AFLP Genome Scan to Detect Genetic Structure and Candidate Loci under Selection for Local Adaptation of the Invasive Weed *Mikania micrantha*

Ting Wang¹, Guopei Chen¹,², Qijie Zan³, Chunbo Wang², Ying-juan Su²⁺

¹ CAS Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, China, ² State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou, China, ³ Shenzhen Wildlife Rescue and Rehabilitation Center, Shenzhen, China

Abstract

Why some species become successful invaders is an important issue in invasive biology. However, limited genomic resources make it very difficult for identifying candidate genes involved in invasiveness. *Mikania micrantha* H.B.K. (Asteraceae), one of the world's most invasive weeds, has adapted rapidly in response to novel environments since its introduction to southern China. In its genome, we expect to find outlier loci under selection for local adaptation, critical to dissecting the molecular mechanisms of invasiveness. An explorative amplified fragment length polymorphism (AFLP) genome scan was used to detect candidate loci under selection in 28 *M. micrantha* populations across its entire introduced range in southern China. We also estimated population genetic parameters, bottleneck signatures, and linkage disequilibrium. In binary characters, such as presence or absence of AFLP bands, if all four character combinations are present, it is referred to as a character incompatibility. Since character incompatibility is deemed to be rare in populations with extensive asexual reproduction, a character incompatibility analysis was also performed in order to infer the predominant mating system in the introduced *M. micrantha* populations. Out of 483 AFLP loci examined using stringent significance criteria, 14 highly credible outlier loci were identified by DfDist and Bayescan. Moreover, remarkable genetic variation, multiple introductions, substantial bottlenecks and character compatibility were found to occur in *M. micrantha*. Thus local adaptation at the genome level indeed exists in *M. micrantha*, and may represent a major evolutionary mechanism of successful invasion. Interactions between genetic diversity, multiple introductions, and reproductive modes contribute to increase the capacity of adaptive evolution.

Introduction

Why some species become successful invaders is an important issue in invasive biology. When species are introduced into a new region, they face two fates. Some species quickly go extinct, whereas others persist and finally become highly competitive invaders, posing a serious threat to native diversity and ecosystems [1, 2]. Successful invasions involve three major phases: introduction, naturalization, and invasion [3, 4]. In the initial introduction phase, invasive species often contain low levels of genetic diversity due to bottleneck and founder effects [5–8]. Then, invaders produce pre-adapted genotypes in response to the abrupt environmental changes during naturalization [9–12]. Finally, exotic species become broadly invasive in the extended period [9, 13]. The rapid population expansion of invaders is expected to promote adaptive evolution, since it has been shown that the rapidly increasing population size is conducive to withstanding (and responding to) strong directional selection [14, 15]. A substantial time lag is involved during the transition from introduction via naturalization to invasion [4]. The occurrence of a lag phase allows populations to adapt to new environmental factors such as ecological niche, temperature, precipitation, soils, frost, and wind speed or growing season length [11, 12, 16–18]. On the other hand, neutral or deleterious alleles, which become favored in new ecological contexts, will contribute to adaptive changes of invasive populations [19]. These changes may increase the survival rate of invasive species [12], making them become gradually dominant in the introduced range [20]. Therefore, pre-adaptation to novel environments is often counted as a premise for successful invasion [3, 4, 21].

Genomic scans are useful to identify potential adaptive loci under selection at the genomic level [22, 23]. All loci across the genome are anticipated to possess similar demography and neutral evolution history of populations, including genetic drift and gene dispersal [24]. If variation of a locus is beyond the genomic pattern with an unusual frame of higher genetic differentiation, it is deemed an “outlier locus” under natural selection [24, 25]. The outlier locus can be identified explicitly in the genes under selection and also in neutral flanking regions due to hitchhiking effects [26, 27]. In model organisms for which whole genomic information is available, it is easy to track the “outlier locus” under selection [28]. However, for non-model organism such as invasive
species, it becomes difficult to identify candidate genes and pinpoint the evolutionary and genetic factors involved in invasiveness because of restricted genomic resources [29,30]. New methods, especially those based on the polymerase chain reaction (PCR) to obtain amplified polymorphisms, have frequently been used to scan genomes in non-model organisms [29,31]. Among these, amplified fragment length polymorphisms (AFLPs) are reported to be the most efficient approach to identify candidate genomic regions under selection (candidate loci or outlier loci) [22,23,31–37]. They can provide several hundred random loci scattered throughout the genome at low cost [37]. So far, the most commonly used analysis approaches for AFLP genome scans are DfDist and the hierarchical Bayesian method (Bayescan). DfDist, which was originally developed by Beaumont & Nichols [38], employs a classical Wright's island model to generate the expected neutral distribution of FST estimates [39–41]. In contrast, Bayescan relies on a logistic regression model [42], representing an extended method devised by Beaumont & Balding [43]. To decrease false positives, DfDist mainly depends on the trimmed mean of the empirical FST distribution for the simulation [44], whereas Bayescan uses a likelihood ratio test to evaluate the most likely of two alternative models, one that includes the effect of selection and another that excludes it [41,42]. Bayescan has been suggested to be more efficient at detecting high selective loci with low false positives [45].

*Mikania micrantha* H. B. K. (Asteraceae) is one of the top ten worst weeds in the world [46]. It is a many-branched climbing perennial vine native to tropical Central and South America where it is a weed of minor importance [46–48]. *M. micrantha* was deliberately introduced into Asia as early as 1900s [49]. In humid sub-tropical China, *M. micrantha* is subjected to new selective pressures, including such abiotic and biotic stresses as new climate, soils, pathogens, herbivores, pollinators, and competitors [50]. By adaptive evolutionary changes, *M. micrantha* survived and reproduced in new environments using strikingly different strategies from those employed in its original niche [19]. It demonstrated the ability to outcompete native plants in utilizing such limited resources as soil nutrients and sunlight and releasing phytotoxic compounds to inhibit growth of neighboring plants [51–54]. Once established, *M. micrantha* became a dominant plant across most regions, influencing ecosystems, biological diversity, and natural communities [50]. By the late 1980s and early 1990s, it had spread extensively in southern China, colonizing agricultural land, orchards, nurseries, lawns, mangroves, secondary forests, scrubland, waste ground, ponds, and seashore [55,56]. Unlike in its native range, in southern China *M. micrantha* grows on dry soils as well as shady sites [56]. Its favorable growth conditions have changed to an average annual temperature higher than 21°C and soil moisture content over 15% [52]. Since spreading at accelerated rates, it is reasonable to postulate that adaptive evolution provides a key mechanism allowing the success of *M. micrantha* in new environments [31,57]. Such adaptive processes may leave specific signatures in the genome of *M. micrantha* [36]. Thus we expected to detect the “outlier locus” signature of such local adaptations under selection at the genomic level. It is particularly important to find the “outlier locus” associated with the local adaptation because, firstly, the information is critical to understanding the introduction history and genetic consequences of introduced populations of *M. micrantha* [2,30]. Second, it may provide fresh insights into the evolutionary potential of *M. micrantha* populations, which is helpful to predict their adaptability in response to management practices [9,58]. Finally, the locus will offer a prime candidate for functional surveys targeting the linked gene and dissecting the molecular mechanism of invasiveness [36].

