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Karyological analysis of the five native Macaronesian *Festuca* (Gramineae) grasses supports a distinct diploid origin of two schizoendemic groups

by

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Abstract

Menezes de Sequeira, M., Díaz-Pérez, A., Santos-Guerra, A., Viruel, J. & Catalán, P. 2009. Karyological analysis of the five native Macaronesian *Festuca* (Gramineae) grasses supports a distinct diploid origin of two schizoendemic groups. *Anales Jard. Bot. Madrid* 66(1): 55-63.

A karyological analysis has been conducted of all five native Macaronesian *Festuca* grasses belonging to fine-leaved *F.* subg. *Festuca* sect. *Aulaxyper* and broad-leaved *F.* subg. *Drymanthele* sect. *Phaeochloa* Loliinae lineages. Chromosomal analyses were made in 30 plants corresponding to 17 populations of the fine-leaved *F. agustinii*, *F. jubata*, *F. francoi* and *F. petraea* and 2 populations of the broad-leaved *F. donax*. All counts except one tetraploid count were diploids, showing $2n = 14$ chromosomes. Diploidy was confirmed for the robust *F. donax*, nested within a clade of relict ancestral fescues as reported in recent phylogenetic studies, and was also found in the more slender *F. agustinii*, *F. jubata*, *F. francoi* and *F. petraea*, which are basal to a recently evolved clade of polyploid red fescues. Karyotypes of the two groups are however distinct, with broad-leaved *F. donax* showing larger and more regular chromosomes and all four fine-leaved taxa showing smaller and more irregular submetacentric chromosomes. Our karyological data indicate that these two groups of diploid fescues correspond to distinct schizoendemics which apparently originated at different times after independent continental colonizations of Macaronesia.

Keywords: diploidy, *Festuca agustinii*, *F. donax*, *F. francoi*, *F. jubata*, *F. petraea*, karyology.

Resumen

Menezes de Sequeira, M., Díaz-Pérez, A., Santos-Guerra, A., Viruel, J. & Catalán, P. 2009. El análisis cariológico de las cinco especies de *Festuca* (Gramineae) autóctonas de Macaronesia sostiene un origen diploide diferente de dos grupos de esquizoendemismos. *Anales Jard. Bot. Madrid* 66(1): 55-63 (en inglés).

Se presenta un análisis cariológico de las cinco especies de *Festuca* L. autóctonas en Macaronesia pertenecientes a linajes de hojas finas (*F.* subg. *Festuca* sect. *Aulaxyper*) y hojas anchas (*F.* subg. *Drymanthele* sect. *Phaeochloa*) de Loliinae. Los recuentos cromosómicos fueron realizados en 30 individuos correspondientes a 17 poblaciones de las especies de hojas finas *F. agustinii*, *F. jubata*, *F. francoi* y *F. petraea* y a 2 poblaciones de la especie de hojas anchas *F. donax*. Todos los recuentos excepto uno tetraploide indicaron que estas especies son diploides, mostrando $2n = 14$ cromosomas. La diploidía fue confirmada para la robusta *F. donax*, emplazada en un clado de festucas relicticas ancestrales, tal como ha sido indicado en recientes estudios filogenéticos, al igual que en las más gráciles *F. agustinii*, *F. jubata*, *F. francoi* y *F. petraea*, resueltas filogenéticamente en una posición basal en un clado recientemente evolucionado de festucas rojas poliploides. Los patrones cariotípicos de los dos grupos son sin embargo distintos. La especie de hojas anchas *F. donax* muestra cromosomas metacéntricos mayores y más regulares, mientras que las cuatro especies de hojas finas muestran cromosomas submetacéntricos más pequeños y más irregulares. Nuestros datos cariológicos y evolutivos indican que estos dos grupos de festucas diploides corresponden a distintas especies esquizoendémicas que aparentemente se originaron en distintos momentos históricos tras colonizaciones independientes de Macaronesia por diversos linajes continentales.

Palabras clave: diploidía, *Festuca agustinii*, *F. donax*, *F. francoi*, *F. jubata*, *F. petraea*, cariología.

