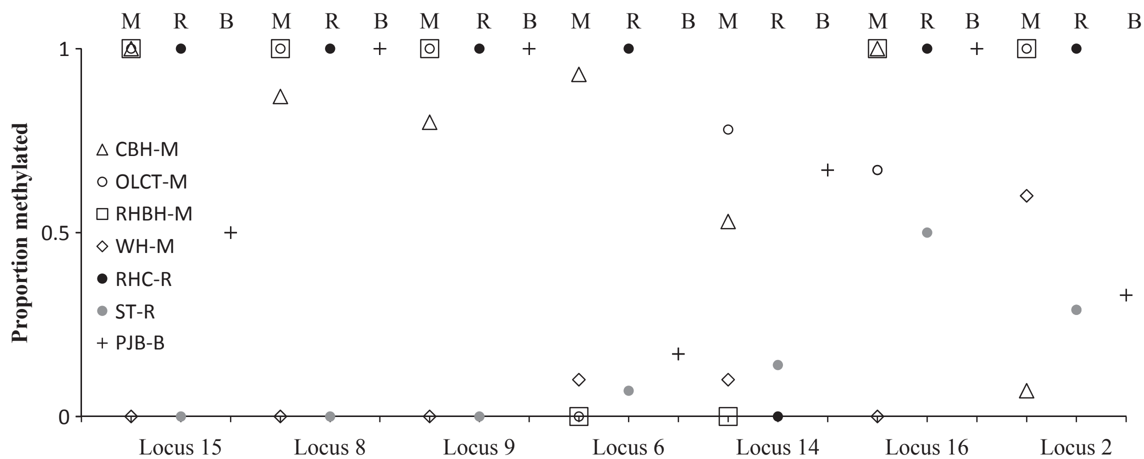

Figure 2 The proportion of *F.* × *bohemica* individuals with methylation at a representative selection of MS-AFLP loci with significant epigenetic differentiation among sites. These individuals had no genetic variation (AFLP-haplotype G). Habitats are designated across the top of the figure; saltmarsh = M, roadside = R and beach = B. Site abbreviations are defined in Table 1.

individuals with methylation at a subset of the significant loci (Fig. 2) shows that the epigenetic loci may allow for fine-scale differentiation to local microhabitat conditions in absence of genetic variation.

DISCUSSION

Although it has often been suspected that epigenetic mechanisms could contribute to the diversity and evolutionary potential of natu-

© 2012 Blackwell Publishing Ltd/CNRS

ral populations (reviewed in Rapp & Wendel 2005; Bossdorf *et al.* 2008b; Jablonka & Raz 2009), these ideas have rarely been tested in natural populations. Epigenetic sources of variation could be particularly important in invasive species given that many invasives appear to do well even with low levels of genetic variation (Hollingsworth & Bailey 2000; Dlugosch & Parker 2008a,b; Richards *et al.* 2008; Loomis & Fishman 2009). Our results confirm that invasive Japanese knotweed populations in and around Long Island, NY have almost no genetic diversity despite the fact that they occur across a diversity of habitats and display significant phenotypic variation (Richards *et al.* 2008; Walls 2010). As such, the Japanese knotweed populations provide a convenient system to test the relevance of epigenetic effects.

The increased interest in understanding the role of epigenetic processes in ecology and evolution has attracted empirical studies across plant, animal and yeast taxa. A variety of studies merge ecological experimental design with chemical manipulation of genome-wide DNA methylation by treatment with 5-azacytidine (Bossdorf *et al.* 2010; Herrera & Bazaga 2012) or screening for MS-AFLPs in response to stress (Verhoeven *et al.* 2010; Herrera *et al.* 2012), sampling from different habitats (Herrera *et al.* 2010; Paun *et al.* 2010; Herrera & Bazaga 2012; Massicotte & Angers 2012; Schrey *et al.* 2012) and surveying natural levels of herbivory (Herrera & Bazaga 2011). Evidence to date supports the idea that epimutations may be more frequent, but also more labile than mutations of DNA sequence (Rapp & Wendel 2005; Johannes *et al.* 2009; Teixeira *et al.* 2009; Verhoeven *et al.* 2010). Moreover, it has been shown that in some sequence contexts, DNA methylation errors are quickly and actively repaired (e.g. through RNA-directed DNA re-methylation; Teixeira *et al.* 2009), whereas in other DNA contexts no active restoration occurs and DNA methylation changes may turn into stable polymorphisms. A recent study showed that even in a constant environment, some DNA methylation differences developed between individual plants (7.5% of the polymorphic DNA methylation loci), that most of these changes were inherited across generations and that new DNA methylation changes occurred in each generation (Verhoeven *et al.* 2010).

