Salsola tragus or *S. australis* (Chenopodiaceae) in Australia - untangling taxonomic confusion through molecular and cytological analyses

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Abstract. *Salsola tragus sensu lato* (Chenopodiaceae) is found throughout Western Australia and is considered to be a weed in both natural and agricultural ecosystems, although the current taxonomic status of this species is not clear. The taxonomic literature reports morphological variation within Australian populations of the weed, indicating that there may be genetically distinct ecotypes or unidentified subspecies present within the species. A genetic and cytological approach was used to detect variation between 22 populations of *S. tragus sensu lato* in the south-west of Western Australia. Out-groups used in this study included a population of *S. tragus* L. from the USA and *Maireana brevifolia* (R.Br.) Paul G.Wilson (Chenopodiaceae) from Lake Grace. Four genetically distinct groups were identified, which were not closely related to the *S. tragus* out-group (approximately 60% similarity). Further, these groups and a *S. australis* R.Br. sample from the USA were all diploid ($2n = 18$), unlike the tetraploid ($2n = 36$) *S. tragus*. The predominant wheat-belt weed, group A, which was previously classified as *S. tragus* ssp. *tragus* L., was identified as *S. australis*. This species is probably native to Australia, given its arrival predated European invasion. Further research is required to clarify the taxonomic status of the other three possible taxa and determine their status in relation to *S. australis*.

Introduction

The genus *Salsola* (Chenopodiaceae), consisting of approximately 100-150 species, is globally distributed, occurring on all continents with the exception of Antarctica (Akhani *et al.* 2007; Kadereit *et al.* 2005; Rilke 1999*b*). Species of this genus are herbs or shrubs, characterised by axillary flowers subtended by prominent bracteoles, a hard perianth (often winged in fruit), and a superior ovary with the embryo frequently forming a conical spiral (Wilson 1984). Accurate classification of species within this genus has proven to be challenging, partly due to the large total number of species and the international distribution of a number of single species (particularly those that are weeds of ruderal habitats). Taxonomic concepts within the genus have also varied widely as some researchers have identified many species on the basis of minor morphological differences, while others have grouped several morphological types into widespread, single species (such as *S. tragus sensu lato*) (reviewed by Akhani *et al.* 2007). Further complications arise due to apparent frequent hybridisation events (Rilke 1999*a*, 1999*b*; Ryan and Ayres 2000).

Within Australia, Kadereit *et al.* (2005) suggested that *Salsola tragus* arrived recently via long distance dispersal from Asia, prior to European settlement. In spite of this apparent recent arrival, there are a wide variety of morphologically distinct forms found throughout the continent, which occur over a diverse range of habitats (Beadle 1981; Rilke 1999*a*). As a result, there is considerable debate about the taxonomic status of *Salsola* in Australia and whether it is native or exotic (Australian Plant Name Index 2008; Bean 2007). Australian populations of *Salsola* were originally classified as *S. kali* L. by Joseph Banks, from samples collected in 1770, which is another widely distributed species (previously recorded in USA, Europe, South Africa and Australia) that is morphologically similar to *S. tragus* (Rilke 1999*a*; Smith *et al.* 1980). In 1802-1804, Brown described *S. australis* and *S. macrophylla* R.Br., predominately based on the five

wings observed on the perianth of both species and the thickness (succulence) of the leaves observed in *S. macrophylla* (1810). In the Flora of Australia treatment, Australian representatives of the genus were all reclassified once again as *S. kali* (Wilson 1984). In a subsequent international review of the genus *Salsola* L. section *Salsola sensu lato*, the Australian populations were reclassified as *S. tragus* by Rilke (1999*a*), as she did not consider that the lectotype of *S. australis* was sufficiently distinct from *S. tragus* to justify the classification by Brown (1810). This review also recognised three subspecies of *S. tragus* within Australia. *Salsola tragus* ssp. *tragus* is found in Asia, Europe, northern Africa and throughout Australia, and is a common agricultural weed. *Salsola tragus* ssp. *grandiflora* Rilke occurs within the subtropical northern regions and is endemic to Australia. *Salsola tragus* ssp. *pontica* (Pall.) Rilke exists in littoral zones on the north-east and south-west coast of Australia and is also present in Europe, south-west Asia and northern Africa (Australian Plant Name Index 2008; Rilke 1999*a*). Akhani *et al*. (2007) recently suggested that, based on molecular sequence data, *S. kali* and *S. tragus* (among other species) should be transferred to the genus *Kali*. It should be noted that in the international studies of the genus *Salsola* by Rilke (1999*a*), Pyankov *et al.* (2001) and Kadereit *et al.* (2005), it is assumed that *S. australis* is synonymous to *S. tragus*, but few Australian specimens were examined in the review by Rilke (1999*a*) (Australian Plant Name Index 2008; Western Australian Herbarium 2007), and the other studies did not include any Australian specimens of *Salsola*. Akhani *et al.* (2007) recognises *S. tragus* as distinct from *S. australis*, but again, did not include Australian material in the study. Within Australia, most Herbaria still accept the classification system proposed by Rilke (1999*a*), and that the three *S. tragus* subspecies are the only taxa of *Salsola* present in Australia (Australian Plant Name Index 2008). For the purpose of this paper, the changes proposed by Akhani *et al.* (2007) were not used, in favour of the current classification recognised by the Western Australian herbarium (2007). Descriptions of this

species in Australia acknowledge the morphological diversity evident within *S. tragus* ssp. *tragus* populations, which is significantly greater than the polymorphism evident within *S. tragus* ssp. *tragus* populations found on other continents (Rilke 1999*a*; Wilson 1984). However, Rilke (1999*a*) attributes these differences to mutations or environmental conditions, and does not attempt to further subdivide the species beyond the three subspecies already recognised.

As stated, *Salsola tragus* ssp. *tragus* is a highly invasive agricultural and environmental weed, surviving in a broad range of environments in Australia (Beadle 1981; Rilke 1999*a*; Wilson 1984). Given the diverse habitat range occupied by *S. tragus* and the morphological diversity apparent between populations, it is possible that other genetically distinct ecotypes or subspecies may exist, beyond those proposed by Rilke (1999*a*). Traditional morphological analysis has struggled to conclusively identify species of the genus *Salsola*, but cytological and genetic methods have proven more successful (Gaskin *et al.* 2006; Ryan and Ayres 2000).