Introduction

Although the Macaronesian region is one of the major hot-spots of plant diversity in oceanic isles (Humphries, 1979; Hansen & Sunding, 1993; Francisco-Ortega & al., 1996; Carine & al., 2004), most of the Poaceae genera present in the region show a relatively low number of Macaronesian endemics (Cope, 1994; Santos-Guerra, 1999; Borges & al., 2005). Conversely to other angiosperm groups that show a high range (8-63) of oceanic taxa (e.g. *Argyranthemum* Webb, *Echium* L., *Micromeria* Benth., *Sonchus* L., *Tolpis* Adans., several genera of the family Crassulaceae –*Aeonium* Webb & Berthel., including *Greenovia* Webb & Berthel., *Aichryson* Webb & Berthel., *Monanthes* Haw., among others–; summarized in Silvertown, 2004, and Carine & al., 2004), the Macaronesian grasses present a small range of endemic species per genus which vary from single species (e.g. *Anthoxanthum* L., *Aristida* L., *Arrhenatherum* P. Beauv., *Avena* L., *Brachypodium* P. Beauv., *Eragrostis* Wolf, *Gaudinia* J. Gay, *Koeleria* Pers., *Lophochloa* Rchb., *Sporobolus* R.Br.) to two to five taxa (e.g. *Agrostis* L., *Dactylis* L., *Deschampsia* P. Beauv., *Festuca* L., *Holcus* L., *Lolium* L., *Melica* L., *Phalaris* L., *Poa* L.) (Hansen & Sunding, 1993; Cope, 1994; Santos-Guerra, 1999; Borges & al., 2005).

Different studies have demonstrated that a high percentage of the Macaronesian endemic flora has a continental Mediterranean origin (Emerson, 2002; Comes, 2004; Carine & al., 2004), and that the potential ancestors were probably located in SW Europe and NW Africa (Carine & al., 2004; Carine, 2005). The existence of large endemic plant groups has been interpreted as the result of either single continental colonization followed by successful adaptive radiation or multiple colonizations followed by in-situ speciation (reviewed in Silvertown, 2004; and Carine & al., 2004). Conversely, the presence of reduced endemic plant groups has been attributed to recent long distance dispersals followed by restricted insular speciation (Díaz-Pérez & al., 2008). However, the ages of the Macaronesian plant lineages are unknown in most cases, and their relative ancestry has been based on karyological and nuclear DNA content assessments (Löve & Löve, 1975; Suda & al., 2003) and on phylogenetic analyses (Francisco-Ortega & al., 1996; Silvertown, 2004; Carine & al., 2004).

Festuca, the main genus of subtribe Loliinae (Poaceae), is present in all main Macaronesian archipelagos except Cape Verde (Catalán, 2006). Despite its large size (c. 500 species) and almost worldwide distribution, it contains only five native taxa in Macaronesia (Inda & al., 2008; Fernández-Prieto & al.,

2008; Díaz-Pérez & al., 2008) (Fig. 1). Four of them, *Festuca agustinii* Lindinger, *F. jubata* Lowe and *F. francoi* Fern. Prieto, C. Aguiar, E. Dias & M.I. Gut and *F. petraea* Guthnick ex Seub., which were previously classified within *F.* subg. *Festuca* sect. *Amphigenes* Janka (Saint-Yves, 1922), have been recently found to be related to the fine-leaved red fescues (*F. rubra* group, *F.* subg. *F.* sect. *Aulaxyper*) (Torrecilla & al., 2003; Catalán, 2006; Inda & al., 2008). By contrast, the fifth species, *F. donax* Lowe, has been always classified within the broad-leaved *F.* subg. *Drymanthele* sect. *Phaeochloa* (Saint-Yves, 1922; Catalán, 2006).

Karyological data are essential to decipher the potential ancestry of species and populations and to support evolutionary hypotheses in angiosperms (Stebbins, 1971). *Festuca* shows a single chromosome base number of $x = 7$ but a large variety of ploidy levels ranging from diploids to duodecaploids (Ainscough & al., 1986; Dubcovsky & Martínez, 1992; Catalán & al., 2004). Chromosomal analysis conducted by Dubcovsky & Martínez (1992) found a world biogeographical distribution of *Festuca* ploidy levels, with almost all relict diploid taxa restricted to the Mediterranean-Eurasian region, whereas polyploid taxa were the only ones present in the southern hemisphere but also extended elsewhere. Dawe (1989), Catalán (2006) and Šmarda & al. (2008) further reported an evolutionary signal of karyotype patterns, ploidy levels and genome sizes within *Festuca*, with diploid races resolved basally in most Loliinae clades (Catalán, 2006).

Because chromosome data are lacking or have been barely investigated in most of the endemic Macaronesian *Festuca* we have conducted a chromosomal analysis of the five native species of this genus, aiming to investigate their ploidy and karyotype patterns, and their evolutionary implications.

