by developing a threshold value of Jaccard's similarity index for assigning individuals to clones or to siblings. Variation in AFLP banding patterns among clones was partitioned into three potential sources; clones, stems within-clones and foliage within-stems. Most of the variation was attributable to clones and then to stems within-clones. To provide an objective means of identifying clones, we developed a method for establishing a threshold similarity index to assign individuals to the same clone. Our method yielded a Jaccard similarity threshold of 0.983 that resulted in a potential pairwise error rate of 8.1% putative clone assigned to siblings and 1.5% sibling assigned to clones. The method was tested on independent clonal and sibling individuals resulting in the same threshold value and similar error rates. We applied our method to assign individuals to clones in a population of S. exigua along the Cosumnes River, California. A total of 11 clones were identified, with

Samples were collected when aments were available, allowing for the identification of each stem's sex. Due to the size of the site it was not possible to identify clones by digging up the root systems, and as a result it was impossible to establish "known" clones for testing purposes.

## DNA isolation and AFLP analyses

### DNA extraction

DNA was extracted according to the Cullings (1992) modification of Doyle and Doyle (1987). DNA concentrations were established by electrophoresis on agarose gels and comparisons with DNA lambda standards of known concentration.

### AFLP analysis

The amplified fragment length polymorphism (AFLP) method developed by Vos et al. (1995) was performed with the following modifications: restriction digestion and ligation were performed simultaneously in a 50 ml solution containing 250 ng of genomic DNA, 5 U of EcoRI, 5 U of MseI, 5 ml of 10 restriction–ligation buffer (100 mM of Tris-Acetate, 100 mM of Mg-acetate, 500 mM of K-acetate, 50 mM of DTT), 1 U of T4 DNA ligase, 0.2 mM of ATP, 1.0 mM of MseI adapter and 0.1 mM of EcoRI adapter. The restriction-ligation reaction was incubated for 4 h at 37 C, then diluted to 200 ml with 1 TE. Preamplification was performed in a 25-ml solution containing 2.5 ml of diluted restriction–ligation product, 0.2 mM of dNTPs, 0.3 mM of each primary amplification primer, 2.5 ml of 10 PCR buffer (100 mM of Tris–HCl, 500 mM of KCl, 20 mM of $MgCl_2$, 13 mg/ml of BSA), and 0.5 U of Taq polymerase. For the primary amplification primers, the EcoRI primer was identical to the adapter sequence, whereas the MseI primer had an extra "C" as a selective nucleotide. The PCR reaction was performed on a Techne Genius thermocycler for 28 cycles using the following cycling parameters: 30 s at 94 C, 60 s at 60 C and 60 s at 72 C. The primary amplification product was then diluted to 250 ml with 1 TE. Selective amplification was performed in a 25-ml solution containing 6.25 ml of diluted primary amplification product, 0.2 mM of dNTPs, 0.06 mM of EcoRI fluoresced selective primer, 0.3 mM of MseI selective primer, 2.5 ml of 10 buffer and 0.5 U of Taq polymerase. We pre-screened 32 selective primer pairs and chose three pairs that were reliable and highly polymorphic for this study (MseI-CCAA/EcoRI-GTA, MseI-CTC/EcoRI-TAC and MseI-CGTG/EcoRI-GTA). The selective PCR reaction had two cycle sets: 13 cycles of 30 s at 94 C, 30 s at 65 C (annealing temperature was lowered 0.7 C at each cycle) and 60 s at 72 C, followed by 18 cycles of 30 s at 94 C, 30 s at 56 C and 60 s at 72 C. Fingerprint data were obtained by running the amplified samples on an ABI Prism 377 DNA Sequencing System using PE Applied Big1(Cturfm5eu(p58.6(90sselecem)-3103.079fied)-5St1.1st571l(had)-lesnalyssing)-2co

We selected 177 polymorphic amplified fragments out of a total of 1,144. These were chosen in an initial screening across siblings, open-pollinated families and stems. Only

similarity threshold criterion (Fig. 2). In almost every case similarity values were mutually supportive in the assignment of clones and siblings (e.g. each sample in clone B had pairwise similarity values above the threshold when

which intra-clonal similarities were not fully consistent.

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