To test our prediction that outlier loci can be found in southern China populations of *M. micrantha* (Figure 1; Table 1) and to detect genetic structure, in the present study we performed a genome scan analysis based on a large number of AFLP polymorphisms. The goals of the study were (1) to identify candidate loci under selection for local adaptation in *M. micrantha* and (2) to understand contributions of interactions between genetic diversity, reproductive modes, bottlenecks, and multiple introductions to the adaptive evolution of *M. micrantha*.

## Results

### Population genetic structure

AFLP analysis of *M. micrantha* was optimized by determining which selective primer sets produced the clearest DNA fragments based on the eight primer pair combinations. The eight primer pairs were polymorphic in each population. A total of 483 clear polymorphic fragments were detected. The proportion of polymorphic loci within a population ranged from 10.57% to 73.47%, with an average value of 41.84% (Table 2). The average number of fragments per individual was 338.39, and 67.75 per primer combination.

At the regional level, either Hong Kong or Zhuhai was consistently found to maintain the highest level of variation according to all the genetic diversity statistics (Table 2). However, populations such as MA106, SZ72, NLD10, NLD26, and NLD30 growing in regions of Macao, Shenzhen, and Neilingding were observed to possess lower amounts of genetic variation than others in the same region (Table 2).

### Linkage disequilibrium and character compatibility

We estimated levels of linkage disequilibrium (LD) and character compatibility among the 483 AFLP loci. Each region was observed to possess significant LD and matrix compatibility obtained by testing the index of association (I_A and F_A) and the incompatibility excess ratio (IER) (Table 2). Likewise, significant LD and matrix compatibility were also detected at the population scale (Table 2).

### Allele frequency distribution and bottleneck signature test

Alleles were defined as rare when they occurred at a frequency of less than 0.05 in the examined populations [59]. In total, 22 rare alleles were identified at the species level. Across regions, 14, 12, and 9 rare alleles were detected in Hong Kong, Shenzhen, and Neilingding, respectively, whereas no rare alleles were found in Macao, Zhuhai, or Dongguan. In addition, no rare allele was detected within any populations at the population level.

Using the stepwise mutation model (SMM) and the infinite allele model (IAM), we identified bottleneck signatures in each population with a heterozygosity excess/deficiency ratio that significantly deviated from the expected ratio (1:1) at mutation-drift equilibrium (Table 3, P<0.05). Moreover, the entire range of *M. micrantha* appeared subject to a significant bottleneck (Table 3).

### Population genetic differentiation and relationship

The genetic differentiation ΦSTS measured by AMOVA analysis was 0.3355; 66.65% of the variation was partitioned within populations, 28.61% was attributed to differences among populations within regions, and only 4.74% of the variation was due to regional differences (all three hierarchical levels were significant with P<0.001) (Table 4). When individual pairs of populations were compared, of the 378 pairwise ΦSTS values, 373 were significant (P<0.001), whereas only five values derived from SZ64
vs. SZ75, SZ64 vs. SZ77, SZ64 vs. DG91, SZ75 vs. SZ77, and SZ75 vs. DG91 were not (P > 0.05), highlighting remarkable differences among populations.

The $f$-free model chosen based on the smallest mean DIC was more suitable than other models for the AFLP data set of the 28 populations. Therefore, $\theta^B = 0.2927$ (95% credible interval: 0.2772–0.3058) was determined to be an unbiased Bayesian estimate of all population genetic differentiation.

No significant associations between geographical distances and pairwise estimates of $h_B$ were observed in the full AFLP data set ($r = 0.0032$, $P = 0.452$). A similar pattern was derived at the regional level. Hence no evidence of “isolation by distance” [60] was revealed by Mantel tests at either the regional or entire range level.

The $AK$ criterion of Evanno et al. [61] was applied to estimate the number of population clusters. The maximal value of $AK$ was $K = 24$, indicating the need to divide the samples into 24 clusters. Bar plots showed varying extents of admixture among populations (Figure 2). Ten clusters including populations NLD10, NLD26, NLD30, NLD31, SZ35, SZ72, HK87, HK90, MA105, and MA106 were detected, although each population also contained a very few individuals from different regions. The substructure of the rest populations was weak and could only be resolved when information about the sampling locations was included. A mix of individuals from other clusters was often observed in each cluster. The results suggested that $M.\ micrantha$ populations in southern China maintained a relatively weak genetic structure.

In addition, the UPGMA tree rooted with $M.\ cordata$ revealed that populations of $M.\ micrantha$ from different locations usually were intermingled (Figure 3), suggesting a lack of geographic pattern. HK87, which was documented as the earliest introduced population in China [49], formed an independent branch, one that first diverged from the other $M.\ micrantha$ populations.

Detection of signatures of positive selection

The AFLP data set of $M.\ micrantha$ was used in a global analysis for outlier detection, both with Dfdist and Bayescan (Table 5). By using Dfdist on the 28 populations of $M.\ micrantha$, 23 out of 483 loci (4.8%) were identified as outlier loci under directional selection at the 99.5% probability level (Figure 4A). Bayescan analysis produced high differentiation loci at a threshold of $\log_{10} PO > 2.0$ (posterior probabilities higher than 0.99), corresponding
to 7.87% of the 483 investigated loci. This approach identified 38 outlier loci potentially under selection or linked to a locus under selection (Table 5; Figure 5A). Notably, Bayescan found 24 outliers (loci 35, 50, 56, 136, 231, 240, 324, 342, and 465) not identified by Dfdist. By contrast, Dfdist detected nine outliers (loci 275, 276, 388, 420, 425, 434, 451, 455, 473, and 483) that were not detected by Dfdist. By contrast, Dfdist detected nine outliers (loci 35, 50, 56, 136, 231, 240, 324, 342, and 465) not identified by Bayescan. By pooling the results of the totally different two