Material and methods

Root tips obtained from planted individuals were used for the chromosomal analysis. Chromosome counts were done in mitotic cells of *Festuca agustinii* (5 populations from Tenerife island, 5 individuals), *F. jubata* (4 populations from Madeira island, 10 individuals), *F. francoi* (5 populations from S. Miguel, S. Jorge, Pico and Flores islands, 8 individuals), *F. petraea* (7 populations from St. Maria, Graciosa, S. Jorge, Pico, Faial and Flores islands, 8 individuals), and *F. donax* (2 populations from Madeira island, 2 individuals) (see Results and Appendix 1). Chromosome counting was carried out following the procedures of Menezes de Sequeira (2004) and Menezes de Sequeira & Castroviejo (2007) standardized for *Hol-*

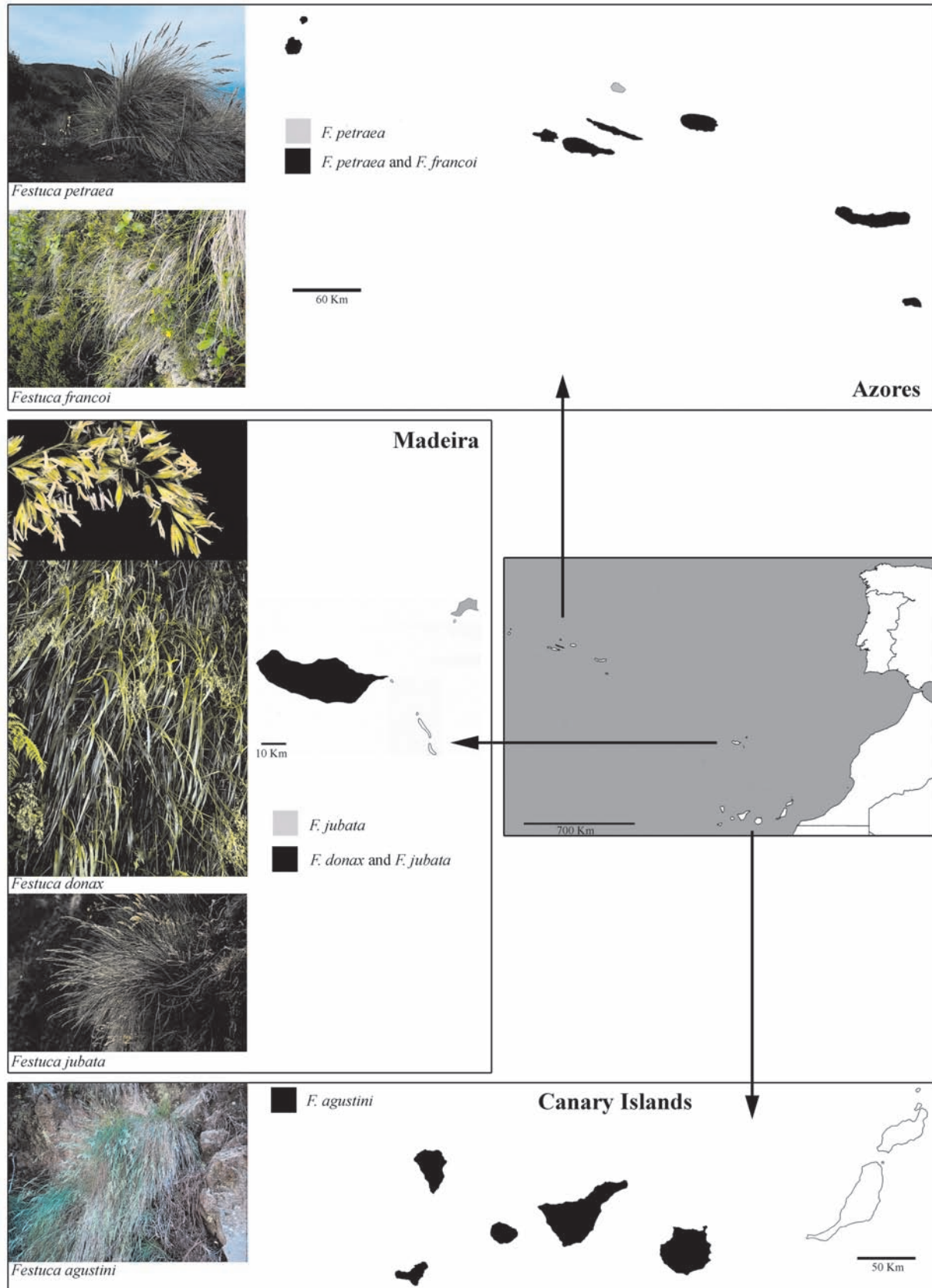


Fig. 1. Map of geographical distribution and illustrations of habits and ecological niches of the five native Macaronesian *Festuca*. Black and grey backgrounds indicate the distribution of the species in the islands of each archipelago. *F. agustinii*: western Canary islands; *F. donax*: Madeira island; *F. jubata*: Madeira and Porto Santo islands; *F. petraea*: Azores islands; *F. francoi*: all Azores islands except Graciosa.

cus L. Root tips (0.5-1.5 cm) were cut and kept in distilled water at 0-4 °C for 24-48 hours. Then they were fixed in Carnoy's solution (3:1 ethanol:acetic acid) overnight and transferred to a staining hydrochloric aceto-carmin solution (saturated) 48 hours before counting. Semi-permanent slides were prepared after a gentle squashing of the root tips in a drop of 45% acetic acid.

