South African fireweed *Senecio madagascariensis* (Asteraceae) in Argentina: relevance of chromosome studies to its systematics

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The systematic identity of *Senecio madagascariensis* is ratified against the opinion that it is conspecific with *Senecio inaequidens*. Both species are native to South Africa and have been merged in the ‘Senecio inaequidens complex’, a group of entities difficult to distinguish from each other. *Senecio madagascariensis* is widespread in South America and Australia, where it is an invasive weed. Mitotic and meiotic studies were conducted on Argentinian material; chromosome counts solved the chromosome number controversy, validating $2n = 20$. The karyotype was symmetrical, composed of ten pairs of metacentric chromosomes varying from 1.62 to 2.38 μm in length. The most frequent number of satellited chromosomes was three, but their position was difficult to assign. Meiosis was regular, with a configuration of ten predominantly open bivalents. Univalents and quadrivalents were rarely observed. High frequencies of secondary associations of bivalents, chromosome asynchrony and bivalent grouping were documented, reinforcing the hypothesis that $x = 5$ is the basic chromosome number. Pollen stainability ranged from 94 to 99%. The relevance of chromosomal studies in the circumscription of *S. madagascariensis* is discussed. Hybridization and polyploidy, as principal evolutionary forces in this genus, explain the systematic difficulties. **© 2008 The Linnean Society of London, Botanical Journal of the Linnean Society, 2008, 158, 613–620.**

**ADDITIONAL KEYWORDS:** basic chromosome number – hybridization – karyotype – meiotic analysis – polyploidy – secondary association of bivalents – *Senecio inaequidens*.

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**INTRODUCTION**

*Senecio madagascariensis* Poir. is an African native plant, described from Madagascar by Poiret (1817). The species is an alien weed, in the sense of Pyšek et al. (2004), in Australia (Sindel, 1996), East Asia (Kinoshita et al., 1999) and South America. This opportunistic perennial herb has a short lifespan and develops three seed morphs differing in dormancy and germination rate, ensuring constant seedling emergence (Verona et al., 1982; Sindel, 1996).

The first Argentinian specimen of *S. madagascariensis* was collected in 1940 by Cabrera, who named it as a new species, *S. incognitus* (Cabrera, 1941). Later, it was again determined erroneously, as *S. burchelli* DC. (Cabrera, 1963). Finally, the name *S. madagascariensis* was adopted by Cabrera & Zardini (1978) following the revision of Asteraceae of Natal Province, South Africa, by Hilliard (1977).

More recently, *S. madagascariensis* has been considered to be part of the ‘*Senecio inaequidens* complex’ and conspecific with the *S. inaequidens* (Lafuma et al., 2003). Although *S. madagascariensis* and *S. inaequidens* are morphologically very similar, Radford
Table 1. Vouchers, the herbaria at which they were deposited and the provenance of the Argentinian material of Senecio madagascariensis studied

<table>
<thead>
<tr>
<th>Voucher</th>
<th>Herbarium</th>
<th>Provenance</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFW 930</td>
<td>SI</td>
<td>Tucumán, Departamento Tafí, San Javier</td>
</tr>
<tr>
<td>AFW 970</td>
<td>BAFC</td>
<td>Buenos Aires, Partido de Avellaneda, Sarandí</td>
</tr>
<tr>
<td>CCX &amp; MGL 3215, 3216, 3217</td>
<td>SI</td>
<td>Córdoba, Departamento Punilla, Sierra Chica</td>
</tr>
<tr>
<td>CCX &amp; MGL 3221, 3222, 3223, 3224</td>
<td>SI</td>
<td>Buenos Aires, Partido de San Pedro, Ciudad</td>
</tr>
<tr>
<td>CCX &amp; MGL 3225, 3226</td>
<td>SI</td>
<td>Buenos Aires, La Plata</td>
</tr>
<tr>
<td>CCX &amp; MGL 3227, 3228, 3229</td>
<td>SI</td>
<td>Distrito Federal</td>
</tr>
<tr>
<td>MGL 48, 49, 50*, 51, 52, 53, 54*</td>
<td>SI</td>
<td>Buenos Aires, Partido de Balcarce, Sierra El Volcán</td>
</tr>
<tr>
<td>MGL, CCX &amp; MNS 179</td>
<td>BAFC</td>
<td>Salta, Departamento Capital, Ciudad</td>
</tr>
</tbody>
</table>

AFW, Arturo F. Wulff; CCX, Cecilia C. Xifreda; MGL, Mariana G. López; MNS, Micaela N. Seo.

