DEVELOPMENT OF 23 POLYMORPHIC MICROSATELLITE LOCI IN INVASIVE SILVER WATTLE, ACACIA DEALBATA (FABACEAE)¹

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Silver wattle, Acacia dealbata Link (Fabaceae), is a tree species native to southeastern Australia, where it is widespread and common. It has been introduced since the 18th century in various parts of the world for ornamental or forestry purposes (Kull et al., 2011). It is now considered an invasive species in southern Europe, South Africa, India, Madagascar, New Zealand, Chile, and California (Kull et al., 2011). In those regions, A. dealbata invades woodland and disturbed environments where it outcompetes native species. Acacia dealbata has a long and complex history of worldwide introductions from Australia, at least since its introduction in the early 18th century in Europe (Kull et al., 2011). Silver wattle is the focus of research both because it is a model for the impact of landscape fragmentation in its native area (Broadhurst and Young, 2006) and because it is a famous invader (Lorenzo et al., 2010), classified on the Delivering Alien Invasive Species Inventories for Europe (DAISIE) list as one of the 100 European invaders with the most significant ecological impacts (DAISIE European Invasive Alien Species Gateway, 2006). In both cases, the use of highly polymorphic genetic markers could help answer questions related to the spatial genetic structure of A. dealbata populations.

Variable genetic markers such as microsatellite markers are a common tool used to infer invasion routes of invading species and specifically to identify the sources of invasive populations (Estoup and Guillemaud, 2010). They are also commonly used in conservation biology studies to decipher the impact of environmental disturbance on the genetic structure of tree species (e.g., Aldrich et al., 1998). Recently, next-generation sequencing technologies have been used to obtain very large numbers of shotgun sequences of genomic DNA from which microsatellites could be isolated (Guichoux et al., 2011). This method requires a large effort of sequencing to obtain a sufficient number of sequences containing microsatellites. Microsatellite enrichment of the genomic DNA prior to sequencing allows a drastic cost reduction (Malausa et al., 2011). Here we present the isolation and properties of 23 polymorphic microsatellite loci of A. dealbata using 454 GS FLX Titanium (Roche Applied Science, Penzberg, Germany) pyrosequencing of a microsatellite-enriched genomic DNA library.

METHODS AND RESULTS

Total genomic DNA was extracted from leaf material of a plant collected in southeastern Australia (34°30’51.498”S, 148°49’53.3892”E) using the DNeasy Plant Kit (QIAGEN, Hilden, Germany). We followed the procedure of Malausa et al. (2011) for enrichment and sequencing. Genomic DNA was submitted to sonication, ligation to standard adapters, and purification on a NucleoFast PCR Plate.

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plate (Macherey-Nagel, Düren, Germany). DNA was denatured and then hyd-
bridized for 20 min at 56°C to eight biotin-labeled oligonucleotides, the se-
quences of which are microsatellite motifs [(AG)_10, (AC)_10, (AAC)_8, (AGG)_8,
(ACO)_6, (AAG)_6, (ACAT)_6, and (ATCT)_6].

We then used primers corresponding to the adapters (5’-GTATTAGGCC-
TAGCTAGCGAAATC-3’ and 5’-GATTCTGCTAGCGACTT-3’) to am-
plify the microsatellite-enriched DNA. Preparation of the enriched library for
emulsion PCR, sequencing, and analytical processing using the 454 GS FLX
Titanium followed the manufacturer’s protocols. We then sorted the obtained
sequences, removed the sequence of the adapters, and selected sequences with
desirable properties using QDD version 1 (Meglécz et al., 2010).

Among the 33,290 sequences obtained, a total of 201 sequences longer than
80 bp, containing microsatellite motifs with at least seven uninterrupted repeats
and flanking regions free of tandem repetitions, were used to design PCR
primer pairs. We tested the 201 primer pairs for amplification on seven indi-
viduals, and then tested the successful primer pairs for fluorescent PCR on 32
individuals collected in southeastern Australia, between latitudes 34°37’43.1”S
and 34°29’08.9”S and between longitudes 148°50’28.3”E and 148°45’23.9”E
(see Appendix 1 for individual coordinates). A representative voucher speci-
men (CANB77329, deposited in the Australian National Herbarium [CANB],
Canberra, Australia) was previously collected in the same region as the samples
studied here.