Chromosome observations were made using a Nikon Eclipse 90i binocular microscope and micrographs were taken using a digital camera (Applied Imaging, Progressive Scann), and treated using the program Cytovision 3.9 (Applied Imaging). Between 4 and 10 chromosome counts per plant were made, totalling 230 metaphase plates studied (50 of *F. agustini*, 40 of *F. jubata*, 50 of *F. francoi*, 70 of *F. petraea*, and 20 of *F. donax*). Chromosome sizes were estimated using the biometric application AxioVision Rel. 4.6 (Carl Zeiss Vision) on the captured images. The best 2-3 metaphase plates from each studied species were used to construct the respective karyograms. Chromosomes were classified according to their size and shape related to the centromere position following Stebbins (1938) and Levan & al. (1965), respectively. Karyotype asymmetry was calculated following the asymmetry index of Stebbins (1971) and the intrachromosomal asymmetry index A1 and interchromosomal asymmetry index A2 of Romero Zarco (1986). The best karyograms were used to construct the ideograms from the mean length of the long and short arms between homologous chromosomes.

Results

Our chromosome analysis indicates that all Macaronesian fine-leaved and broad-leaved native *Festuca* are diploid, showing $2n = 14$ chromosomes (Table 1; Fig. 2), except for one individual of *F. francoi* that showed $2n = 28$ chromosomes (Table 1). Chromosome numbers are reported for the first time for the

Canarian *F. agustini* and the Azorean *F. francoi*. Our extensive survey also confirms previous diploid counts reported for the Madeiran *F. jubata* (Dalgaard, 1986) and *F. donax* (Malik & Thomas, 1966) and for the Azorean *F. petraea* (Devesa & Gómez, 1988).

Chromosome size and shape vary between the fine-leaved and broad-leaved studied taxa (Table 1). Chromosomes of the fine-leaved group are overall medium-small (mean values < 5 µm) and those of the broad-leaved *F. donax* are medium-large (mean > 5 µm). Chromosomes of the fine-leaved *Festuca agustini*, *F. jubata*, *F. francoi* and *F. petraea* range between 1.95-3.37 µm with a mean size and standard deviation of 2.7 ± 0.47 µm in *F. agustini* (Fig. 2a), $2.16-3.65$ µm and 2.66 ± 0.46 µm in *F. jubata* (Fig. 2b), $2.65-4.67$ µm and 3.47 ± 0.61 µm in *F. petraea* (Fig. 2c), and $2.29-3.59$ µm and 2.83 ± 0.47 µm in *F. francoi* (Fig. 2d). By contrast, chromosomes of the broad-leaved *F. donax* range between 5.43-11.08 µm, with a mean size and standard deviation of 7.18 ± 1.09 µm (Fig. 2e). The ideograms constructed for these species are shown in Figs. 2a-e. The total haploid complement lengths are 18.91 µm in *F. agustini*, 18.60 µm in *F. jubata*, 24.31 µm in *F. petraea*, 19.85 in *F. francoi*, and 50.28 µm in *F. donax*.

Karyotypes also differ between the two *Festuca* groups. They tend to be more symmetrical in broad-leaved *F. donax* and more asymmetrical in the four fine-leaved taxa. *F. agustini* shows an ideogram formula of $10 m + 4 sm$, an asymmetry of type 2A and asymmetry indices A1 and A2 of 0.28 and 0.17, respectively (Fig. 2a). *F. jubata* has an ideogram formula of $10 m + 4 sm$, asymmetry of type 1A and asymmetry indices A1 and A2 of 0.30 and 0.17 (Fig. 2b). *F. petraea* shows an ideogram of $10 m + 4 sm$, asymmetry of type 2A and asymmetry indices A1 and A2 of 0.29 and 0.18 (Fig. 2c). *F. francoi* has an ideogram formula of $10 m + 4 sm$, asymmetry of type 2A and asymmetry indices A1 and A2 of 0.32 and 0.17 (Fig. 2d), and *F. donax* shows an ideogram formula of $4 M + 10 m$, asymmetry of

Table 1. Karyological data of Macaronesian *Festuca*. Asymmetry index of Stebbins (1975) and coefficients A1 and A2 of Romero Zarco (1986).