*Only pollen stainability.

e.t. al. (2000) differentiated them, basing their conclusions on the micromorphology of the cypsela surface. Another distinctive feature is the chromosome number. Although this is 2n = 40 for S. inaequidens (Chichiricco, Frizzi & Tammaro in Goldblatt, 1984; Harland in Radford, Liu & Michael, 1995), two chromosome numbers have been published for S. madagascariensis, namely n = 10 (Turner & Lewis, 1965, as S. pellucidus DC.; Verona et al., 1982; Radford et al., 1995) and n = 20 (Hunziker et al., 1989), 2n = 20 and 2n = 40, respectively.

In order to clarify the identity and status of the species, we undertook a study of the chromosome number and ploidy level in S. madagascariensis, with special emphasis on Argentinian representatives. Our data are discussed in comparison with the concepts of Lafuma et al. (2003), and our different point of view is considered with regard to the model of evolution of the genus.

MATERIAL AND METHODS

PLANT MATERIAL

The studied material and its provenance are summarized in Table 1. Vouchers were deposited at the herbaria SI or BAFC. Additional geographical distribution data of Argentinian material (Fig. 1) were obtained from the literature and from specimen labels in the herbaria SI, BA, BAA, BAB, BAF, LP, LIL, MCNS and CORD.

CHROMOSOME STUDIES

Young capitula were collected from 22 plants from eight different localities (Table 1). The inflorescences were fixed in situ in ethanol–chloroform–glacial acetic acid (6 : 3 : 1) for at least 24 h, transferred into 70% ethanol (v/v) and stored at 4–5 °C until use. Immature anthers were squashed in a drop of 2% propionic acid–haematoxylin solution, using ferric citrate as a mordant (Núñez, 1968). Photographs of meiosises were taken using a Leica DMLB photomicroscope and a Leitz camera. Open (IIo) and closed (IIc) bivalents per cell were recorded, and the mean and standard deviation of the frequencies were calculated.

For mitotic studies, seeds were germinated in humidity chambers and incubated under constant light at room temperature until the appearance of the root tips. The cell cycle was synchronized by the incubation of germinated seeds at 4 °C for 24 h. Afterwards, root tips were treated as follows: 2 h 30 min at room temperature, incubation for 2 h at 37 °C, transfer to 2 mM 8-hydroxyquinoline solution for 2 h at room temperature, followed by 1 h at 4 °C. Root tips were finally fixed in an ethanol–glacial acetic acid (3 : 1) solution for at least 24 h and stored at 4–5 °C until required. Prior to slide preparation, root tips were hydrolysed for 40 min in 5 M HCl at room temperature, rinsed once in distilled water and stained with 2% propionic acid–haematoxylin solution. Slide preparations were photographed as described above. The karyotype was determined from 18 cells at metaphase belonging to nine different individuals from Balcarce. For each metaphase, the absolute lengths of the short (s) and long (l) chromosome arms, whole chromosome length (c) and haploid karyotype length (HKL) were measured. Relative values of c, s and l were calculated to minimize the error caused by variation in the amount of chromosome contraction, considering HKL as 100%. The measurements were made from photographs, using a Zeiss stereoscopic microscope and an eyepiece micrometer. The centromere position in each chromosome was obtained using the arm ratio index (r = l/s), according to Levan, Fredga & Sandberg (1964).

POLLEN STAINABILITY

In order to estimate pollen grain fertility, anthers from fixed material were dissected and stained using
Alexander’s differential method (Alexander, 1969). The individuals examined were: CCX & MGL 3215, 3216, 3217, 3222, 3223, 3224, 3225, 3227, 3228, 3229; MGL 48, 49, 50, 52, 53, 54; AFW 930 (Table 1).