In this paper we use genetic and cytological analysis to investigate the possibility of genetically distinct populations of *S. tragus* in south-western Australia. Genetic variability was investigated using the RAMP (random amplified microsatellite polymorphism) method. The RAMP method has been used in other studies to determine the genetic relationships of plant species, from species to cultivar level (Davila *et al.* 1999; Richardson *et al.* 1995; Wu *et al.* 1994; Yuan *et al.* 2005). A RAMP assay combines microsatellites and RAPD markers to detect a high level of polymorphisms (Richardson *et al.* 1995; Wu *et al.* 1994). This method was employed because we did not know how closely related Western Australian populations of *S. tragus* ssp. *tragus* are, and therefore, what level of variability would be needed to distinguish between them.

Cytological analysis of species of the *Salsola* genus indicate a base chromosome number of $x = 9$, with polyploidy evident in both weedy and non-weedy species. Diploid, tetraploid and hexaploid forms are reported both from the native range of the *Salsola* genus in Europe and Asia,

and the introduced range of North America and Canada, with *S. tragus* and *S. kali* reported as tetraploid (2*n* = 36) (Crompton and Bassett 1985; Löve 1970; Mosyakin 1996; Pohl and Gillespie 1959; Wojnicka-Poltorak *et al.* 2000). This research examined genetic diversity and chromosome number in Western Australian populations of *S. tragus* and compared them with specimens of the genus *Salsola* from North America (California).

Materials and methods

Plant material

Salsola tragus ssp. *tragus* plants were sampled from 22 sites in south-western Australia during March and April 2005 (over an area approximately 550 by 550 km, Fig. 1). At each site seeds were harvested from 15 randomly selected plants. Site locations were recorded using GPS (WGS84) and at least one voucher specimen was taken from each population where plants were still alive (plants to the north east of the sampling area were often completely senesced at the time of sampling). Selected voucher specimens have been lodged at the Western Australian Herbarium (PERTH).

Fig. 1

Genetic analysis

Seed samples from five plants per site were selected and 200 seeds from each sample were sown in pots (116 mm diameter, 117 mm tall) containing potting mix (50% mulched pine bark, 25% sand, 35% peat moss), at a depth of 1 cm. When seedlings reached the four leaf stage, leaves were harvested to acquire 0.1 g of leaf material, directly before DNA isolation commenced. The out-groups used in the analysis were *S. tragus* from Davis, California USA, (previously classified using genetic analysis, Ryan and Ayres 2000) and *Maireana brevifolia* (R.Br.) Paul G.Wilson

(subfamily Salsoloideae, Chenopodiaceae) from Lake Grace, Western Australia (33.127^{o}S, 118.482^{o}E). The *S. tragus* seeds from California were germinated on damp filter paper in a physical containment level 2 facility (to prevent possible escape of the imported seeds), and leaf material was harvested as above. Branches from *M. brevifolia* plants were harvested in April 2005 and stored at -80^{o}C.

The DNA was isolated from 93 seedlings (five or less per population) using the QIAGEN DNeasy® Plant Mini DNA isolation kit (QIAGEN 2004). This method produced 100 µL DNA solutions, which were stored at -20^{o}C. A solution of 10.5 µL sterile water, 9 µL 5 x kinase buffer, 1.5 µL T4 polynucleotide kinase, 9 µL of γ-33P ATP and 30 ng µL^{-1} of SSR primer stock (Table 1) was incubated in a thermocycler (Hybaid OmniGene) at 37^{o}C for 1 hour and then 70^{o}C for 10 min. Forty five µL of this solution was added to 50 µL of RAPD primer stock (Table 1) and 800 µL of PCR mix (containing 2000 µL polymerisation buffer, 800 µL $MgCl_2$, 90 µL Taq polymerase and 4610 µL sterile water; Fisher Biotec, Perth). A labelling stock was prepared by mixing 2.1 µL sterile water, 1.8 µL 5 x kinase buffer, 0.3 µL T4 polynucleotide kinase and 1.8 µL of γ-33P ATP with 3 µL ladder stock and 180 µL TBE buffer and incubating as above. The concentration of each DNA sample was adjusted to between 5 and 25 ng µL^{-1} by diluting with sterile water (Water for Injections BP, Astra Pharmaceuticals Pty. Ltd.). The DNA amplification was carried out by incubating solutions containing 1.5 µL of a DNA sample and 8.5 µL of the PCR solution in the thermocycler, using the PCR cycles described by Wu *et al*. (1994). The PCR products were resolved on a polyacrylamide denaturing sequencing gel and the amplified marker bands were detected by autoradiography according to the method described by Yuan (2005). For each primer combination, the experiment was replicated twice, with samples in different combinations.

Table 1

Fig. 2

Statistical analysis

The polymorphic RAMP markers that appeared in both replicates of the analysis were scored (by eye) as 1 for present and 0 for absent, to construct a binary dataset. A regression analysis ($r^2 = 1$) to compare the size of the ladder bands and the distance they moved was used to determine the size of the RAMP markers. Morphological differences between plants at the 22 sites (identified by visual assessment during collection) allowed populations to be separated into four groups prior to genetic analysis (A, B, C and D, Fig. 3). The binary data set was used to calculate a simple matching similarity matrix and produce an MDS (non-metric multi-dimensional scaling) analysis (with 50 restarts and a stress of 0.16 for the best two dimensional configuration), with data points labelled according to group. The variation among the four groups (indicated by the MDS) was assessed using ANOSIM (analysis of similarity), with 9999 permutations. ANOSIM is an approximate analogue of the standard univariate ANOVA, testing the null hypothesis that there are no assemblage differences among groups. A group averages dendrogram (likewise based on the simple matching similarity matrix) was constructed, and a SIMPROF (similarity profile) test with 1000 permutations at a significance level of 1% was conducted on the dendrogram (PRIMER 6.1.6) (Clarke and Gorley 2006). A distance matrix based on total character difference was calculated based on the original dataset and dendrograms were constructed using UPGMA (unweighted pair-group method with arithmetic averages) and Neighbour-Joining. Bootstrap analysis with the UPGMA search was based on 2000 replications (PAUP) (Swofford 2001).