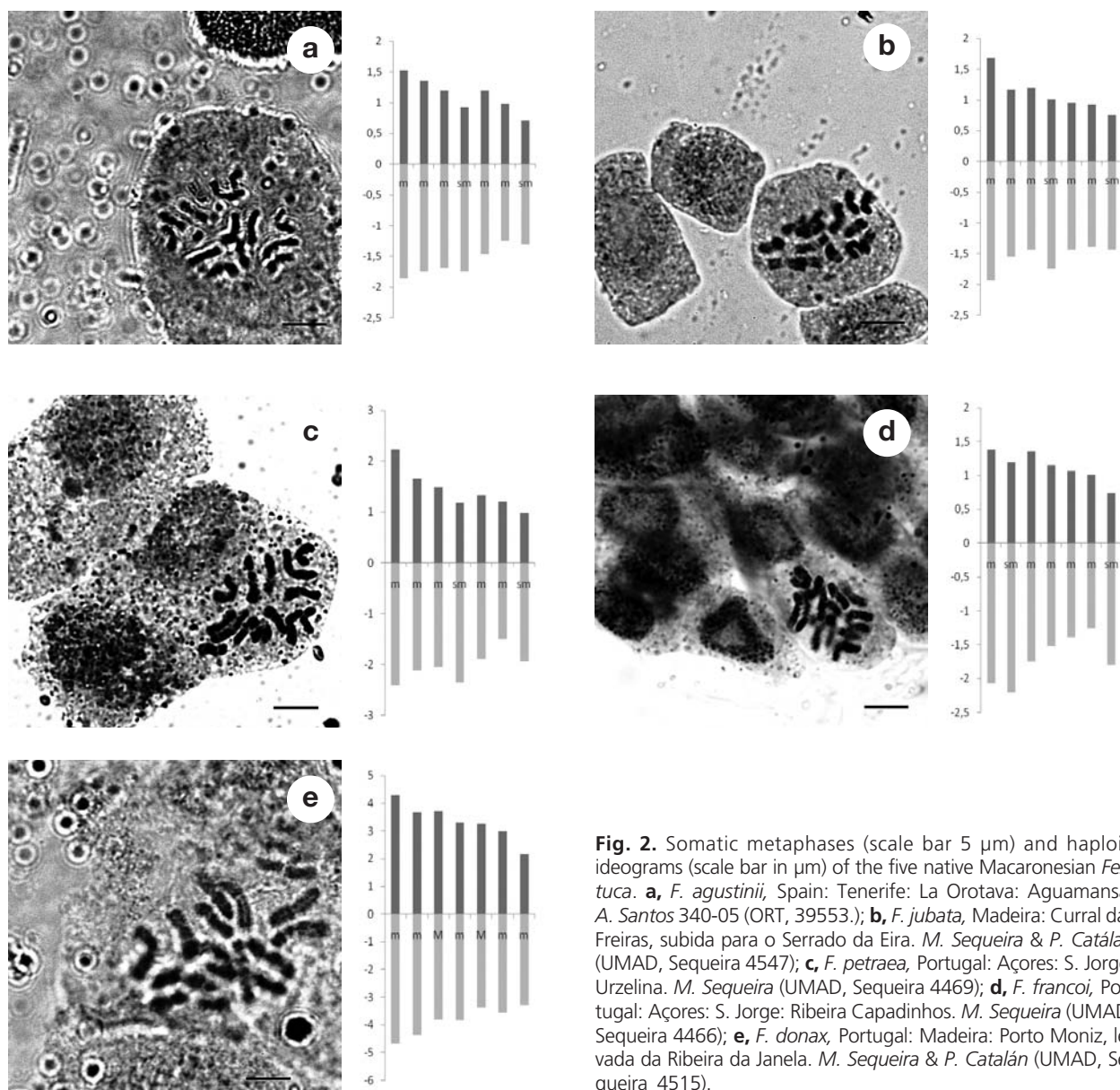
Taxon	No. of studied individuals	2n	Chromosome length (µm)	Idiogrammatic formula	Asymmetry index	Coefficient A1	Coefficient A2
Fine-leaved <i>Festuca</i>							
<i>Festuca agustini</i> Lindinger	5	14	1.95-3.37	10 m + 4 sm	2A	0.28	0.17
<i>Festuca jubata</i> Lowe	10	14	2.16-3.65	10 m + 4 sm	1A	0.30	0.17
<i>Festuca francoi</i> Fern. Prieto, C. Aguiar, E. Dias & M.I. Gut	8	14 (28)	2.65-4.67	10 m + 4 sm	2A	0.29	0.18
<i>Festuca petraea</i> Guthnick ex Seub.	8	14	2.29-3.59	10 m + 4 sm	2A	0.30	0.17
Broad-leaved <i>Festuca</i>							
<i>Festuca donax</i> Lowe	2	14	5.43-11.08	4 M + 10 m	1A	0.13	0.15