RESULTS

The Argentinian *S. madagascariensis* specimens studied here had a sporophytic chromosome number of 2n = 20 (Figs 2–4) with a symmetrical karyotype composed of ten pairs of metacentric chromosomes. Detailed chromosome measurements are shown in Table 2 and the idogram is illustrated in Figure 5. Metaphase cells exhibited one to six satellited chromosomes (Fig. 2), with three being the most frequent number (Figs 3, 4). Because of the morphological and dimensional similarities of the chromosomes, only one pair of satellites could be unambiguously located on the first chromosome pair (Fig. 5).

Meiotic analysis of 200 cells is shown in Table 3. Diakinesis or metaphase I revealed the uniform gametic number n = 10 (Figs 6–14). The main meiotic configuration was ten bivalents (II) (Fig. 6), although two univalents (I) or one quadrivalent (IV) per cell were sometimes observed (Fig. 7). Open bivalents appeared to be more frequent than closed bivalents (Table 3; Fig. 8).

Although the meiotic behaviour was regular (Figs 6–14), the bivalents showed a peculiar distribution, exhibiting secondary association. All the bivalents were associated in pairs in 8.59% of the cells studied (Table 4), whereas eight bivalents were associated in 25% of meiocytes (Table 4, Figs 9, 12). Six was the most frequent number of associated bivalents observed (Table 4, Fig. 7), with the remaining four not or doubtfully associated. Only 10 of 128 cells displayed no associations, whereas four cells showed indeterminate association. Some of the latter cells exhibited unusual behaviour. In one, two groups of five bivalents were observed, each at different disassociation states (Fig. 10). In another, two distinctive groups of five bivalents each were found (Fig. 11). In addition, fused prometaphase IIIs and large pollen grains (Fig. 15) were also observed, although at low frequency. A high degree of pollen grain stainability was observed in all individuals studied, ranging from 94 to 99%.

### Table 2. Chromosome measurements of the specimens of *Senecio madagascariensis* studied

<table>
<thead>
<tr>
<th>Chromosome pair number</th>
<th>Chromosome length (c)</th>
<th>Short arm length (s)</th>
<th>Long arm length (l)</th>
<th>Arm ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute (μm)</td>
<td>Relative (% HKL)</td>
<td>Absolute (μm)</td>
<td>Relative (% HKL)</td>
</tr>
<tr>
<td></td>
<td>± SD</td>
<td>(±)</td>
<td>± SD</td>
<td>(±)</td>
</tr>
<tr>
<td></td>
<td>x ± SD</td>
<td>x ± SD</td>
<td>x ± SD</td>
<td>x ± SD</td>
</tr>
<tr>
<td>1</td>
<td>2.38 ± 0.38</td>
<td>12.06</td>
<td>1.06 ± 0.21</td>
<td>5.38</td>
</tr>
<tr>
<td>2</td>
<td>2.23 ± 0.32</td>
<td>11.28</td>
<td>0.98 ± 0.12</td>
<td>4.95</td>
</tr>
<tr>
<td>3</td>
<td>2.12 ± 0.28</td>
<td>10.77</td>
<td>0.93 ± 0.14</td>
<td>4.73</td>
</tr>
<tr>
<td>4</td>
<td>2.06 ± 0.28</td>
<td>10.42</td>
<td>0.90 ± 0.13</td>
<td>4.55</td>
</tr>
<tr>
<td>5</td>
<td>2.00 ± 0.28</td>
<td>10.13</td>
<td>0.87 ± 0.16</td>
<td>4.38</td>
</tr>
<tr>
<td>6</td>
<td>1.94 ± 0.26</td>
<td>9.82</td>
<td>0.83 ± 0.12</td>
<td>4.22</td>
</tr>
<tr>
<td>7</td>
<td>1.87 ± 0.26</td>
<td>9.49</td>
<td>0.79 ± 0.14</td>
<td>4.00</td>
</tr>
<tr>
<td>8</td>
<td>1.80 ± 0.26</td>
<td>9.14</td>
<td>0.80 ± 0.11</td>
<td>4.06</td>
</tr>
<tr>
<td>9</td>
<td>1.72 ± 0.25</td>
<td>8.70</td>
<td>0.75 ± 0.12</td>
<td>3.79</td>
</tr>
<tr>
<td>10</td>
<td>1.62 ± 0.26</td>
<td>8.19</td>
<td>0.67 ± 0.11</td>
<td>3.40</td>
</tr>
</tbody>
</table>

HKL, haploid karyotype length; SD, standard deviation; x, mean value.