Cytological analysis

Seeds from 18 sites were used for cytological analysis (some sites were omitted as seed samples rapidly lost viability and could not be included). A population of *S. australis* from Santa Nella, California USA, was included for comparison (previously classified using morphological analysis, Dr. Fred Hrusa, pers. comm.; Ryan and Ayres 2000), as well as the *S. tragus* sample from California used in the genetic analysis. It should be noted that the *S. australis* sample was not available at the time the genetic analysis was performed. One hundred seeds from one or more plants per site were placed on damp filter paper in petri dishes. The Petri dishes were sealed in air tight bags and left for 1 to 7 days at 25^{o}C. Once seeds had germinated and the roots (not the radicle) were 0.5 to 5 cm in length, 10-15 seedlings were removed, placed in water at 4^{o}C and stored in a refrigerator at 4^{o}C for 18-24 h. Seedlings were subsequently hydrolyzed with 1 M HCl at 60^{o}C for 8 min and then stained with Feulgen stain for 2 h. The first 1-3 mm tip of each root was macerated and exposed to FLP orcein on a glass slide. Cover slips were placed on the slide, and each slide was heated gently over a flame and squashed. Chromosome counts were made from multiple cells from the roots of ten seedlings from each collection site. Only cells with fully contracted, clearly observable chromosomes were used (Shepherd and Yan 2003). Representative images of mitotic cells at metaphase were recorded with a Zeiss AxioCam (MRm) microscope.

Results

Genetic analysis

Each of the eight primer combinations used in the RAMP analysis produced from 5 to 90 polymorphic bands. In total, 422 polymorphic bands were identified (Table 1), ranging in size from 120 to 2873 bases. A section of the amplified products for selected samples, from the primer combination MF 52 and OPC 18, is shown in Fig. 2. Twenty-three markers that were specific to one of the four morphological groups were identified from seven of the primer

combinations (Table 2). The primer combination MF 153 OPC 20 provided specific markers for group B, C and D and the combination MF 155 OPC 07 provided specific markers for group A, B and C. None of the primer combinations produce specific markers for all four groups.

Table 2

The MDS, ANOSIM and dendrogram cluster analyses based on RAMP data clustered the 22 populations into four genetically distinct groups, corresponding to the four morphologically distinct groups referred to as group A, B, C and D (Fig. 3, 4, 5). Plants were initially grouped according to morphological differences readily apparent in the field. Fruiting perianth, commonly used to distinguish between species of the genus *Salsola*, were similar between groups. All plants had a perianth with four to five papery wings of similar size, and no reduced wings. Group A was distributed over most of the broad scale cropping region (wheat-belt), at sites 1, 3, 6, 13, 16, 19, 20, 21 and 22 (Fig. 1). These plants were characterised by single seeds (within the fruiting perianth) at the base of the leaves on each branch (Fig. 3). Group B existed in the rangelands found to the north-east of the wheat-belt, at sites 7, 8, 9, 10, 11 and 12. These plants were distinguished from group A plants by the fruiting structures. Fruit formed at the base of each leaf, as for group A. However, fruits congested together on globular spikes attached to branches were also apparent (Fig. 3). Group C was found at two isolated locations in the broad scale cropping region (wheat-belt), sites 5 and 15. These plants had all fruiting structures on globular spikes attached to the main branches (Fig. 3). Plants of group A, B and C appeared to be annuals, while those from group D appeared to be biennials or short lived perennials. Following senescence, plants from groups A, B and C detached from their root systems to become mobile, as usual for *S. tragus* plants (Mitchell and Wilcox 1988). In contrast group D plants, which were found on sand dune systems along the coast on the north-west side of the wheat-belt (sites 2, 4, 14, 17 and 18), showed no sign of becoming mobile at senescence. These plants were also a darker green in

colour, with flowers attached to the main branches and shorter, more succulent bracts and leaves (Fig. 3). However, given that the groups (except group A and C) were geographically distinct, it is possible that some of the observed morphological variation resulted from environmental factors.

Fig. 3

The MDS and ANOSIM analysis indicated that the samples clustered strongly according to morphological groups (Fig. 4 and Table 3; R: 0.967, $P < 0.01$). There was slightly more variation between samples classified as group C than between samples within groups A, B and D, but there were also few samples within group C. Group B and D had slightly more variation within the groups than group A. However, most of this variation can be accounted for by a single outlier in each of these populations (site 2, plant 5 in group D and site 10, plant 2 in group B).

Table 3

Fig. 4

The group average dendrogram confirmed that the samples could be separated into four groups and showed that group A, B and C were more closely related to each other than to group D (Fig. 5). The four groups were approximately 71% similar. The UPGMA and Neighbour-Joining dendrograms showed almost identical relationships between samples in the four groups, and so were not included. The bootstrap analysis indicated that grouping of samples into genetically distinct groups was strongly supported. The SIMPROF analysis confirmed that the relationships between the populations within a group and among the four groups were significant. All four groups were approximately 60% similar to the out-groups *S. tragus* and *M. brevifolia*, with 100% bootstrap support (Fig. 5). The samples from a single site were usually more closely related to each other than to samples from other sites, a grouping supported by both the bootstrap

and SIMPROF analysis. However, sample 6-3, 16-4 and 10-2 were not similar to the other plants of the same site.

Fig. 5

Cytological analysis

Somatic chromosome counts were recorded for populations collected from Western Australia and from *S. tragus* and *S. australis* from the USA. A count of 2*n* = 18 chromosomes was recorded for 18 of the Western Australian populations, as well as the sample of *S. australis* from the USA, and 2*n* = 36 chromosomes was recorded for the *S. tragus* population from the USA (Table 4, Fig. 6).

Table 4

Fig. 6

Discussion

At least four genetically, morphologically, and geographically distinct populations of the Salsola genus were present in the south-west of Western Australia. The genetic analyses indicated that groups A, B and C were more similar to each other than to group D. Groups A, B and C were annual plants that detached from their root systems to become mobile following senescence while group D were biennials or short-lived perennial plants that did not detach from their roots. All groups were diploid (2n = 18), in contrast to the tetraploid (2n = 36) *S. tragus* from the USA. Further, all groups were genetically and cytologically distinct from *S. tragus* (as recognised in the USA).