type 1A and asymmetry indices A1 and A2 of 0.13 and 0.15 (Fig. 2e). No differences have been found in A2 indices between fine and broad-leaved groups, indicating that similar interchromosomal asymmetry relative to the respective sizes of the chromosomes exists in both cases. Contrastingly, the intrachromosomal asymmetry, which is related to the branches' length and the Stebbins' type of asymmetry, clearly separates both groups, indicating that the broad-leaved *F. donax* has more metacentric chromosomes than the fine-leaved group.

Discussion

Our karyological analysis conducted in individuals

from different islands and populations of the five native Macaronesian *Festuca* indicate that these endemic taxa are mostly diploid. Diploid individuals have been found in all Madeiran *F. jubata* populations examined, including the low altitudinal population from Curral das Freiras, which showed a genetic AFLP profile distinct from that of the remaining populations from the highest peaks (Díaz-Pérez & al., 2008). Diploidy is a common phenomenon within the Macaronesian flora (Suda & al., 2003) and a genomic trait related to ancestry within *Festuca* (Dawe, 1989; Dubcovsky & Martínez, 1992). Evolutionary studies have demonstrated that the more ancestral broad-leaved Loliinae lineages are totally or predominantly diploids, whereas the more advanced fine-leaved lin-



eages tend to show some basal and subbasal diploid elements that are usually outnumbered by the more diverse and recently evolved polyploid taxa (Catalán, 2006; Inda & al., 2008). The diploidy observed in the endemic Macaronesian *Festuca* agree with their respective phylogenetic placements. However, whereas the robust *F. donax* is nested within a relict diploid clade of paraphyletic broad-leaved *F.* subg. *Drymanthele* sect. *Phaeocloa* taxa, the slender diploid *F. agustinii*, *F. jubata*, *F. francoi* and *F. petraea*, together with the single continental diploid relative *F. rivularis*, are placed in an unresolved basal position within the otherwise recently evolved clade of high-polyploid fine-leaved *F.* subg. *Festuca* sect. *Aulaxyper* taxa (Catalán, 2006; Inda & al., 2008).

The karyotypical differences observed between *F. donax* and the group of slender Macaronesian *Festuca* also suggest a distinct origin. The usually large sized chromosomes of *F. donax* ($7.18 \pm 1.09 \mu\text{m}$) correspond to the mean values observed within *F.* subg. *Drymanthele* sect. *Phaeocloa* ($8.66 \mu\text{m}$), whereas the smaller sized chromosomes of *F. agustinii*, *F. jubata*, *F. francoi* and *F. petraea* ($2.7 \pm 0.47 \mu\text{m}$, $2.66 \pm 0.46 \mu\text{m}$, $2.84 \pm 0.47 \mu\text{m}$ and $3.47 \pm 0.61 \mu\text{m}$, respectively) fall within the values reported for different sectional representatives of fine-leaved *Festuca* ($3.45\text{--}3.47 \mu\text{m}$ in *F.* sects. *Eskia*, *Festuca* and *Aulaxyper*; Dawe, 1989). The 2.5-fold range decrease in chromosome size from primitive broad-leaved *Festuca* subg. *Drymanthele* taxa to the more advanced fine-leaved *Festuca* subg. *Festuca* taxa reported by Dawe (1989) has been confirmed in the studied Macaronesian taxa.

Furthermore, the karyotype profile obtained for *F. donax*, with large metacentric chromosomes agrees well with that reported for the central European *F. drymeja* (Dawe, 1989). The close affinities between these two vicariant species of *F.* subg. *Drymanthele* sect. *Phaeochloa* were first suggested by successful interspecific artificial crosses (Borrill & al., 1977, 1980) and have been later reaffirmed by their sister relationships recovered from phylogenetic analyses (Catalán, 2006; Inda & al., 2008). On the other hand, the karyotypes of *F. agustinii*, *F. jubata*, *F. francoi* and *F. petraea*, which tend to have medium-small submetacentric chromosomes, repeated the pattern described for the more recently evolved *F. rubra* (Dawe, 1989). Karyotypes of ancestral broad-leaved *F.* subg. *Drymanthele* were characterized by large near-metacentric chromosomes with very little heterochromatin distributed in a few centromeric and subcentromeric bands, whereas those of most recently evolved fine-leaved *F.* sect. *Aulaxyper* were characterized by smaller submetacentric chromosomes with high contents