### Table 1. Locations of *Mikania micrantha* and *Mikania cordata* populations surveyed in this study.

| Species     | Region     | Population | Location                                           | Latitude | Longitude | Sample size | Altitude (m) |
|-------------|------------|------------|---------------------------------------------------|----------|-----------|-------------|--------------|
| *M. micrantha* | Hong Kong | HK81       | Hong Kong Island, Stubbs Road, in shrubs          | 22°16'08"N | 114°10'49"E | 15          | 74           |
|             |            | HK82       | Hong Kong Island, Barker Road, in tussock         | 22°16'09"N | 114°09'50"E | 13          | 300          |
|             |            | HK84       | Hong Kong Island, Mount Gough, in tussock         | 22°16'05"N | 114°09'43"E | 16          | 315          |
|             |            | HK85       | Hong Kong Island, Victoria Peak, in tussock       | 22°16'30"N | 114°08'57"E | 15          | 503          |
|             |            | HK87       | Hong Kong Zoological and Botanical Gardens, under bamboo forest | 22°16'55"N | 114°09'23"E | 12          | 372          |
|             |            | HK88       | New Territories, Hok Tau, in tussock              | 22°29'53"N | 114°10'49"E | 15          | 46           |
|             |            | HK89       | New Territories, Luk Keng, Pat Sin Leng Country Park, rivulet-side | 22°31'26"N | 114°12'57"E | 12          | 8            |
|             |            | HK90       | Kowloon, Hong Kong Baptist University, slope      | 22°20'07"N | 114°10'57"E | 19          | 25           |
| *M. micrantha* | Macao     | MA101      | Hác Sá Beach, wasteland                          | 22°07'08"N | 113°34'04"E | 15          | 3            |
|             |            | MA105      | Hác Sá Village, roadside                         | 22°07'02"N | 113°34'03"E | 15          | 1            |
|             |            | MA106      | Hác Sá Beach, building site                       | 22°07'16"N | 113°34'10"E | 14          | 8            |
| *M. micrantha* | Shenzhen  | SZ34       | Fairy Lake Botanical Garden, Liang Yi Ting, in tussock | 22°34'49"N | 114°10'08"E | 14          | 88           |
|             |            | SZ35       | Fairy Lake Botanical Garden, Desert Plant Section, roadside | 22°35'10"N | 114°10'26"E | 16          | 42           |
|             |            | SZ64       | The office of Mangrove Natural Reserve, roadside | 22°31'58"N | 114°00'01"E | 11          | 19           |
|             |            | SZ72       | Meili Park, Huanshan Road, slope                  | 22°33'59"N | 114°01'27"E | 16          | 83           |
| *M. micrantha* | Nellingding | NLD1     | Management station, roadside                     | 22°23'48"N | 113°49'09"E | 15          | 3            |
|             |            | NLD10      | Dong Jiao Zui Wan, slope, in shrubs               | 22°24'06"N | 113°48'44"E | 14          | 51           |
|             |            | NLD15      | Dong Jiao Zui Wan, slope, under Spiny date palms | 22°24'21"N | 113°48'42"E | 17          | 145          |
|             |            | NLD26      | Nan Wan, in shrubs                               | 22°23'41"N | 113°48'52"E | 14          | 3            |
|             |            | NLD29      | Dong Wan, ravine                                 | 22°23'48"N | 113°49'38"E | 15          | 15           |
|             |            | NLD30      | Bei Wan Ma Guan, in shrubs                       | 22°25'12"N | 113°47'17"E | 15          | 3            |
|             |            | NLD31      | Management station East, ravine                  | 22°24'01"N | 113°49'59"E | 14          | 8            |
| *M. cordata*  | Zhuhai     | ZH43       | Q’ao Island, roadside                            | 22°24'37"N | 113°38'38"E | 15          | 2            |
|             |            | ZH50       | Q’ao Island, No Jia Le, roadside                 | 22°24'03"N | 113°37'40"E | 12          | 2            |
| *M. cordata*  | Dongguan   | DG91       | Da Ling Shan forestry centre, Shan Zhuo           | 22°51'51"N | 113°46'21"E | 16          | 174          |
|             |            | DG92       | Da Ling Shan forestry centre, Chang Keng Kou     | 22°51'38"N | 113°46'27"E | 15          | 114          |
| *M. cordata*  | Hainan Island | HN1  | Xinglong, in arbors                             | 18°42'04"N | 110°13'23"E | 12          | 49           |
|             |            | HN2        | Xinglong, in tussock                            | 18°42'03"N | 110°13'22"E | 5           | 44           |

doi:10.1371/journal.pone.0041310.t001
detection approaches, 14 outlier loci (3, 17, 25, 57, 116, 132, 177, 183, 184, 219, 325, 347, 381 and 426) were identified by Dfdist and Bayescan. The 14 outliers represented truly adaptive loci (not false positives) because the two approaches used different algorithms and assumptions, and very stringent significance criteria were considered (99.5% probability level for Dfdist, posterior probability \( P > 0.90 \) for Bayescan) in the study. Additionally, in the two populations of *M. cordata*, no outlier locus under selection was detected by either Dfdist or Bayescan (Figures 4B and 5B). Nevertheless, this lack of detection of any outliers may also be due to lower sample size.

**Discussion**

To the best of our knowledge, this study is the first report on detection of candidate loci under selection by genome scan in the invasive weed *M. micrantha* since its introduction to southern China. To avoid spurious outlier loci, several authors advocated employment of two or more outlier detection methods and a conservative significance level [25,28,62–65]. In this study, the AFLP explorative genome scan has revealed 14 loci as under selection among a total of 483 loci in *M. micrantha*. These 14 loci are considered to possess high credibility because they were picked...
Table 3. Genetic bottleneck of *Mikania micrantha* populations from six introduced regions in southern China.

| Region      | SMM             | IAM             | P     | trimming mean F<sub>ST</sub> | trimming mean F<sub>ST</sub> |
|-------------|-----------------|-----------------|-------|-----------------------------|-----------------------------|
|             | He/H<sub>D</sub> | P               | He/H<sub>D</sub> | P |                               |                               |
| HK81        | 268/45          | 0.00000         | 268/45          | 0.00000            |                               |                               |
| HK82        | 230/43          | 0.00000         | 230/43          | 0.00000            |                               |                               |
| HK83        | 101/24          | 0.00000         | 102/23          | 0.00000            |                               |                               |
| HK85        | 156/32          | 0.00000         | 156/32          | 0.00000            |                               |                               |
| HK87        | 75/20           | 0.00000         | 75/20           | 0.00000            |                               |                               |
| HK88        | 172/33          | 0.00000         | 172/33          | 0.00000            |                               |                               |
| HK89        | 202/41          | 0.00000         | 202/41          | 0.00000            |                               |                               |
| HK90        | 175/28          | 0.00000         | 175/28          | 0.00000            |                               |                               |
| Hong Kong   | 277/165         | 0.00000         | 302/140         | 0.00000            |                               |                               |
| MA101       | 177/60          | 0.00000         | 177/60          | 0.00000            |                               |                               |
| MA105       | 138/35          | 0.00000         | 138/35          | 0.00000            |                               |                               |
| MA106       | 30/9            | 0.000448        | 30/9            | 0.00000            |                               |                               |
| Macao       | 199/119         | 0.00000         | 242/76          | 0.00000            |                               |                               |
| SZ34        | 149/27          | 0.00000         | 149/27          | 0.00000            |                               |                               |
| SZ35        | 72/13           | 0.00000         | 72/13           | 0.00000            |                               |                               |
| SZ64        | 174/38          | 0.00000         | 174/38          | 0.00000            |                               |                               |
| SZ72        | 52/6            | 0.00000         | 52/6            | 0.00000            |                               |                               |
| SZ75        | 230/37          | 0.00000         | 230/37          | 0.00000            |                               |                               |
| SZ77        | 212/31          | 0.00000         | 212/31          | 0.00000            |                               |                               |
| Shenzhen    | 243/123         | 0.00000         | 289/77          | 0.00000            |                               |                               |
| NLD1        | 171/25          | 0.00000         | 171/25          | 0.00000            |                               |                               |
| NLD10       | 41/11           | 0.00032         | 41/11           | 0.00000            |                               |                               |
| NLD15       | 149/31          | 0.00000         | 149/31          | 0.00000            |                               |                               |
| NLD26       | 50/9            | 0.00000         | 50/9            | 0.00000            |                               |                               |
| NLD29       | 186/37          | 0.00000         | 186/37          | 0.00000            |                               |                               |
| NLD30       | 42/5            | 0.00000         | 42/5            | 0.00000            |                               |                               |
| NLD31       | 119/22          | 0.00000         | 119/22          | 0.00000            |                               |                               |
| Neilingding | 222/128         | 0.00000         | 238/112         | 0.00000            |                               |                               |
| ZH43        | 250/40          | 0.00000         | 250/40          | 0.00000            |                               |                               |
| ZH50        | 221/46          | 0.00000         | 221/46          | 0.00000            |                               |                               |
| Zhubai      | 311/65          | 0.00000         | 323/53          | 0.00000            |                               |                               |
| DG91        | 146/38          | 0.00000         | 146/38          | 0.00000            |                               |                               |
| DG92        | 113/58          | 0.00000         | 115/56          | 0.00000            |                               |                               |
| Dongguan    | 201/40          | 0.00000         | 214/27          | 0.00000            |                               |                               |
| Total       | 309/174         | 0.00000         | 337/146         | 0.00000            |                               |                               |