Because epigenetic variation is to some extent environmentally labile and reversible, it follows that patterns of epigenetic differentiation measured among individuals collected in natural field populations confound the reversible epigenetic effects that are due to within-generation phenotypic plasticity (Richards *et al.* 2010b; Richards 2011). For this reason, growth in a common garden is necessary to distinguish between plastic and persistent components of variation and firmly establish that epigenetic effects could contribute to the process of adaptation. While a few studies have found a correlation between epigenetic variation and different habitats (e.g. Herrera & Bazaga 2010, 2011; Herrera & Bazaga 2012; Lira-Medeiros *et al.* 2010; Paun *et al.* 2010; Massicotte & Angers 2012), or greater epigenetic than genetic variation among different habitats (Massicotte & Angers 2012; Schrey *et al.* 2012), these studies have invariably confounded environmentally labile and stable epigenetic polymorphisms. Our study accounts for this problem as we grew material from rhizomes in a common environment to minimise the environmentally induced nature of methylation changes. Growing the material in the greenhouse from rhizomes for 6 months and sampling leaf material that was produced in the greenhouse should have minimised the labile differences induced among sites and maximised our ability to detect stable methylation changes that persist through clonal reproduction (Rapp & Wendel 2005; Bossdorf *et al.* 2008b; Richards *et al.* 2010a,b). Even though we cannot say that the epigenetic marks that we detected are truly heritable (i.e. surviving the process of sexual reproduction), *F. japonica* has been shown to disperse entirely by clonal propagation across the invasive range (Hollingsworth & Bailey 2000). The persistence of epigenetic marks that we found is therefore meaningful for the success of the Japanese knotweed invasion and expansion. Moreover, while the process of meiosis involves extensive epigenetic remodelling in animals, this remodelling is not universal in animals and even less extensive in plants (Feng *et al.* 2010).

The number of natural field studies of epigenetic effects is limited, but several controlled environment studies suggest that environmental induction of epigenetic variation can be stably inherited across generations and could be particularly important in plant invasions. Multigeneration experiments have shown that parental exposure to biotic or abiotic stresses resulted in modified DNA methylation in unexposed offspring (e.g. Boyko *et al.* 2010; Verhoeven *et al.* 2010). In dandelion, MS-AFLP showed that plants with identical genotypes exposed to different stresses had up to 30% change in polymorphic methylation-sensitive markers compared to control (Verhoeven *et al.* 2010). Some of these methylation differences reverted back to the original in the next generation of plants grown in a common environment, but this study provides some of the first evidence that the majority of the changes in methylation are inherited in the next generation.

In our study, we expected that the limited genetic diversity across 16 populations of *Fallopia* would allow us to explore the structure and potential importance of epigenetic variation for establishment of this successful invasive in diverse habitats. Indeed, we found low sequence polymorphism with only four polymorphic sites out of 200 yielding only 8 haplotypes that showed largely differentiation by taxa and to a lesser degree differentiation by site. In contrast, epigenetic variation was much higher (19 polymorphic sites out of 180 creating 129 epi-genotypes), and nearly all sites were differentiated. This pattern was present regardless of whether we looked across the whole collection of Japanese knotweed, just the *F. japonica,* the hybrid *F.* × *bohemica* or the most common hybrid haplotypes (E and G). These findings support the idea that the epigenetic marks locally differentiate faster and among more sites than genetic markers, which suggests that local habitats elicit persistent changes. Not only was epigenetic differentiation greater than genetic differentiation, but pairwise comparisons showed that epigenetic differentiation occurs independently of genetic differentiation. Remarkably, the epigenetic structure is even stronger in response to sites than the differences between the two most common hybrid haplotypes, which were not significantly differentiated across the epigenetic marks.

Analysis of individuals with no genetic variation (*F. japonica* and *F.* × *bohemica* with haplotype G) further illustrates how epigenetic marks can differentiate independently of genetic ones. We found that some epigenetic loci in *F. japonica* show differentiation among habitats, although this could be an artefact of low replication of sites within habitats. In *F.* × *bohemica* with haplotype G, several loci showed a similar pattern of differentiation among habitats, but in every case at least one site had the opposite pattern of methylation from the trend. These findings suggest that epigenetic marks may be associated more specifically to each unique location, or that

© 2012 Blackwell Publishing Ltd/CNRS

factors other than our characterisation of 'beach', 'marsh' and 'roadside' habitats may play an important role in shaping epigenetic differentiation to local conditions. For example, Massicotte & Angers (2012) recently reported that methylation patterns in natural populations of the clonal fish *Chrosomus eos-neogaeus* did not correlate with their broadly defined habitats, but instead correlated more specifically with variation in pH.