These 32 individuals were used to test the amplification of these loci according
to the following procedure: PCR amplifications were performed in a 10-μL
volume containing 1× QIAGEN Multiplex Master Mix, 0.2 μM of each primer,
and 20 ng of genomic DNA extracted from individual leaves using the
DNeasy Plant Kit (QIAGEN). The amplification reactions were performed in an
Eppendorf (Hamburg, Germany) Mastercycler thermocycler and included
a 15-min denaturation step at 95°C; followed by 35 cycles of 30 s at 94°C,
1.5 min at 56°C, and 1 min at 72°C; followed by a final extension step at 60°C
for 30 min. Fifty primer pairs gave positive PCR amplification of the predicted
size for the seven individuals. They were then used in fluorescent PCR to amplify

| Locus | Primer sequences (5’–3’) | Repeat motif | Allele size range (bp) | 5’ end-labeled dye | Multiplex marker set | GenBank accession no. |
|-------|--------------------------|--------------|-----------------------|-------------------|-------------------|----------------------|
| Ad-33 | F: GAGAAGAGAAAGGGGATG   | (GA)_k       | 159–169               | FAM               | 2                  | KP702738             |
|       | R: CCACTATTATTATTGACTGC  |              |                       |                   |                   |                      |
| Ad-41 | F: TGAAGTTATATGCTCTCTGT  | (GA)_k       | 102–108               | PET               | 2                  | KP702739             |
|       | R: AAAAACAGCTTCTTATTTT   |              |                       |                   |                   |                      |
| Ad-45 | F: TCTAATAAAAAGAAGCGGCTA| (AC)_k       | 74–87                 | NED               | 2                  | KP702740             |
|       | R: AATTGTTTTGATGATGAG    |              |                       |                   |                   |                      |
| Ad-47 | F: CCCACAATGAAAGAAAATGTGA| (AG)_k       | 89–95                 | PET               | 1                  | KP702741             |
|       | R: CCGGAGAAAAGAAATGAGAT |              |                       |                   |                   |                      |
| Ad-48 | F: TACCTTGTCTGGAGCTCTTT | (AC)_k       | 196–214               | VIC               | 2                  | KP702742             |
|       | R: ACACATCGAAGATGCTGAG   |              |                       |                   |                   |                      |
| Ad-49 | F: CCTTCAAGGCAAAAGAGGAC| (AG)_k       | 168–184               | VIC               | 1                  | KP702743             |
|       | R: CAGGCTATGTTAGTTAATAA |              |                       |                   |                   |                      |
| Ad-54 | F: TGACCCAGAAAGTGAATG    | (TTT)_k      | 131–143               | NED               | 1                  | KP702744             |
|       | R: GGAAGAACAAAGAGAAAGCC |              |                       |                   |                   |                      |
| Ad-59 | F: CCGAGTCAAAGCGCTTAA   | (TC)_k       | 167–175               | NED               | 1                  | KP702745             |
|       | R: CTGCTCAACACAGAAAGT    |              |                       |                   |                   |                      |
| Ad-63 | F: CTTTTTCCCCACATTCTCTCT| (TC)_k       | 120–133               | VIC               | NA                 | KP702746             |
|       | R: CGTCCCCGATTTCTTATGT   |              |                       |                   |                   |                      |
| Ad-66 | F: TGAGGACCAAGAGCAAGAC  | (CA)_k       | 161–165               | VIC               | 1                  | KP702747             |
|       | R: TGTGAGCAAGCACTGTGAC  |              |                       |                   |                   |                      |
| Ad-70 | F: CTCCCACTGAGCTTGGGC    | (GT)_k       | 131–132               | PET               | 1                  | KP702748             |
|       | R: CGGCAGAATTTCCACCTTCTC|              |                       |                   |                   |                      |
| Ad-86 | F: TTTGAAAGGATGCTCCATTCT| (AC)_k       | 106–222               | VIC               | 1                  | KP702749             |
|       | R: GCTTTTCTTGGTATGAACTC  |              |                       |                   |                   |                      |
| Ad-89 | F: TCAATCTGAGCTATTTCTCT| (CTT)_k      | 80–96                 | FAM               | 1                  | KP702750             |
|       | R: CACGAGGGCTTTGATTTGATCTT| (CA)_k     | 92–96                 | PET               | 1                  | KP702751             |
| Ad-97 | F: SCAGGAACCCATCCTGAAGGC| (GT)_k       | 127–135               | VIC               | NA                 | KP702752             |
|       | R: CTGACTTTGACCTTCAAGTTG|              |                       |                   |                   |                      |
| Ad-116| F: TGTGTTGAGATTTCTCTCA  | (AC)_k       | 112–128               | VIC               | 2                  | KP702753             |
|       | R: GTATGCTTCGAGGATGTTG  |              |                       |                   |                   |                      |
| Ad-126| F: TTAGCAAGAACATGTAAGA  | (AG)_k       | 112–121               | NED               | 1                  | KP702754             |
|       | R: CAGGGTCCCATAAACACTTG  |              |                       |                   |                   |                      |
| Ad-127| F: GATGTTATGTGTGGCTCAAG | (GT)_k       | 106–136               | VIC               | 1                  | KP702754             |
|       | R: GCCCTAAACCACAGGTTGA  |              |                       |                   |                   |                      |
| Ad-137| F: ACCCTCAACCCATGTCTCT  | (AC)_k       | 148–128               | NED               | 2                  | KP702755             |
|       | R: AACCTCAACCTCGGACATGT|              |                       |                   |                   |                      |
| Ad-145| F: CGTAAAAAGGCTCGGTATGGT| (AG)_k       | 234–284               | NED               | 1                  | KP702756             |
|       | R: CTTCAATCTCACTTGACCTTC| (CTC)_k      | 150–160               | NED               | 2                  | KP702757             |
| Ad-173| F: TCTCAACCTCAAACTGCAA | (TG)_k       | 114–130               | NED               | 1                  | KP702758             |
|       | R: TCTTATTTAAAAACATGGCAATAGA| (AC)_k   | 149–151               | PET               | 1                  | KP702759             |
| Ad-201| F: GACGAGTCTCGGATGTTT  | (TG)_k       | 197–210               | VIC               | 1                  | KP702760             |