P values are determined by a sign test under the stepwise mutation model (SMM) and the infinite allele model (IAM). $H_e/H_d$ the heterozygosity excess/deficiency ratio. doi:10.1371/journal.pone.0041310.t003

Table 4. Analysis of molecular variance (AMOVA) of 483 AFLP loci for 28 *Mikania micrantha* populations from six introduced regions in southern China.

| Variance components | Percentage of total variation | P     | ϕ statistics |
|---------------------|-------------------------------|-------|--------------|
| Among regions       | 2.884                         | 4.74  | <0.001        |
|                     |                               |       | ϕ<sub>ST</sub> = 0.0474 |
| Among populations within regions | 17.404                          | 28.61 | <0.001        |
|                     |                               |       | ϕ<sub>SC</sub> = 0.3004 |
| Within populations  | 40.538                        | 66.65 | <0.001        |
|                     |                               |       | ϕ<sub>ST</sub> = 0.3335 |

The P-value was calculated by a permutation procedure based on 1023 replicates. doi:10.1371/journal.pone.0041310.t004
ability and pace of proliferation of invasive species and are able to
enhance adaptive evolutionary responses to novel environmental
selection [10,12,19,75]. Based on the UPGMA and STRUC-
TURE analysis results, closely related populations often are from
different geographical locations (Figures 2 and 3), while individuals
from the same location do not group together. Thus multiple
introductions are inferred among populations of *M. micrantha*. In
addition, the significant genetic differentiation detected among *M.
micrantha* populations is also consistent with the expectation that
each population has experienced severe bottleneck (Table 3). It is

Figure 2. Bayesian assignment proportions for *K*=28 clusters determined in STRUCTURE 2.3.3. Each vertical bar represents one individual.
doi:10.1371/journal.pone.0041310.g002
generally suggested that bottlenecks tend to increase genetic differentiation among populations by changing allelic frequencies [76,77] and they are perceived to reduce the potential for adaptive evolution as well [29,78]. Nevertheless, multiple introductions can ameliorate this effect and accelerate the speed of adaptive evolution [8]. Such phenomena have been recorded in the populations of other invasive species including *Heracleum mantegazzianum* [79], *Heracium sphondylium* [80], and *Alliaria petiolata* [81]. Furthermore, no geographical signature is revealed in the AFLP variation pattern among the introduced populations of *M. micrantha* at either regional or entire range scale. Repeated or secondary introductions, which are induced by long-distance dispersals of copious amounts of fine and fluffy seeds, pollination or propagules mediated by humans, insects, and violent winds [82], may further complicate the geographic consequences of the population genetic variation of *M. micrantha*. Therefore, multiple introductions can add to the successful invasiveness of *M. micrantha* by increasing genetic variation, preventing genetic bottlenecks, and strengthening the capacity of adaptive evolution.

Adaptive evolution of invasive species is also determined by the reproductive system such as the amount of sexual and asexual reproduction and the patterns of mating [19]. *Mikania micrantha* mainly propagates by seeds, but its local spread mostly results from vegetative propagation [56,83,84]. When reproducing vegetatively, *M. micrantha* generates shoots from small stem fragments and rosettes [83,84]. In this study, significant LD and locus compatibility have been identified in a majority of *M. micrantha* populations (Table 2), indicating that asexual reproduction is the predominant propagation system. In addition, self-fertilization (selfing) has also been reported in the southern China populations of *M. micrantha* [85]. The results are in accord with the findings that most invasive plants reproduce asexually or by selfing [86–88], and reproductive systems may be subject to evolutionary modification during invasion such as switching to higher levels of inbreeding or vegetative reproduction [5,19,89–91]. For species that are able to reproduce both sexually and clonally, sexual reproduction would promote allelic recombinations that result in high genetic variation, whereas clonal reproduction preserves successful combinations and maintains genetic variation by occasionally creating new alleles through somatic mutations [92–95]. The specific reproductive modes (asexual and selfing) of *M. micrantha* are likely to enhance its adaptive potential in southern China.

In summary, this study is the first report to identify outlier loci under selection in the genome of the invasive weed *M. micrantha* without reference to coding vs. noncoding sequences, or specific models of selection by explorative genomic scans [25]. We find that 2.9% candidate loci are under selection, indicating that local adaptation has indeed occurred in *M. micrantha* since its introduction to southern China. This study also demonstrates high genetic variability, strong genetic differentiation, severe bottlenecks, and multiple introductions, as well as primarily asexual reproduction in the introduced populations. The interactions between genetic diversity, multiple introductions, and reproductive modes are revealed to be involved in the response of *M. micrantha* to the new local environment, allowing a more precise understanding of its successful invasion. In future studies, we will isolate and sequence the outlier AFLP bands to identify their genomic locations and neighboring genes to further detail the adaptive molecular mechanisms of *M. micrantha*.

---

**Figure 3.** UPGMA dendrogram derived from AFLP data by Nei’s [105] unbiased genetic distances. It shows the relationships among 28 examined populations of *Mikania micrantha*. Populations of *Mikania cordata* are used as the root. Numbers above branches indicate bootstrap values (% of 1000 replicates). Only values larger than 40% are displayed. Branch lengths are proportional to genetic distances (see scale at the bottom of figure).

doi:10.1371/journal.pone.0041310.g003
Materials and Methods

Ethics statement

This study was conducted in accordance with all People’s Republic of China laws. No specific permits were required for the described field studies. No specific permissions were required for the locations/activities described in this study. The location is not privately owned or protected in any way. The field studies did not involve endangered or protected species.