Although we controlled for environmentally induced effects by growing the plant material in a common garden, our findings lead to the question of whether the epigenetic differences allowed the colonising individuals to succeed in these variable environments or if instead they were induced by the environment once the individuals had arrived. In a series of studies of wild *Viola cazorlensis,* DNA methylation variation was correlated with both flower morphology and natural levels of herbivory in plants collected from their natural habitat (Herrera & Bazaga 2010, 2011). While the authors of these studies were unable to control for genetic variation or environmentally induced epigenetic variation, they were able to model the interaction among diversity at AFLP loci, MS-AFLP loci and ecologically important traits as expressed in natural habitats. Both DNA sequence and methylation patterns had direct and indirect effects on response to herbivory, but path analysis was unable to discriminate alternative models that would indicate whether the epigenetic effects were the cause or the effect of differential herbivory. Similarly, in our study, it could be that exposure to the novel habitat induced epigenetic variation, which then became correlated with the greater success of the individual knotweed plants that survived. Alternatively, *potential* for greater epigenetic variation could be adaptive for invasive plants. Further manipulative studies with replicates of epi-genotypes are required to tease apart these possibilities (Bossdorf *et al.* 2008b; Richards *et al.* 2010a,b).

ACKNOWLEDGEMENTS

We thank the Wendel, Pigliucci, Purugganan and True labs (especially Ryan Percifield, Jennifer Hawkins and Shia-Ren Liou) for advice and support on running AFLP. We thank Ramona Walls for invaluable assistance in finding field sites and making connections with local government agencies and organisations, and John Bailey at the University of Leicester and Amy Litt at the New York Botanical Gardens for arranging for the import of the Japanese specimens. We also thank Larry Gottschamer for assistance in collecting rhizomes in the field as well as Stony Brook University greenhouse staff Mike Axelrod and John Clumpp for maintaining the plants in the greenhouse. Jonathan Wendel and Koen Verhoeven provided valuable feedback on the manuscript. This work was partially supported by New York SEA Grant (CLR & MP), the Research Foundation of the State University of New York (CLR & MP) and the University of South Florida (CLR & AWS).

STATEMENT OF AUTHORSHIP

CLR & MP developed the ideas for the study. CLR designed the study, collected and cultivated plant material and ran AFLP and MS-AFLP protocols, AWS performed AFLP and MS-AFLP analyses. CLR wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

REFERENCES

Bailey, J.P. (2003) Japanese knotweed *s.l.* at home and abroad. In: *Plant Invasions: Ecological Threats and Management Solutions* (eds Child, L.E., Brock, J.H., Brundu, G., Prach, K., Pysek, P., Wade, P.M. & Williamson, M.). Backhuys Publishers, Leiden, The Netherlands, pp. 183–196.

Bailey, J.P., Bímová, K. & Mandák, B. (2009). Asexual spread versus sexual reproduction and evolution in Japanese Knotweed s.l. sets the stage for the "Battle of the Clones". *Biol. Invasions*, 11, 1189–1203.

Baker, H.G. (1965) Characteristics and modes of origin of weeds. In: *The Genetics of Colonizing Species* (eds Baker, H.G. & Stebbins, G.L.). Academic Press, New York, pp. 147–169.

Benham, J., Jeung, J.-U., Jasieniuk, M., Kanazin, V. & Blake, T. (1999). Genographer: a tool for automated AFLP and microsatellite analysis. *J. Agric. Genomics*. 4: article 3. Available at: http://hordeum.oscs. montana.edu/genographer/. Last accessed 11 April 2011.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W.E., Siemann, E. & Prati, D. (2005). Phenotypic and genetic differentiation between native and introduced populations. *Oecologia*, 144, 1–11.

Bossdorf, O., Lipowsky, A. & Prati, D. (2008a). Selection of preadapted populations allowed *Senecio inaequidens* to invade Central Europe. *Divers. Distrib.*, 14, 676–685.

Bossdorf, O., Richards, C.L. & Pigliucci, M. (2008b). Epigenetics for ecologists. *Ecol. Lett.*, 11, 106–115.

Bossdorf, O., Arcurri, D., Richards, C.L. & Pigliucci, M. (2010). Experimental alteration of DNA methylation affects the phenotypic plasticity of ecologically relevant traits in Arabidopsis thaliana. *Evol. Ecol.*, 24, 541–553.