in heterochromatin primarily located in telomeric bands (Dawe, 1989; Catalán, 2006). Šmarda & al. (2008) have recently reported a similar 2.52-fold variation in genome size and GC content between the highest values of broad-leaved *F.* subg. *Drymanthele* (c. 5 pg; > 75%) and the smallest values of fine-leaved *F.* sects. *Festuca* and *Aulaxyper* (c. 2 pg; approx. 50%). These data, altogether with the close evolutionary relationship found between the slender Macaronesian fescues and the *F. rubra* group (Torrecilla & al., 2003; Catalán & al., 2006; Inda & al., 2008), definitively separates them from the intermediate to broad-leaved *F.* sect. *Amphigenes*, proposed by Saint-Yves (1922), and places them firmly within *F.* sect. *Aulaxyper* (Torrecilla & al., 2003; Catalán & al., 2007).

Differences in genome size and karyotype evolution in the native Macaronesian fescues are therefore concordant with hypotheses recovered from molecular data by Catalán (2006) and Inda & al. (2008). These authors indicate that these two groups of schizoendemics, i.e. relict endemic diploid taxa with similar karyotype profiles (cf. Favarger & Contandriopoulos, 1961; Contandriopoulos, 1988), originated independently and probably at different times. This has been further reassessed by divergence time calculations of those lineages estimated from Bayesian relaxed-clock phylogenies of Loliinae (Inda & al., 2008). These authors dated the divergence event that led to *F. donax* from its common ancestor with *F. drymeja* c. 3.6 ± 1.6 Mya, whereas the radiation of *F. agustinii*, *F. jubata*, *F. francoi* and *F. petraea* from the ancestor of the *F. rubra* group took part approximately 2.5 ± 0.9 Mya.

The older origin of *F. donax* would probably be the result of an earlier long distance dispersal event of broad-leaved *F.* subg. *Drymanthele* founders from Europe to Madeira (Inda & al., 2008). The nemoral ecology of *F. donax* seems to agree with an early colonization of Madeira, possibly with other paleoendemic plants such as the Lauraceae. In fact, *F. donax* is one of the few grasses occurring in the deep shaded laurel forest of this volcanic island (Capelo & al., 2004), which arose ca. 5.3 My ago (Gelmacher & al., 2000). By contrast, the recent origin of *F. agustinii*, *F. jubata*, *F. francoi* and *F. petraea* likely resulted from more recent colonizations of the Macaronesian archipelagos from near western Mediterranean diploid *F.* sect. *Aulaxyper* ancestors. In their molecular phylogeographical study, Díaz-Pérez & al. (2008) suggested the existence of three relatively recent long distance continental colonizations to the Canaries, Madeira and Azores archipelagos that resulted in *F. agustinii*, *F. ju-*

bata, and the sister taxa *F. francoi* and *F. petraea*, respectively. The ecological preferences for open high altitudinal habitats shown by *F. agustinii*, *F. jubata* and *F. francoi* (Saint-Yves, 1922; Díaz-Pérez & al., 2008) correlate well with a more recent establishment of these grasses in the high mountain cliffs of these archipelagos, whereas the recent derived origin of *F. petraea* from *F. francoi* (1.1 ± 0.6 Mya; cf. Inda & al., 2008) has been interpreted as the results of ecological in-situ adaptation to seashore ecosystems (Díaz-Pérez & al., 2008).

Despite the predominant diploid state found in all native Macaronesian *Festuca*, one tetraploid count of $2n = 28$ chromosomes was observed in one individual of the Azorean Pico island population of *F. francoi*. The existence of polyploid endemic Macaronesian grasses is a rather common phenomenon, as exemplarized by the cases found in the genera *Dactylis* (e.g. tetraploid Madeiran *D. smithii* Link subsp. *hylopedes* Parker (Parker, 1972), *Holcus* (e.g. tetraploid Azorean *H. rigidus* Hochst. (Menezes de Sequeira & Castroviejo, 2007) and Canarian *Holcus mollis* L. subsp. *hierrensis* Stierst. (Stierstorfer, 2001) and pentaploid *Holcus azoricus* M. Seq. & Castrov. (Menezes de Sequeira & Castroviejo, 2007), and *Koeleria* (e.g. the high 25-ploid Madeiran *K. loweana* Quintanar, Catalán & Castrov. [syn. *Parafestuca albida* (Lowe) E.B. Alexeev] (Quintanar & al., 2006). These polyploid Macaronesian endemics would correspond to neo-endemics (or apo-endemics) in the sense of Favarger & Contandriopoulos (1961) and Contandriopoulos (1988), and probably derived very recently from polyploid mainland colonizers. Nonetheless, the single case of tetraploidy detected in the otherwise largely diploid *Festuca francoi* and the long geographical distances that separate the Azores archipelago from any other land mass precludes any hypothesis of secondary colonization. It rather suggests an in-situ origin of autotetraploid races from older and previously established diploid races in the Pico island. This potential euploidy should be confirmed, however, through a more extensive cytological survey of populations of this Azorean fescue.