Plant material

For *M. micrantha*, individuals were sampled from 28 populations in six regions (Hong Kong, Macao, Shenzhen, Neilingding, Zhuhai, and Dongguan) representing different habitats (Figure 1A; Table 1). In each population, we collected fresh leaf material from 12 to 19 randomly selected individuals (Table 1). These samples represented a large number of individuals of *M. micrantha* throughout its introduced range in southern China. To further confirm that the outlier loci under selection in *M. micrantha* were caused by novel environments, we tested whether AFLP loci can demonstrate evidence of selection in the congeneric species *M. cordata*, which is native to sub-tropical China. For *M. cordata*, five to 12 individuals were collected from its two populations (HN1 and HN2) located on Hainan Island (Figure 1B; Table 1). Leaves were preserved in silica gel. Voucher specimens (*M. micrantha*: CGP1-409; *M. cordata*: CGP410-426) were deposited at the herbarium of Sun Yat-sen University (SYS), Guangzhou, China.

DNA extraction

Total genomic DNA was extracted from ground tissue, following the modified CTAB protocol [96]. The quality and quantity of the DNA were determined with a spectrophotometer (Pharmacia 2000 UV/Visible, Amersham Pharmacia Biotech, Piscataway, NJ) and on 0.8% agarose gels.

AFLP protocols

AFLP analyses were performed according to Vos et al. [97] with the following modifications: genomic DNA (50 ng) was digested with 2 U EcoRI and 4 U MseI (New England Biolabs, Ipswitch, MA) for 3 h at 37°C in 1×NE buffer and incubated at 70°C for

Figure 4. Results of the simulations with Dfdist for outlier detection. Plots representing $F_{ST}$ values are against heterozygosity. Each dot indicates an AFLP locus. The lower, intermediate, and higher lines represent the 0.5%, 50%, and 99.5% confidence intervals, respectively. Loci above the 99.5% line are regarded as outlier loci. (A) The result of *Mikania micrantha*. The 23 outlier loci under selection are represented by red dots accompanied by the locus number. (B) The result of *Mikania cordata*. No outlier locus under selection is detected.
doi:10.1371/journal.pone.0041310.g004
20 min. To ligate the resulting fragments to the corresponding adapters, 10 μl of restriction products were added into a 20 μl reaction mixture containing 0.1 μM EcoRI adapter, 1 μM MseI adapter, and 60 U T4 DNA ligase (New England Biolabs). After being incubated at 20°C for 3 h, the samples were diluted 10 times with ddH2O, then 2 μl of each sample was used as a template to conduct the pre-amplification in a final volume of 20 μl that contained 1×PCR buffer, 100 nM each of EcoRI+A and MseI+C primers, 0.24 mM dNTPs, and 1.5 U Taq DNA polymerase. The preamplification reaction was carried out for 20 cycles of 30 s at 94°C, 1 min at 56°C and 1 min at 72°C. After that, preamplified product was diluted 1:50 with ddH2O, and then used as template for the selective PCR amplifications to generate AFLPs.

**Table 5.** Comparison of outlier loci of *Mikania micrantha* under selection using Bayescan, Dfdist, and both with Dfdist and Bayescan, respectively.

| Approach          | Outlier loci identified |
|-------------------|-------------------------|
| Bayescan          | 3, 4, 5, 11, 14, 17, 25, 43, 54, 57, 59, 116, 132, 153, 166, 167, 173, 177, 183, 184, 209, 219, 224, 228, 275, 325, 347, 381, 388, 420, 425, 426, 434, 451, 455, 473, 483 |
| Dfdist            | 3, 17, 25, 35, 50, 56, 57, 116, 132, 136, 177, 183, 184, 219, 231, 240, 324, 325, 342, 347, 381, 426, 465 |
| Bayescan and Dfdist | 3, 17, 25, 57, 116, 132, 177, 183, 184, 219, 325, 347, 381, 426 |

doi:10.1371/journal.pone.0041310.t005
Table 6. Percentage of polymorphic loci (PL), Nei’s total gene diversity (H), G\textsubscript{ST}, D\textsubscript{ST}, and I\(\theta^B\) obtained from the populations of different weedy species based on AFLP data.

| Species                     | PL   | H\textsubscript{r} | G\textsubscript{ST} | D\textsubscript{ST} | I\(\theta^B\) | Reference                  |
|-----------------------------|------|---------------------|----------------------|----------------------|---------------|-----------------------------|
| Guizotia scabra ssp. scabrai| 0.8455 | 0.32               | 0.18                 | 0.22                 |               | Geleta et al. [130]         |
| Guizotia scabra ssp. schimperi| 0.9002 | 0.32               | 0.19                 | 0.17                 |               | Geleta et al. [130]         |
| Guizotia villosa             | 0.8393 | 0.33               | 0.19                 | 0.26                 |               | Geleta et al. [130]         |
| Nicotiana attenuata         | 0.961  | 0.114              | 0.0549               | 0.31                 |               | Bahulikar et al. [131]      |
| Scalesia affinis            | 0.549  | 0.34               | 0.1808               | 0.435                |               | Haldimann et al. [134]      |
| Oxalis pes-caprae*           | 0.8843 | 0.23               |                      |                      |               | Rottenberg & Parker [94]    |
| Festuca pratensis           | 0.9320 | 0.31               |                      |                      |               | Fjellheim & Rognli [133]    |
| Senecio vulgaris            | 0.34   | 0.1808             |                      |                      |               |                             |
| Ranunculus glacialis        | 0.99   | 0.7442             |                      |                      |               | Schönswetter et al. [135]   |
| Ranunculus carpatioca       | 0.8594 | 0.279              |                      |                      |               | Paun et al. [136]           |
| Arabidopis thaliana         | 1.00   | 0.179              | 0.23                 |                      |               | Jørgensen & Mauricio [137]  |
| Lolium perenne              | 0.886  | 0.4736             |                      |                      |               | Treuner et al. [138]        |
| Alopecurus myurosoides      | 0.905  | 0.3335             | 0.2927               |                      |               | Menchari et al. [139]       |
| Mikania micrantha           | 1.00   | 0.3376             | 0.4736               | 0.2927               |               | This Study                  |

*Invasive weed.

doi:10.1371/journal.pone.0041310.t006

Selective amplification was performed in a final volume of 10 µl containing 1× PCR buffer, 125 nM EcoRI primer, 125 nM 6-FAM-EcoRI primer, 250 nM MseI primer, 0.2 mM dNTPs, 0.75 U Tag DNA polymerase and 2 µl diluted pre-amplified DNA sample. The reaction was conducted for 13 cycles of 30 s at 94°C, 30 s at 65°C and 1 min at 72°C. The annealing temperature was reduced by 0.7°C per cycle. Then 25 cycles consisting of 30 s at 94°C, 30 s at 56°C, and 1 min at 72°C were performed.