Boyko, A., Blevins, T., Yao, Y.L., Golubov, A., Bilichak, A., Ilnytskyy, Y. *et al.* (2010). Transgenerational adaptation of Arabidopsis to stress requires DNA methylation and the function of dicer-like proteins. *PLoS One*, 5, e9514.

Davidson, A.M., Jennions, M. & Nicotra, A.B. (2011). Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecol. Lett.*, 14, 419–431.

Dlugosch, K.M. & Parker, I.M. (2007). Molecular and quantitative trait variation across the native range of the invasive species Hypericum canariense: evidence for ancient patterns of colonization via pre-adaptation? *Mol. Ecol.*, 16, 4269–4283.

Dlugosch, K.M. & Parker, I.M. (2008a). Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol. Ecol.*, 17, 431–449.

Dlugosch, K.M. & Parker, I.M. (2008b). Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. *Ecol. Lett.*, 11, 701–709.

Durka, W., Bossdorf, O., Prati, D. & Auge, H. (2005). Molecular evidence for multiple introductions of invasive garlic mustard (Alliaria petiolata, Brassicaceae) to North America. *Mol. Ecol.*, 14, 1697–1706.

Feng, S., Jacobsen, S.E. & Reik, W. (2010). Epigenetic reprogramming in plant and animal development. *Science*, 330, 622–627.

Gammon, M.A. & Kesseli, R. (2010). Haplotypes of *Fallopia* introduced into the US. *Biol. Invasions*, 12, 421–427.

Gammon, M.A., Grimsby, J.L., Tsirelson, D. & Kesseli, R. (2007). Molecular and morphological evidence reveals introgression in swarms of the invasive taxa *Fallopia japonica, F. sachalinensis,* and *F.* × bohemica (Polygonaceae) in the United States. *Am. J. Bot.*, 94, 948–956.

Grimsby, J.L., Tsirelson, D., Gammon, M.A. & Kesseli, R. (2007). Genetic diversity vs, sexual reproduction in *Fallopia* spp. (Polygonaceae). *Am. J. Bot.*, 94, 957–964.

Herrera, C.M. & Bazaga, P. (2010). Epigenetic differentiation and relationship to adaptive genetic divergence in discrete populations of the violet *Viola cazorlensis*. *New Phytol.*, 187, 867–876.

Herrera, C.M. & Bazaga, P. (2011). Untangling individual variation in natural populations: ecological, genetic and epigenetic correlates of long-term inequality in herbivory. *Mol. Ecol.*, 20, 1675–1688.

Herrera, C.M., Pozo, M.I. & Bazaga, P. (2012). Jack of all nectars, master of most: DNA methylation and the epigenetic basis of niche width in a flower-living yeast. *Mol. Ecol.*, 21, 2602–2616.

Hollingsworth, M.L. & Bailey, J.P. (2000). Evidence for massive clonal growth in the invasive weed *Fallopia japonica* (Japanese knotweed). *Bot. J. Linn. Soc.*, 133, 463–472.

© 2012 Blackwell Publishing Ltd/CNRS

Jablonka, E. & Raz, G. (2009). Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Q. Rev. Biol.*, 84, 132–176.

Johannes, F., Porcher, E., Teixeira, F.K., Saliba-Colombani, V., Simon, M., Agier, N. *et al.* (2009). Assessing the Impact of transgenerational epigenetic variation on complex traits. *PLoS Genet.*, 5, e1000530.

Keller, S.R. & Taylor, D.R. (2008). History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. *Ecol. Lett.*, 11, 852–866.

Lavergne, S. & Molofsky, J. (2007). Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proc. Nat. Acad. Sci.*, 104, 3883–3888.

Lira-Medeiros, C.F., Parisod, C., Fernandes, R.A., Mata, C.S., Cardoso, M.A. & Ferreira, P.C.G. (2010). Epigenetic variation in mangrove plants occurring in contrasting natural environment. *PLoS One*, 5, e10326.

Loomis, E.S. & Fishman, L. (2009). A continent-wide clone: population genetic variation of the invasive plant *Hieracium aurantiacum* (Orange hawkweed; asteraceae) in North America. *Int. J. Plant Sci.*, 170, 759–765.

Mandák, B., Bímová, K., Pysek, P., Stepánek, J. & Placková, I. (2005). Isoenzyme diversity in *Reynoutria* (Polygonaceae) taxa: escape from sterility by hybridization. *Plant Syst. Evol.*, 253, 219–230.

Massicotte, R. & Angers, B. (2012). General-Purpose Genotype or how epigenetics extend the flexibility of a genotype. *Genetics Research International* 2012, 1–7: Article ID 317175.