aRepeat motifs are those of the pyrosequenced alleles.
bSize ranges are based on the allele scoring performed on capillary electrophoresis data.
cTwo sets of possible multiplex reactions are listed. NA indicates that the primer pair produces unspecific products in multiplex.

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TABLE 2. Variability of the 23 microsatellite loci developed for *Acacia dealbata* based on 32 individuals sampled in southeastern Australia.

| Locus | A   | \(H_o\) | \(H_e\) | \(H_e/H_o\) |
|-------|-----|---------|---------|-------------|
| Ad-33 | 6   | 0.677   | 0.651   |
| Ad-41 | 4   | 0.344   | 0.579   |
| Ad-45 | 4   | 0.438   | 0.588   |
| Ad-47 | 4   | 0.656   | 0.613   |
| Ad-48 | 6   | 0.5     | 0.569   |
| Ad-49 | 9   | 0.813   | 0.858   |
| Ad-54 | 5   | 0.438   | 0.449   |
| Ad-59 | 5   | 0.29    | 0.457   |
| Ad-63 | 5   | 0.406   | 0.458   |
| Ad-66 | 3   | 0.531   | 0.478   |
| Ad-70 | 2   | 0.125   | 0.119   |
| Ad-86 | 7   | 0.531   | 0.472   |
| Ad-89 | 6   | 0.4     | 0.525   |
| Ad-97 | 3   | 0.29    | 0.263   |
| Ad-116| 4   | 0.625   | 0.668   |
| Ad-126| 6   | 0.625   | 0.58    |
| Ad-127| 8   | 0.633   | 0.791   |
| Ad-137| 7   | 0.563   | 0.606   |
| Ad-145| 11  | 0.296   | 0.876   |
| Ad-173| 6   | 0.594   | 0.555   |
| Ad-176| 6   | 0.313   | 0.427   |
| Ad-177| 2   | 0.438   | 0.437   |
| Ad-201| 5   | 0.531   | 0.58    |