An initial screening of 48 combinations of selective primers, in which six were EcoRI [EcoRI]+AAG, +ACT, +AGC, +ACA, +AAC, +ACC] and eight were MseI [MseI+CAG, +CTC, +CTA, +CAA, +CAG, +CTG, +CAT, +CTT], were performed. Eight combinations were selected that generated clear and evenly distributed bands: EcoRI+ACT/MseI+CAG, EcoRi+AAC/MseI+GAG, EcoRI+ACT/MseI+GAG, EcoRI+ACT/MseI+CTG, EcoRI+ACT/MseI+CTG, EcoRI+ACT/MseI+CTA, EcoRI+ACT/MseI+CTA. Therefore, the eight primer combinations were chosen for selective amplifications of all samples. PCR reactions were conducted on a PTC-100 Peltier Thermal Cycler (MJ Research, St. Bruno, Quebec). The selective PCR products were separated by electrophoresis on 6.5% polyacrylamide gels on an ABI 377 automated sequencer (Applied Biosystems, Carlsbad, CA). An ROX-500-labeled internal size standard (Applied Biosystems) was added to each sample to size fragments.

Data analysis

Software GeneScan 3.7 (Applied Biosystems) and genographer (version 1.6.0; http://hordeum.oscs.montana.edu/genographer) were utilized to collect and score raw fluorescent AFLP data. The presence (1) or absence (0) of data from unambiguous AFLP bands was used to establish the matrix of genetic identity of the sampled individuals. Genetic diversity statistics, including percentage of polymorphic loci and Nei’s gene diversity [98], were calculated using POPGENE32 software [99]. Shannon’s index of phenotypic diversity was quantified as $S = - \sum p_i \log p_i$, where $p_i$ is the frequency of a given AFLP band in the population [100].

Analysis of Molecular Variance (AMOVA) based on a Euclidean squared distance matrix was hierarchically calculated to estimate the allocation of genetic variation among and within populations by ARLEQUIN 3.0 [available at http://cmpg.unibe.ch/software/arlequin3] [101]. In this analysis, the AFLP data set was partitioned at three levels: regional, among-population, and within-population. One thousand random permutations were used to infer the significance of the variance components [101]. Also using the same software, a Mantel test was performed to investigate the correlation between genetic differentiation and geographic distances (km) among populations. The matrix of genetic differentiation was composed by pairwise $\theta^B$ estimates.

Holsinger et al. [102] proposed a Bayesian approach to estimating genetic structure for dominant and codominant markers. The nearly unbiased parameter estimations of heterozygosity, genetic distance, and population differentiation can be obtained using the Bayesian method [102]. Its $f$ and $\theta^B$ are equivalent to the inbreeding coefficient ($F_{IS}$) and the fixation index ($F_{ST}$) of $F$-statistics, respectively. The posterior distributions of $f$ and $\theta^B$ were estimated through Markov Chain Monte Carlo (MCMC) methods by HICKORY v1.0, with a burn-in of 250 000 iterations and a sampling run of 250 000 iterations from which every fiftieth sample was retained for posterior calculations [102]. The analysis model was chosen based on the deviance information criterion (DIC). The “f-free” model, where $f$ was not estimated but was chosen at random from the prior distribution, was decided due to its smaller DIC than for other models. Alleles were deemed rare if their frequencies were ≤ 0.05 in the sampled populations [59].

To gain further perspectives on genetic structure, we also employed the Bayesian clustering method to infer the pattern of population structure by implementing the STRUCTURE 2.2.3 program [103,104]. To determine the best number of clusters, 30 independent runs of $K$ ($K = 1$ to 30) were performed with an admixture model at 100 000 MCMC iterations and a 10 000 burn-in period. We used $DAK$, the second-order rate of change in $\ln \mathbf{P}(K|\mathbf{K})$ for successive values of $K$ to determine the number of clusters [61]. The distribution map of STRUCTURE was plotted according to $K$ value at the highest log likelihood. Moreover, based
on Nei’s genetic distance [105], an unweighted pair group method with arithmetic mean analysis (UPGMA) was used to generate a dendrogram of the relationships among the populations by TFPGA 1.3 [106]. One thousand bootstrap replicates were performed to assess the reliability of the UPGMA dendrogram.

We further tested whether populations have suffered a bottleneck using the program BOTTLENECK [107]. The heterozygosity (Heq) expected at mutation-drift equilibrium was calculated based on both the stepwise mutation model (SMM) and the infinite allele model (IAM) [108,109]. The significance of heterozygosity excess was determined by the sign test [110].

Linkage disequilibrium, which is an association of alleles at different loci on chromosomes in a population, is mainly affected by population size [111]. The dependency of population size can be removed by multinucleotide linkage disequilibrium with both indices of association I_S [112–114] and its modified measure FST [115]. These indices and their significance by randomization were computed using the software Muhilocus v1.2 (http://www.bio.ic.ac.uk/evolve/software/multilocus/).

A character incompatibility analysis was carried out to probe the predominant mating system in the populations at the molecular level [116–118]. In a pair of binary character data, such as the presence or absence of AFLP bands at two loci, the presence of all four possible combinations of characters (0/0, 1/0, 0/1, 1/1) is more parsimoniously explained by sexual recombination than by three mutation events. Once all four character combinations arise, an incompatibility is said to occur, and can be used as a measure of recombination [116]. The incompatibility excess ratio (IER) was calculated using PICA 4.0 by comparing the observed incompatibility count for the original data and mean incompatibility count for randomly permuted data [118]. If none or a small fraction of incompatible loci occur, asexual reproduction is implied to have happened [80,119].