Muth, N.Z. & Pigliucci, M. (2007). Implementation of a novel framework for assessing species plasticity in biological invasions: responses of *Centaurea* and *Crepis* to phosphorus and water availability. *J. Ecol.*, 95, 1001–1013.

Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A., Finnegan, E.J., Mathesius, U. *et al.* (2010). Plant phenotypic plasticity in a changing climate. *Trends Plant Sci.*, 15, 684–692.

Paun, O. , Bateman, R.M., Fay, M.F., Hedrén, M., Civeyrel, L. & Chase, M.W. (2010). Stable epigenetic effects impact adaptation in allopolyploid orchids (*Dactylorhiza*: Orchidaceae). *Mol. Biol. Evol.*, 27, 2465–2473.

Peakall, R. & Smouse, P.E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes*, 6, 288–295.

Prentis, P.J., Wilson, J.R.U., Dormontt, E.E., Richardson, D.M. & Lowe, A.J. (2008). Adaptive evolution in invasive species. *Trends Plant Sci.*, 13, 288–294.

Rapp, R.A. & Wendel, J.F. (2005). Epigenetics and plant evolution. *New Phytol.*, 168, 81–91.

Richards, E.J. (2011). Natural epigenetic variation in plant species: a view from the field. *Curr. Opin. Plant Biol.*, 14, 204–209.

Richards, C.L., Bossdorf, O., Muth, N.Z., Gurevitch, J. & Pigliucci, M. (2006). Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol. Lett.*, 9, 981–993.

Richards, C.L., Walls, R., Bailey, J.P., Parameswaran, R., George, T. & Pigliucci, M. (2008). Plasticity in salt tolerance traits allows for invasion of salt marshes by Japanese knotweed s.l. (Fallopia japonica and F. × bohemica, Polygonaceae). *Am. J. Bot.*, 95, 931–942.

Richards, C.L., Bossdorf, O. & Pigliucci, M. (2010a). What role does heritable epigenetic variation play in phenotypic evolution? *Bioscience*, 60, 232–237.

Richards, C.L., Bossdorf, O. & Verhoeven, K.J.F. (2010b). Understanding natural epigenetic variation. *New Phytol.*, 187, 562–564.

Rosenthal, D.M., Ramakrishnan, A.P. & Cruzan, M.B. (2008). Evidence for multiple sources of invasion and intraspecific hybridization in *Brachypodium sylvaticum* (Hudson) Beauv. in North America. *Mol. Ecol.*, 17, 4657–4669.

Salmon, A., Ainouche, M.L. & Wendel, J.F. (2005). Genetic and epigenetic consequences of recent hybridization and polyploidy in *Spartina* (Poaceae). *Mol. Ecol.*, 14, 1163–1175.

Salmon, A., Clotault, J., Jenczewski, E., Chable, V. & Manzanares-Dauleux, M.J. (2008). *Brassica oleracea* displays a high level of DNA methylation polymorphism. *Plant Sci.*, 174, 61–70.

Schrey, A.W., Coon, C.A.C., Grispo, M.T., Awad, M., Imboma, T., McCoy, E.D. *et al.* (2012). Epigenetic variation may compensate for decreased genetic variation with introductions: a case study using house sparrows (Passer domesticus) on two continents. *Genetics Research International*, 2012, 1–3: Article ID 979751.

Scoville, A.G., Barnett, L.B., Bodbyl-Roels, S., Kelly, J.K. & Hileman, L.C. (2011). Differential regulation of a MYB transcription factor predicts transgenerational epigenetic inheritance of trichome density in *Mimulus guttatus*. *New Phytol.*, 191, 251–263.

Simberloff, D., Dayan, T., Jones, C. & Ogura, G. (2000). Character displacement and release in the small Indian mongoose, *Herpestes javanicus*. *Ecology*, 81, 2086–2099.

Teixeira, F.K., Heredia, F., Sarazin, A., Roudier, F., Boccara, M., Ciaudo, C. *et al.*2009). A role for RNAi in the selective correction of DNA methylation defects. *Science*, 323, 1600–1604.

Verhoeven, K.J.F., Jansen, J.J., van Dijk, P.J. & Biere, A. (2010). Stress-induced DNA methylation changes and their heritability in asexual dandelions. *New Phytol.*, 185, 1108–1118.

Walls, R.L. (2010). Hybridization and plasticity contribute to divergence among coastal and wetland populations of invasive hybrid Japanese knotweed *s.l.* (Fallopia spp.). *Estuaries Coasts*, 33, 902–918.

Editor, Mark Vellend
Manuscript received 30 April 2012
Manuscript accepted 28 May 2012

© 2012 Blackwell Publishing Ltd/CNRS