Group A which was classified as *S. tragus* ssp. *tragus* corresponds to the species *S. australis* R.Br. Rilke (1999*a*) visually examined the original voucher specimen of *S. australis* (The Natural History Museum, London Department of Botany, 000016765, BM) collected by Robert Brown in

1802 and concluded that the typical, southern inland form of *Salsola* found throughout Australia (group A) was the same as *S. australis*, but concluded that *S. australis* and *S. tragus* were similar enough to be considered the same species. However, work by Ryan and Ayres (2000) indicated that within California there were three genetically distinct Salsola species, *S. tragus*, *S. paulsenii* Litvinov and an unidentified species that was morphologically similar to *S. tragus*. It was originally concluded by Gaskin *et al*. (2006) that this species was identical to *S. kali* ssp. *austroafricana*. However, unpublished work by Dr Hrusa (Californian Department of Food and Agriculture Herbarium) has indicated that the unclassified species identified by Ryan and Ayres (2000) and group A identified here are the same species, and his comparison of these species with the voucher specimen of *S. australis* confirmed that both are *S. australis* (as the name *S. australis* predates *S. kali* ssp. *austroafricana*) (F. Hrusa, pers. comm.). While *S. australis* is an introduced species in the USA it may in fact be native to Australia.

Cytological and genetic evidence from this study, combined with the morphological comparison of Australian material with the *S. australis* lectotype, lead us to reclassify the common Western Australian wheat-belt weed *S. tragus* ssp. *tragus* as *S. australis* (Dr. Fred Hrusa, pers. com., Ryan and Ayres 2000). Thus, voucher specimens of *S. tragus* ssp. *tragus* at the Western Australian herbarium that morphologically resemble *S. australis* have been redetermined and the species is recognized on the Western Australian census (Western Australian Herbarium 2007). *Salsola australis* is mainly found in the wheat-belt region (Geraldton to Esperance) in Western Australia, but further populations are distributed throughout the central and northern regions of the state (Western Australian Herbarium 2007).

The other three groups (B, C and D) determined in this study remain unclassified. Neither group matches the description of *S. tragus* ssp. *pontica* or ssp. *grandiflora*, although this survey did not cover the northern reaches of Western Australia where *S. tragus* ssp. *grandiflora* is found

(Rilke 1999*a*). Group D matches Robert Brown's description of *S. macrophylla* as an erect, woody plant (Brown 1810). However, the original voucher specimen cited by Brown (1810) was collected in Queensland (Thirsty Sound, 22.133°S 150.85°E) and was not available for comparison to the Western Australian material. Rilke (1999*a*) stated that *S. macrophylla* was not a separate species and concluded that plants previously classified as *S. macrophylla* were actually *S. tragus* with unusually succulent leaves and bracts (diameter > 2 mm), a result of growing in highly saline environments. Based on the similarity index of the dendrogram, group B and C are more similar to group A than to group D, and both match the description of the former *S. kali* var. *strobilifera* Benth. recognised by Wilson (1984), which was previously applied to specimens with fruits congested on globular or ovoid spikes. However, it was previously unclear if these plants formed a distinct taxon. It was hypothesised that the ovoid spikes could be the result of a single, simple mutation, which would suggest that these plants were not a genetically distinct species or subspecies (Wilson 1984; Rilke 1999*a*). As there has not been a formal taxonomic revision of these potentially new taxa they are still labelled as *S. tragus* subsp. *tragus* within the Western Australian Herbarium (Western Australian Herbarium 2007).

The origin of *Salsola* in Australia remains unclear. The number of distinct groups in Western Australia and the close relationship between them indicates that at least some of these groups are likely to be native, rather than introduced species. The historical evidence indicates that species of the genus *Salsola* were established in Australia prior to European invasion (Brown 1810; Smith *et al.* 1980), and were recorded in all states of Australia during the 19th century (Domin 1921; Rilke 1999*a*). This suggests that species of the genus *Salsola* were native throughout Australia. Alternatively, it may be that some species were native to the eastern states of Australia, and their range increased with the expansion of agriculture throughout the continent. Populations morphologically similar to *S. australis* have also been described in South Africa, although the

first specimen was not recorded until 1885, significantly later than in Australia (Brown 1810; Rilke 1999*a*; Smith *et al.* 1980). A recent review of origin status for non-endemic plant species in Australia by Bean (2007) concluded that a species found in littoral habitats (like *S. australis*) should be considered native if the historical evidence indicated that it predated European invasion, even if ecological and phytogeographical data for status are equivocal. Based on this evidence, *S. australis* should be classified as native (Beadle 1981; Bean 2007; Bentham 1870; Brown 1810; Smith *et al.* 1980).

The widespread sampling of *Salsola* plants in south-western Australia (completed during this research) lead us to believe that *S. tragus* was not present, which has several significant implications for weed management. Once current taxa (i.e. the other groups) are reclassified to confirm that they are not *S. tragus*, this species should be assessed for its quarantine risk status and then excluded from Western Australia in the future to protect the State from this highly invasive weed. Secondly, if future research concludes that the taxa identified within this paper are native to Western Australia rather than exotic invasive species then the *Salsola* species found in natural ecosystems do not need to be eradicated or controlled. However, even if native, *S. australis* is a successful agricultural weed requiring management. *Salsola australis* (referred to as *S. tragus* ssp. *tragus*) was recorded as an agricultural weed throughout Australia by 1921, and is also an introduced weed in the USA (Bentham 1870; Domin 1921; Rilke 1999*a*; Ryan and Ayres 2000).

In conclusion, this research showed that there are four distinct groups from the genus *Salsola* in south-west Australia, none of which are the species *S. tragus* ssp. *tragus* (as it is recognised in the USA). Group A has been positively identified as *S. australis* (through comparison of collected specimens to the *S. australis* lectotype), but the other three groups cannot be matched to recognized species, and the origins of all four groups are ambiguous. The literature indicates that

there is a large degree of polymorphism evident in this genus throughout Australia, which implies that similar studies in other States may reveal other species of the genus *Salsola*. The RAMP analysis proved to be a very sound technique for identifying genetic variation within the genus *Salsola*. A large number of marker bands were identified, which were unambiguous and easy to score on the autoradiographs. The resulting multivariate analysis was highly robust. The group specific markers identified can be used to distinguish between these groups and other *Salsola* taxa in the future.