*HKL value is 19.74 ± 2.78 μm.

DISCUSSION

The karyotype of *S. madagascariensis* (2n = 20) is presented here for the first time. It displays a high level of inter- and intrachromosomal symmetry. All chromosomes are metacentric, a feature held in common with other species of this genus (Dematteis & Fernández, 1998; López et al., 2002a). Because the chromosomes were similar and small in size, the identification of pairs was difficult. Our results suggest the existence of at least six satellited chromosomes, but only one pair could be identified with confidence. The difficulties in assigning the correct positions of the secondary constrictions have already been documented by Stace (2000).

The present cytological study of Argentinian representatives of *S. madagascariensis* confirms the previous reports (2n = 20) for Africa (Turner & Lewis, 1965), Australia (Radford *et al.*, 1995) and Argentina (Verona *et al.*, 1982). Unfortunately, the latter authors did not refer their counts to any voucher specimen, preventing us from comparing our results with theirs.

By contrast, Hunziker *et al.* (1989) published the only known record of 2n = 40 in material from Balcarce (Sa. de Volcán). Our results showed 2n = 20 for many individuals from the same geographical region. We re-examined herbarium material and original chromosome drawings of the 2n = 40 specimen in Hunziker *et al.* (1989) and his field diary, and found that the plant with 2n = 40 is a different
native Senecio species [Senecio brasiliensis (Spreng.) Less.], establishing the chromosomal uniformity of S. madagascariensis.

Our meiotic analysis in Argentinian specimens of S. madagascariensis revealed a high frequency of secondary associations of bivalents, i.e. their occurrence together, in pairs or groups, at metaphase I, as described in wheat (Riley, 1960). This phenomenon has been interpreted as evidence of residual homology or homoeology between chromosomes (Poggio, Naranjo & Jones, 1986; Naranjo, Molina & Poggio, 1990; Argimón, Wulff & Xifreda, 1999), and suggests the possible existence of an ancient polyploid condition. Moreover, the quadrivalent observation in Argentinian S. madagascariensis, although rare, reinforces the palaeo-tetraploid condition of this species. The rare appearance of multivalents in the meiosis of suspected polyploids is referred to as ‘diploidized’ meiotic behaviour (Riley & Chapman, 1958; López, Wulff & Xifreda, 2002b), because the predominant occurrence of bivalents resembles the meiosis of diploids. This behaviour could be attributed to Ph-like genes, which suppress multivalent formation and avoid this source of sterility in polyploids (Moore, 1998; Sybenga, 1999), or to the low chiasma frequency (revealed by the high frequency of IIo). The diploidized meiotic behaviour explains the regularity of the meiotic process in this species and, consequently, the high level of pollen fertility (López et al., 2005).

This ‘polyploidy camouflage’ raises some difficulties in basic chromosome number determination, and our detailed analysis of meiotic chromosomes in S. madagascariensis highlights this matter. By contrast with the previous view establishing a basic number of x = 10 in Senecio (Ornduff et al., 1963), the evidence revealed above strongly supports our previous hypothesis of x = 5 as the basic chromosome number (López et al., 2005). This is also strengthened by the existence of Senecio species with 2n = 10 (Lawrence, 1980). In addition, chromosome asynchrony and bivalent groupings of five are evidence of the co-existence of two genomes in the same nucleus (Poggio, Rosato & Naranjo, 1997). Otherwise, the existence of fused prometaphase II is common in polyploids as a source of large pollen grains (i.e. non-reduced gamete formation).