To detect outlier loci under selection for local adaptation of *M. micrantha* and *M. cordata*, two complementary methods were applied to our AFLP data set. We first used Program Dfdist, which is the most popular software for detecting candidate loci [120]. Dfdist software (http://www.rubic.rdg.ac.uk/~mab/stuff/) was recently modified from Beaumont and Balding [43] to analyze dominant data, which is a hierarchical Bayesian approach based on summary statistics in a symmetric island model [24,39]. Outlier loci were detected by comparing empirical FST values for each locus against a null distribution of FST values expected from a neutral drift model [34]. A potential defect of the Dfdist approach for detecting outlier loci is the possibility of false positives [121–123]. The statistical power was enhanced to avoid false positives by setting a conservative constraint [22]. A null distribution of FST close to the empirical distribution was acquired by 50 000 coalescent simulations. Simulations were computed with a mean FST similar to the trimmed mean FST, which was calculated by excluding 30% of the most extreme FST values observed in the empirical dataset [71,124]. The 4Nµ parameter value was set to 0.04 in all simulations. A global analysis was done for *M. micrantha* and *M. cordata* using 28 populations and 2 populations, respectively. The threshold for outliers was set to the more conservative 0.005, estimated from simulated FST values to control for false positives [20,33,125]. In addition, Dfdist assumes that populations are at migration-drift equilibrium, which does not often occur in natural populations [124]. Hence, to cross-check the reliability of the outlier loci detected by Dfdist, we also ran Bayescan software (http://www-leca.ujf-grenoble.fr/logiciels.htm), which better handles dominant marker data by directly estimating the posterior probability of a given locus to be under selection [42]. Assuming that allele frequencies within populations follow a Dirichlet distribution [126–129], the Bayesian method not only permits for different demographic scenarios and different amounts of genetic drift between populations when estimating population-specific FST coefficients, but also considers all loci in the analyses [124]. The Bayesian approach also disposes of the problem of multiple testing of a large number of genomic loci through prior distribution [124]. In our genome scan, the log10 PO>2.0 was considered a threshold value for determining loci under selection according to Jeffreys’ interpretation [129], which is a logarithmic scale for model choice as follows: log10 PO>0.5 (substantial); log10 PO>1.0 (strong); log10 PO>1.5 (very strong); and log10 PO>2.0 (decisive support for accepting a model) [36]. We used 10 pilot runs of 5000 iterations to estimate model parameters. A burn-in of 50 000 iterations was employed to cover the MCMC. The sample size was set to 3000 and the thinning interval to 20, resulting in a total chain length of 150 000 iterations [43]. The loci were ranked according to their estimated posterior probability and all loci with a value over 0.993 were retained as outliers. This corresponds to log10 PO>2.0, which provides decisive support for acceptance of the model. Outliers identified by both Dfdist and Bayescan are likely to be truly adaptive regions of the genome, because the two approaches differ in algorithms and assumptions [27].

Acknowledgments

We thank Zhanming Ying, Zhenyu Li, Junsheng Yu, Binguhi Wei, and Mingzhao Yang of the School of Life Sciences, Sun Yat-sen University, for assistance with the collection of plant materials.

Author Contributions

Conceived and designed the experiments: TW YS. Performed the experiments: TW GC QZ YS. Analyzed the data: TW YS. Wrote the paper: TW YS CW.