Future molecular work to confirm the status of the groups identified here should include samples from the two recognised subspecies of *S. tragus*, and should include an examination of type material for these subspecies and *S. macrophylla*. This will determine if group B, C and D are new or previously recognised species. Further, this work needs to be extended to include northern and eastern Australian material. Possibly genetic analysis of the genus *Salsola* worldwide needs to be extended to determine the status of Australian taxa. The morphology of the genus *Salsola* needs to be re-examined and complemented with taxonomic work to provide names for the unidentified Australian material. A study of the associated pathogens and fauna might help identify which groups are native, and provide potential biological control agents for use in North America where *S. australis* is an invasive weed (Ryan and Ayres 2000).

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Figures list

Fig. 1. *Salsola tragus* sensu lato collection sites in Western Australia, where circles indicate collection sites and squares indicate the location of major cities/towns (scale bar, 100 km).

Fig. 2. Section of a RAMP amplification profile obtained with the primer combination MF 52/OPC 18, for selected DNA samples. The lanes are labelled with collection site number for each plant population (top row) and the number assigned to individual plants at each site (bottom row). The size, in bases, of selected polymorphic bands is indicated by the arrows.

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Fig. 6. Somatic chromosomes from a root tip squash of (A) *Salsola tragus* from the USA.: $2n$ = 36 chromosomes and (B) *Salsola australis* from plant 1, site 5: $2n$ = 18 chromosomes.

Table 1. The combinations of SSR and RAPD primers that produced polymorphic bands in DNA samples from *Salsola tragus sensu lato*

SSR primer	Sequence (5´-3´)	RAPD primer	Sequence (5´-3´)	Number of polymorphic bands
MF 11	$GGAC(CT)_6$	OPC 03	GGGGGTCTTT	5
MF 43	$CCTC(AAG)_5$	OPC 04	CCGCATCTAC	46
MF 43	$CCTC(AAG)_5$	OPC 08	TGGACCGGTG	74
MF 52	$GGG(AAG)_4$	OPC 01	TTCGAGCCAG	77
MF 52	$GGG(AAG)_4$	OPC 18	TGAGTGGGTG	90
MF 153	$CCTT(AC)_6$	OPC 20	ACTTCGCCAC	50
MF 155	$CAAC(TG)_6$	OPC 07	GTCCCGACGA	58
MF 155	$CAAC(TG)_6$	OPC 09	CTCACCGTCC	22

Table 2. Specific markers for groups of the genus *Salsola* in south Western Australia generated by RAMP

Each marker is indicated by the primer combination which generated it and its size (in base pairs)

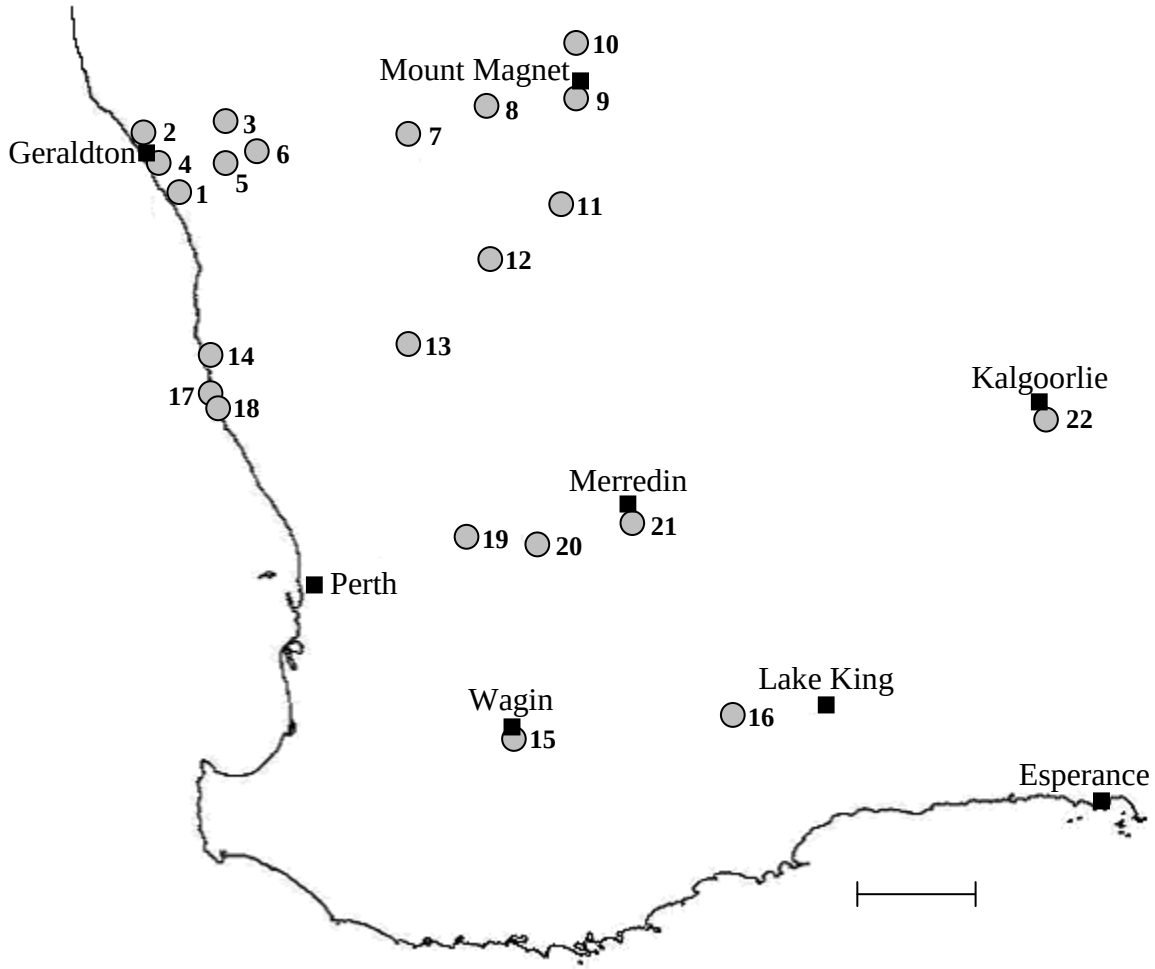
Group	Number of specific markers found	Marker details
A	8	MF 52 OPC 01, 1152; MF 52 OPC 18, 725; MF 155 OPC 07, 1896; MF 155 OPC 07, 1431; MF 155 OPC 07, 845; MF 155 OPC 07, 830; MF 155 OPC 07, 526; MF 155 OPC 07, 517
B	5	MF 43 OPC 08, 868; MF 153 OPC 20, 1299; MF 155 OPC 07, 2181; MF 155 OPC 07, 1865; MF 43 OPC 04, 1210
C	6	MF 52 OPC 18, 440; MF 153 OPC 20, 369; MF 155 OPC 07, 120; MF 43 OPC 04, 2253; MF 43 OPC 04, 1299; MF 155 OPC 09, 1601
D	4	MF 52 OPC 01, 2308; MF 52 OPC 01, 409; MF 52 OPC 18, 1117; MF 153 OPC 20, 2146