The clarification of the chromosome number of S. madagascariensis contributes to the taxonomic controversy over this species and the related S. inaequidens. The two species differ from each other by leaf morphology, cypsela anatomy and micromorphology (M. G. López et al., unpubl. data), but, despite these differences, Lafuma et al. (2003) considered them to be conspecific, being cytotypes of S. inaequidens, based on research performed in South Africa. Three points sustain their conclusion: (1) the

### Table 3. Meiotic analysis of the Argentinian Senecio madagascariensis individuals

<table>
<thead>
<tr>
<th>Meiotic configuration (N = 200 cells)</th>
<th>Meiotic figures per cell ( \bar{x} \pm SD )</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>IIo</td>
</tr>
<tr>
<td>20 II</td>
<td>0.02 ± 0.21</td>
</tr>
</tbody>
</table>

I, univalents; IIc, closed bivalents; IIo, open bivalents; IV, quadrivalents; SD, standard deviation; \( \bar{x} \), mean value.

### Table 4. Secondary association of bivalents observed in Argentinian Senecio madagascariensis individuals

<table>
<thead>
<tr>
<th>Number of bivalents associated per cell</th>
<th>Diakinesis Number (%)</th>
<th>Metaphase I Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7 (5.47)</td>
<td>7 (5.47)</td>
</tr>
<tr>
<td>2</td>
<td>2 (1.56)</td>
<td>8 (6.25)</td>
</tr>
<tr>
<td>4</td>
<td>1 (0.78)</td>
<td>18 (14.07)</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>42 (32.81)</td>
</tr>
<tr>
<td>8</td>
<td>1 (0.78)</td>
<td>31 (24.22)</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>11 (8.59)</td>
</tr>
</tbody>
</table>

The percentage values (of all 128 cells observed) are given in parentheses (see text).
morphological similarities between the two species; (2) ploidy level, and (3) molecular differences.

MORPHOLOGICAL SIMILARITIES
The species are similar, but there are some differences that separate them, i.e. leaf and cypsela morphology (Sindel, 1996; Radford et al., 2000). They have different distributions. Both occur in South Africa, but S. inaequidens is a weed restricted to Europe (Ernst, 1998), whereas S. madagascariensis has dispersed to America and Australia (see ‘Introduction’). They are part of a polyploid complex that also includes S. harveianus MacOwan, S. burchellii DC. and S. pellucidus DC. In South Africa, species recognition is difficult, mostly because of hybridization amongst the members of the complex. This evolutionary force, widespread in Senecio (Hodálová, 1999; López, 2001; López et al., 2005), results in a morphological continuum, confusing the separation of the taxa (Soltis & Soltis, 1999). Following this idea, the entities mentioned by Lafuma et al. (2003) as ‘undefined’ could be hybrids between members of the complex or introgressed forms. Thus, species identification must be conducted carefully in South Africa.

PLOIDY LEVEL
Two ploidy levels were recognized for S. inaequidens in South Africa (Lafuma et al., 2003). As this result was not obtained by chromosome observations, but from DNA content analysis, and was performed on a
complex of hybrids (see above), it must be interpreted with caution (Stace, 2000; Suda et al., 2006). Only a chromosomal study could confirm the two numbers proposed. Although this result suggests the existence of two cytotypes within S. inaequidens, it is not evidence of conspecificity with S. madagascariensis.

**MOLECULAR DIFFERENCES**

More molecular differences were found within S. madagascariensis from different locations (South Africa, Madagascar and Australia) than the variation observed between S. madagascariensis and S. inaequidens from South Africa (Scott, Congdon & Playford, 1998). These findings were interpreted by Lafuma et al. (2003) as evidence of conspecificity between the two species. Conversely, we believe that these similarities could again be consequences of hybridization within the S. inaequidens complex, which maintains a mixed gene pool in South Africa, blurring the differences between species.

The situation in South Africa will be solved only through an extensive research programme including cytological studies. Special attention should be given to species identification, ploidy level and chromosome number assignment in the species complex.

Finally, the sum of the morphological, chromosomal and geographical distribution differences provides sufficient evidence to maintain S. madagascariensis and S. inaequidens as separate species. There is abundant evidence to suggest that polyploidy and hybridization have been important processes in the evolution of the genus Senecio. These processes, with their reticulate as opposed to divergent evolution, could explain the systemic difficulties encountered in the group.

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