References

1. Holt RD (2009) Up against the edge: invasive species as testbeds for basic questions about evolution in heterogeneous environments. Mol Ecol 18: 4347–4349.
2. Xu CY, Julien MH, Fatemi M, Girod C, Van Klinken RD, et al. (2010) Phenotypic divergence during the invasion of *Phylloсетus canadensis* in Australia and France: evidence for selection-driven evolution. Ecol Lett 13: 32–44.
3. Richardson DM, Pyke D, Rejmánek M, Barbour MG, Panetta FD, et al. (2000) Naturalization and invasion of alien plants: concepts and definitions. Divers Distrib 6: 93–107.
4. Lachnath S, Durka W, Schurr FM (2010) The making of a rapid plant invader: genetic diversity and differentiation in the native and invaded range of *Suaeda antiquata*. Mol Ecol 19: 3952–3967.
5. Barrett SCH, Husband B (1996) The genetics of plant migration and colonization. In: Brown AHD, Clegg MT, Kahler AL, Weir BS, editors. Plant population genetics, breeding, and genetic resources. Sunderland: Sinauer & Associates. pp. 254–277.
6. Novak SJ, Mack RN (1993) Genetic variation in *Bromus tectorum* (Poaceae): comparison between native and introduced populations. Heredity 71: 167–176.
7. Milne RI, Abbott RJ (2000) Origin and evolution of invasive naturalized material of *Rhododendron ponticum* L. in the British Isles. Mol Ecol 9: 541–556.
8. Dlugosch MK, Parker MI (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17: 431–449.
9. Sakai AK, Allendorf FW, Holt JS, Lodge DM, Morofsky J, et al. (2001) The population biology of invasive species. Annu Rev Ecol Syst 32: 505–332.
10. Lee CE (2002) Evolutionary genetics of invasive species. Trends Ecol Evol 17: 396–391.
11. Holt RD, Barfield M, Gomulkiewicz R (2005) Theories of niche conservatism and evolution: could exotic species be potential tests? In: Sax DF, Stachowicz JJ, Gaines SD, editors. Species invasions: insights into ecology, evolution, and biogeography. Sunderland: Sinauer & Associates. pp. 259–290.
37. Tollenaere C, Duplantier JM, Rahalison L, Ranjalahy M, Brouat C (2011)
36. Fischer MC, Foll M, Excoffier L, Heckel G (2011) Enhanced AFLP genome
31. Mealor BA, Hild AL (2006) Potential selection in native grass populations by
29. Prentis PJ, Wilson JRU, Dormontt EE, Richardson DM, Lowe AJ (2008)
28. Galindo J, Morales P, Rola³n-Alvaren E (2009) Comparing geographical genetic
25. Murray MC, Hare MP (2006) A genomic scan for divergent selection in a
23. Meyer CL, Vitalis R, Saumitou-Laprade P, Castric V (2009) Genomic pattern
19. Barrett SCH, Colautti RI, Eckert CG (2008) Plant reproductive systems and
15. Whitney KD, Gabler CA (2008) Rapid evolution in introduced species, "invasive traits" and recipient communities: challenges for predicting invasive potential. Divers Distrib 14: 569–580.
21. Kudatian SR, Smouse PE (2001) Comparing indigenous and introduced populations of Senecio squarrosus (Caryophyllaceae): response of seedlings to water and pH levels. Oecologia 127: 487–494.
12. Keller SR, Taylor DR (2008) History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. Ecol Lett 11: 852–866.
13. Allan E, Pannell JR (2009) Rapid divergence in physiological and life-history traits between north-western and southern populations of the British introduced weed species, Senecio vulgaris. Oikos 118: 1053–1061.
11. Reznick DN, Ghalambor CK (2003) The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. Genetica 118: 183–190. Evol Ecol 19: 2267–2276.
10. Whitney KD, Gabler CA (2008) Rapid evolution in introduced species, "invasive traits" and recipient communities: challenges for predicting invasive potential. Divers Distrib 14: 569–580.
9. Kauffmann SR, Smouse PE (2001) Comparing indigenous and introduced populations of Senecio squarrosus (Caryophyllaceae): response of seedlings to water and pH levels. Oecologia 127: 487–494.
8. Broennimann O, Treier UA, Muller-Schwarzer H, Thullier W, Peterson AT, et al. (2007) Evidence of climatic niche shift during biological invasion. Ecol Lett 10: 701–709.
7. Kanarek AR, Webb CT (2010) Allele effects, adaptive evolution, and invasion success. Evol Appl 3: 122–135.
6. Barrett SCH, Colautti RI, Eckert CG (2008) Plant reproductive systems and
5. Prentis PJ, Wilson JRU, Dormontt EE, Richardson DM, Lowe AJ (2008)
4. Galindo J, Mora³n P, Rola³n-Alvaren E (2009) Comparing geographical genetic
3. Prentis PJ, Wilson JRU, Dormontt EE, Richardson DM, Lowe AJ (2008)
2. Whitney KD, Gabler CA (2008) Rapid evolution in introduced species, "invasive traits" and recipient communities: challenges for predicting invasive potential. Divers Distrib 14: 569–580.
1. Whitney KD, Gabler CA (2008) Rapid evolution in introduced species, "invasive traits" and recipient communities: challenges for predicting invasive potential. Divers Distrib 14: 569–580.
73. Noel P, Funk DJ, Ortiz-Barrientos D (2009) Divergent selection and heterogeneous genomic divergence. Mol Ecol 18: 375–402.
74. Leroux S, Feve K, Vignoles F, Bouchez O, Klopp C, et al. (2010) Non PCR-amplified transcripts and AFLP® fragments as reduced representations of the eukaryotic genome for 414 Titanium sequencing. BMC Res Note 3: 214.
75. Novak SJ, Mack RN (2005) Genetic bottlenecks in alien plant species: influences of mating systems and introduction dynamics. In: Sax DF, Stachowicz JJ, Games SD, editors. Species invasions: insights into ecology, evolution, and biogeography. Sunderland: Sinauer & Associates. pp. 201–229.
76. Herdick PW (1999) Perspective: highly variable loci and their interpretation in evolution and conservation. Evolution 53: 313–318.
77. Herdick PW (2000) Genotypes of Populations. Sudbury: Jones and Bartlett Publishers.
78. Van Buskirk J, Willi Y (2006) The change in quantitative genetic variation with inbreeding. Evolution 60: 2240–2434.
79. Walker NF, Hulme PE, Hoelzel AR (2003) Population genetics of an invasive species, Heracleum mantegazzianum: implications for the role of life history, demographics and independent introductions. Mol Ecol 12: 1747–1756.
80. Chapman H, Robinson B, Pearson ML (2004) Population genetic structure of a colonising, triploid weed, Mikania micrantha. Heredity 92: 182–188.
81. Durka W, Bozoorlou P, Prat D, Augé H (2005) Molecular evidence for multiple introductions of garlic mustard (Alliaria petiolata, Brassicaceae) to North America. Mol Ecol 14: 1697–1706.
82. Zhou XM, Huang BQ (2001) The invasion and control of Mikania micrantha in South China. Weed Res 47: 280–283.
83. Price SC, Jain SK (1981) Are inbreeders better colonizers? Oecologia 49: 283–286.
84. Husband BC, Barrett SCH (1997) Colonization history and population genetic structure of Eutrochium nicaeense in Jamaica. Proc Natl Acad Sci USA 94: 287–292.
85. Amundsrud L, Noyer JL, Harris-Mickey M (2001) Evidence for a switch in the reproductive biology of Rubus allegheniensis (Rosaceae) towards apomixis, within its native range and area of introduction. Am J Bot 88: 2283–2291.
86. Burdon JJ, Marshall DR (1981) Biological and the reproductive mode of Hordeum spontaneum populations of Mikania micrantha and its companion species. J Trop Subtrop Bot 8: 139–146.
87. Hong L, Shen H, Ye WH, Cao HL, Wang ZM (2007) Self-incompatibility in Mikania micrantha, a colonising, triploid weed, in China. Weed Res 47: 280–293.
88. Haubold B,ieder M, Sankoff D (2001) Indices of multilocus linkage disequilibrium. Mol Ecol Notes 1: 101–102.
89. Amsellem L, Noyer JL, Hossaert-Mckey M (2001) Evidence for a switch in the reproductive biology of Rubus allegheniensis (Rosaceae) towards apomixis, within its native range and area of introduction. Am J Bot 88: 2283–2291.
90. Brown AHD, Feldman MW, Nevo E (1980) Multilocus structure of natural populations of Senecio vulgaris. Science 209: 323–333.
91. Kim YJ, Feng S, Zeng ZB (2008) Measuring and partitioning the high-order heterogeneity in microsatellite data. Mol Ecol 17: 5378–5390.
92. Klekowski EJ Jr (1997) Somatic mutation theory of clonality. In: de Kroon H, van Groenendael J, editors. The ecology and evolution of clonal plants. Leiden: Backhuys Publishers. pp. 227–241.
93. Kozikowski EJ Jr (1997) Somatic mutation theory of clonality. In: de Kroon H, van Groenendael J, editors. The ecology and evolution of clonal plants. Leiden: Backhuys Publishers. pp. 227–241.
94. Jeffers H (1963) Theory of probability (third edition). Oxford: Oxford University Press.
95. Lavergne S, Molofsky J (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. Proc Natl Acad Sci USA 104: 1694–1699.
96. Miller MP (1997) Tools for Populations Genetic Analyses (TFPGA) 1.3: A computer program for genetic data analysis. Silwood Park: University of Marlow.
97. Nei M (1978) Estimation of average heterozygosity and genetic distance from a sample of diploid DNA. Genetics 90: 583–590.
98. Miller JP (1997) Tools for Populations Genetic Analyses (TFPGA) 1.3: A computer program for genetic data analysis. Silwood Park: University of Marlow.
99. Yeh FC, Yang R (1999) POPGENE version 1.31. Dept of Renewable Resources, University of Alberta. Available: http://www.cimMY.gl.ubc.ca/~fryeh.
100. Jeffers H (1963) Theory of probability (third edition). Oxford: Oxford University Press.
101. Yeh FC, Yang R (1999) POPGENE version 1.31. Dept of Renewable Resources, University of Alberta. Available: http://www.cimMY.gl.ubc.ca/~fryeh.
102. Haubold B,ieder M, Sankoff D (2001) Indices of multilocus linkage disequilibrium. Mol Ecol Notes 1: 101–102.
103. Mes THM (1996) Characteristic compatibility of molecular markers to distinguish axenic and sexual reproduction. Mol Ecol 7: 1719–1727.
104. Haubold B,ieder M, Sankoff D (2001) Indices of multilocus linkage disequilibrium. Mol Ecol Notes 1: 101–102.
105. Nei M (1978) Estimation of average heterozygosity and genetic distance from a sample of diploid DNA. Genetics 90: 583–590.
136. Paun O, Grislhuber J, Temsch EM, Horandl E (2006) Patterns, sources and ecological implications of clonal diversity in apomictic *Ranunculus carpaticola* (*Ranunculus auricomus* complex, Ranunculaceae). Mol Ecol 15: 897–910.

137. Jørgensen S, Mauricio R (2004) Neutral genetic variation among wild North American populations of the weedy plant *Arabidopsis thaliana* is not geographically structured. Mol Ecol 13: 3403–3413.

138. Treuren RV, Bas N, Goossens PJ, Jansen J, Van Soest LJM (2005) Genetic diversity in perennial ryegrass and white clover among old Dutch grasslands as compared to cultivars and nature reserves. Mol Ecol 14: 39–52.

139. Menchari Y, Délye G, Le Corre V (2007) Genetic variation and population structure in black-grass (*Alopecurus myosuroides* Huds.), a successful, herbicide-resistant, annual grass weed of winter cereal fields. Mol Ecol 16: 3161–3172.