Table 3. Global R statistic and pair-wise comparisons from ANOSIM performed on the samples divided into the four genetic and morphological groups (group A, B, C and D). The number of permutations was 9999 and the significance level for each test was 0.01. ANOSIM was based on a simple matching similarity matrix and the R statistic indicates assemblage differences between groups (i.e. R = 0 where all samples are similar and R = 1 where all samples within a group are more similar to each other than to any samples from other groups)

Groups	R statistic
Global test	0.983
A, C	0.999
A, D	0.992
A, B	0.997
C, D	0.896
C, B	0.979
D, B	0.941

Table 4. The somatic chromosome number of the groups of the genus *Salsola* from Western Australia, as well as *S. tragus* and *S. australis* from the USA. The table includes the site number, the number of seedlings from which chromosome assessments were made (N), the population group designation, collection location, GPS coordinate (WGS84), herbarium voucher specimen where available (samples lodged in PERTH herbarium) and chromosome number. Sites for which chromosome number was not assessed are also included in the table, to indicate location and specimen ID

Site	N	Group	Location	GPS coordinate	Specimen ID	2*n*
1		A	Greenough	28.9510°S, 114.7524°E	07265239	
2	11	D	Chapman Valley	28.5517°S, 114.5654°E	07265409	18
3	10	A	Chapman Valley	28.4599°S, 115.1069°E	07265387	18
4	10	D	Greenough	28.8015°S, 114.6178°E	07265379	18
5	12	C	Mullewa	28.6908°S, 115.1509°E	07265360	18
6	10	A	Mullewa	28.6275°S, 115.3560°E	07265352	18
7	10	B	Morawa	28.8351°S, 115.7828°E		18
8	10	B	Yalgoo	28.2989°S, 117.1394°E		18
9	15	B	Mt Magnet	28.1104°S, 117.7990°E		18
10	12	B	Mt Magnet	27.7875°S, 117.9189°E	07265344	18
11	10	B	Yalgoo	29.0519°S, 117.7463°E	07265336	18
12	10	B	Yalgoo	29.5166°S, 117.1732°E		18
13		A	Dalwallinu	30.1296°S, 116.5454°E	07265492	
14	10	D	Dandaragan	30.2861°S, 115.0458°E	07265476	18
15	12	C	Wagin	33.3024°S, 117.3923°E	07265468	18
16	14	A	Lake Grace	33.0954°S, 119.0676°E	07265441	18
17	10	D	Dandaragan	30.5109°S, 115.0690°E	07188307	18
18	10	D	Dandaragan	30.5641°S, 115.1066°E	07265255	18
19		A	Cunderdin	31.6015°S, 117.0081°E		
20		A	Tammin	31.6323°S, 117.5673°E	07265433	
21	11	A	Merredin	31.5026°S, 118.2277°E	07265247	18
22	10	A	Kalgoorlie	30.7342°S, 121.4670°E		18
USA	10	*S. australis*	Santa Nella, CA	37.0619°N, 121.0167°W		18
USA	10	*S. tragus*	Davis, CA	38.5472°N, 121.7122°W		36



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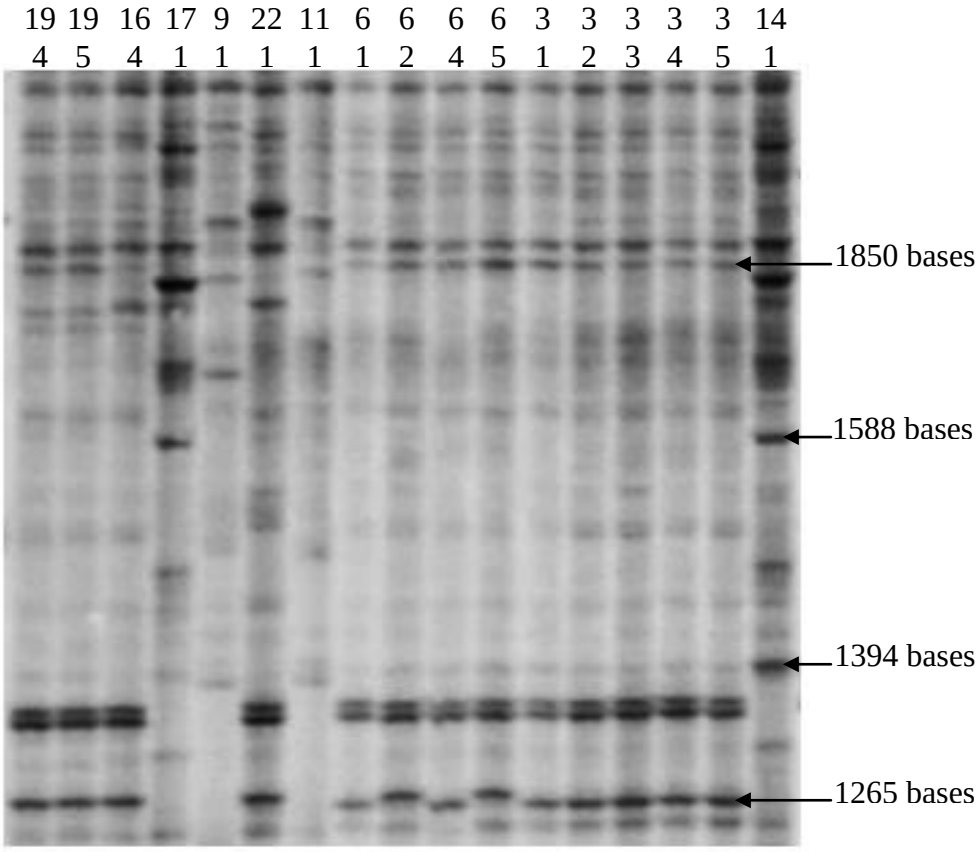