Note: A = number of alleles; \(H_o\) = expected heterozygosity of Nei (1987); \(H_e\) = observed heterozygosity; HW = first type error of the probability test of Hardy–Weinberg equilibrium.

An asterisk (*) indicates a significant test at the 5% level, and “FDR” indicates that the test is significant after the false discovery rate adjustment for multiple testing.

CONCLUSIONS

We chose to present all 23 primer pairs although some of them may display Hardy–Weinberg or linkage disequilibrium because those disequilibria may be specific to the population sampled for the current study. The microsatellite markers developed here for *A. dealbata* display a moderate to large level of polymorphism in individuals sampled in natura in the native area of the species. This level of polymorphism should, however, be large enough for population genetic analyses to provide valuable information regarding the biology and worldwide invasion routes of *A. dealbata*.

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APPENDIX 1. Sampling information of the 32 *Acacia dealbata* individuals used in this study.

| Sample name | GPS coordinates                  |
|-------------|----------------------------------|
| AD100       | 34°31'45.4"S, 148°48'09.4"E     |
| AD120       | 34°31'52.5"S, 148°48'53.2"E     |
| AD14-1      | 34°32'44.5"S, 148°50'28.3"E     |
| AD140       | 34°31'39.4"S, 148°49'15.3"E     |
| AD156       | 34°29'46.9"S, 148°49'32.8"E     |
| AD161       | 34°29'45.0"S, 148°49'33.4"E     |
| AD181       | 34°30'32.2"S, 148°49'56.7"E     |
| AD200       | 34°30'39.1"S, 148°49'39.7"E     |
| AD220       | 34°30'38.8"S, 148°49'31.0"E     |
| AD240       | 34°30'27.7"S, 148°50'05.9"E     |
| AD250       | 34°29'46.0"S, 148°48'42.1"E     |
| AD261       | 34°30'03.8"S, 148°48'39.2"E     |
| AD270       | 34°30'07.0"S, 148°48'38.0"E     |
| AD290       | 34°30'55.3"S, 148°48'24.6"E     |
| AD310       | 34°32'18.2"S, 148°48'51.7"E     |
| AD311       | 34°32'21.4"S, 148°48'53.2"E     |
| AD330       | 34°37'42.5"S, 148°47'12.2"E     |
| AD340       | 34°37'43.1"S, 148°47'12.2"E     |
| AD360       | 34°33'22.8"S, 148°49'40.6"E     |
| AD380       | 34°34'31.0"S, 148°48'20.0"E     |
| AD400       | 34°34'30.6"S, 148°48'19.0"E     |
| AD410       | 34°34'47.8"S, 148°48'44.8"E     |
| AD440       | 34°32'44.9"S, 148°50'27.8"E     |
| AD460       | 34°32'28.2"S, 148°48'59.8"E     |
| AD480       | 34°32'56.8"S, 148°49'09.8"E     |
| AD500       | 34°29'08.9"S, 148°45'23.9"E     |
| AD520       | 34°30'40.5"S, 148°46'13.3"E     |
| AD540       | 34°30'43.9"S, 148°46'13.2"E     |
| AD560       | 34°30'44.6"S, 148°46'23.7"E     |
| AD581       | 34°30'57.2"S, 148°45'35.1"E     |
| AD600       | 34°30'57.2"S, 148°45'31.7"E     |
| AD612       | 34°30'59.0"S, 148°45'31.6"E     |

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