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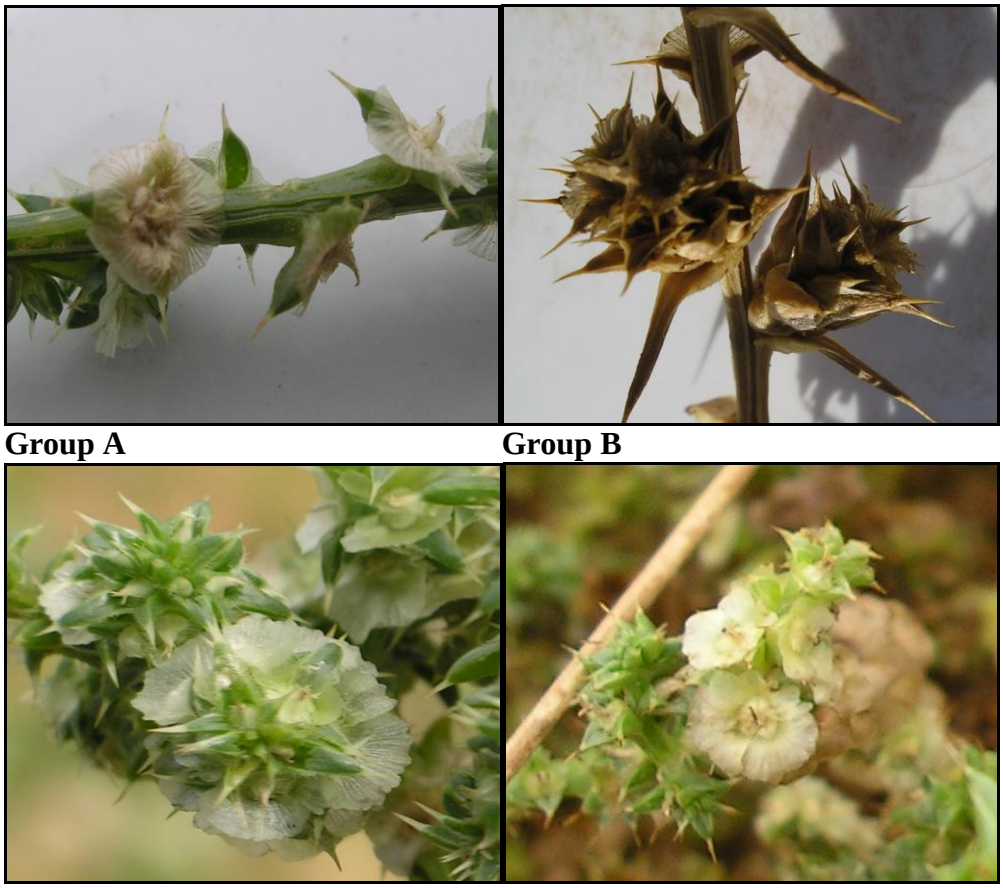

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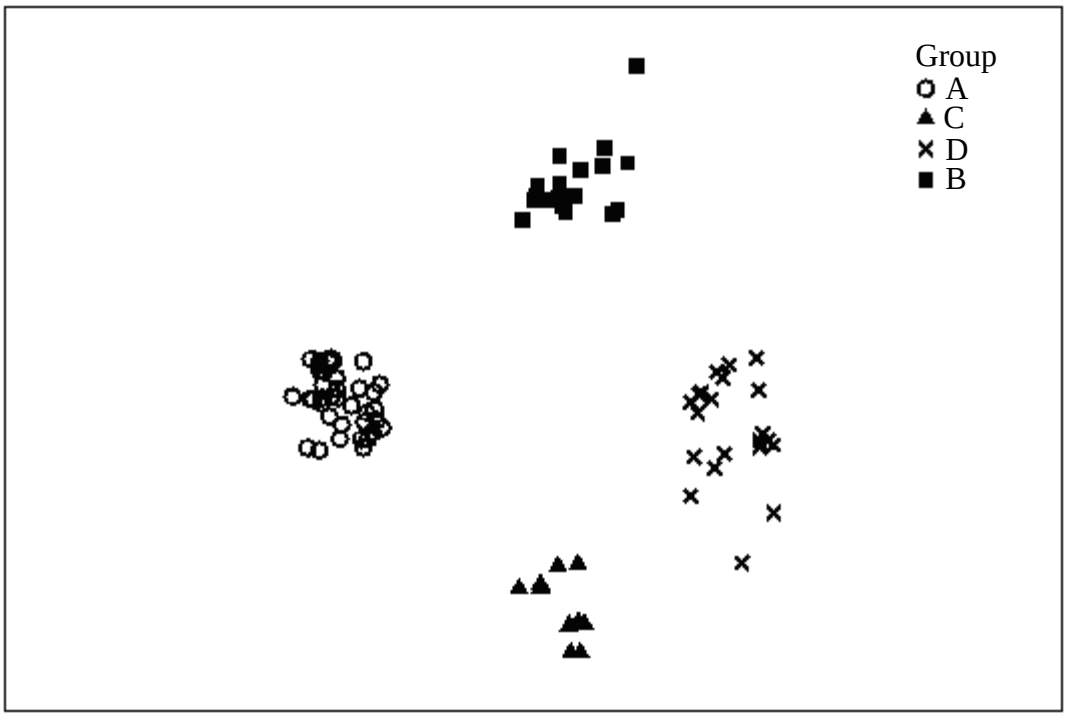

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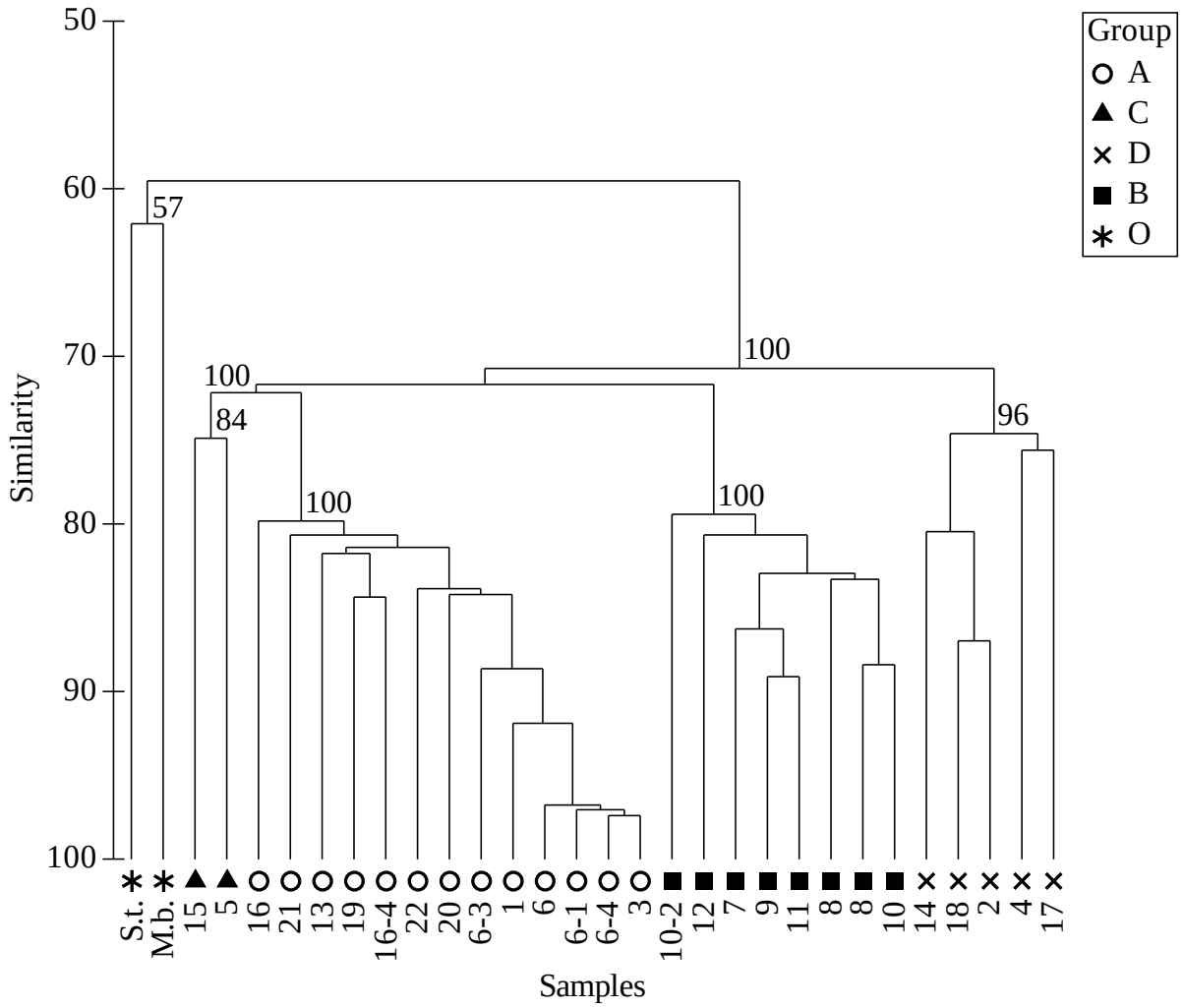

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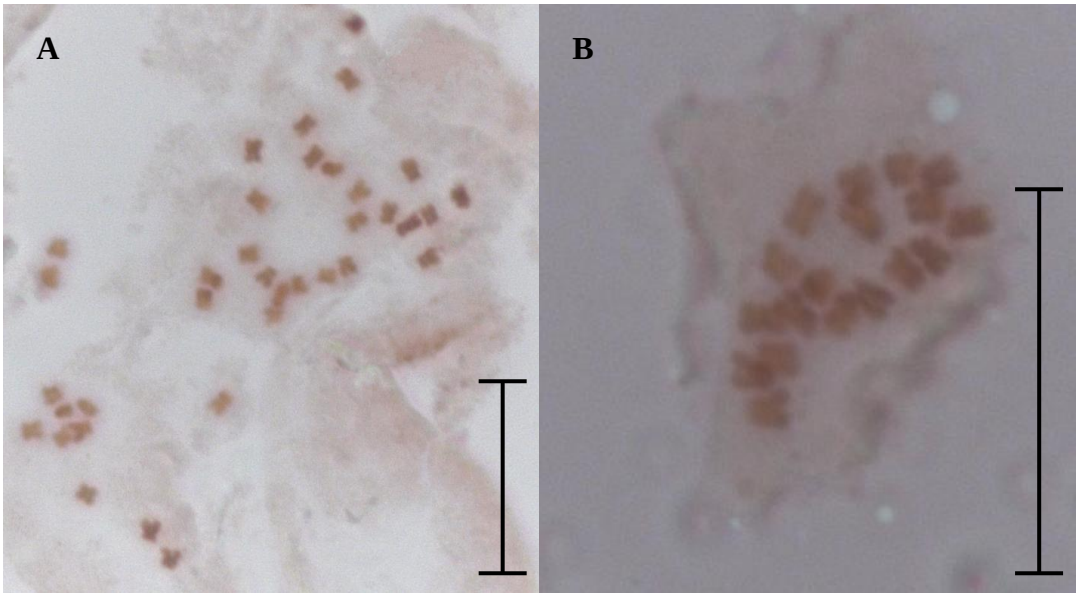

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