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Appendix 1. Chromosome numbers found in the studied specimens of native Macaronesian *Festuca*.

Voucher	Chromosome number	Location
Fine-leaved <i>Festuca</i>		
<i>Festuca agustinii</i> Lindinger		
ORT 39555	2n = 14	Spain: Tenerife: Anaga: Bailadero 1. (Taludes terrosos junto a bordes de montes de laurisilva, localmente frecuente). c. 700 masl., Santos
ORT 36825	2n = 14	Spain: Tenerife: Anaga: Bailadero 2. (Taludes basálticos en zonas de laurisilva muy húmedas). c. 700 masl. Santos
ORT 40198	2n = 14	Spain: Tenerife: El Rosario: Las Lagunetas. Comunidades rupícolas en pinares secos con <i>Aeonium spathulatum</i> . c.800 masl, Santos
ORT 39553	2n = 14	Spain: Tenerife: La Orotava: Aguamansa. (Taludes de piroclastos en pinares secos de <i>Pinus canariensis</i> con <i>Aeonium aureum</i>). c. 1,200 masl., Santos
ORT 39554	2n = 14	Spain: Tenerife: Buenavista (Teno): Bujamé. Taludes terrosos junto a montes aprovechados de fayal-brezal con <i>Erica arborea</i> , <i>Myrica faya</i> e <i>Ilex canariensis</i> . c. 500 masl., Santos
<i>Festuca jubata</i> Lowe		
UMAD s.n.	2n = 14	Portugal: Madeira: Bica da Cana: vereda para o Caramujo. Comunidade casmo-comófito. 1,456 masl. Declive 90°, Exp. ESE, Sequeira & Catálan, Sequeira 4541
UMAD s.n.	2n = 14	Portugal: Madeira: Estrada para o Pico do Arieiro, circa Poça da Neve, nos taludes rochosos na orla de <i>Polystichum falcinelli</i> - <i>Ericetum arboreae</i> degradado com <i>Erica scoparia</i> , <i>Rosa mandoni</i> , <i>Sorbus madeirensis</i> , etc., 1,535 masl. Declive 90°, Exp. N, Sequeira & Catálan, Sequeira 4520
UMAD s.n.	2n = 14	Portugal: Madeira: vereda Pico Ruivo- Encumeada, Pico Eirinhas, comunidade casmo-comófito na orla de uma urzal da <i>Polystichum falcinelli</i> - <i>Ericetum arboreae</i> , com <i>Tolpis macrorhiza</i> , <i>Orchis scopulorum</i> e <i>Koeleria loweana</i> , 1,625 masl. Declive 90°, Exp. NNW 339°, Sequeira & Catálan, Sequeira 4531
UMAD s.n.	2n = 14	Portugal: Madeira: Curral das Freiras, subida para o Serrado da Eira. Nos taludes rochosos, comunidade casmo-comófito com <i>Deschampsia argentea</i> , em pequenas acumulações terrícolas. na orla de uma formação com <i>Erica scoparia</i> e <i>Genista tenera</i> , 880 masl. Declive 90°, Exp. NNW 341°, Sequeira & Catálan, Sequeira 4547
<i>Festuca francoi</i> Fern. Prieto, C. Aguiar, E. Dias & M.I. Gut		
UMAD s.n.	2n = 14	Portugal: Açores: S. Miguel: prope Lagoa do Fogo. Num talude com <i>Deschampsia foliosa</i> ., 639 masl. Declive 80°, Exp. SSE 155°, Sequeira 4496
UMAD s.n.	2n = 14	Portugal: Açores: S. Jorge: Ribeira Capadinhos. Num talude, 690 masl. Declive 90°, Exp. SSE 153°, Sequeira 4466
UMAD s.n.	2n = 28	Portugal: Açores: Pico: ca. Lagoa do Caiado. Nas comunidades herbáceas em mosaico com o urzal, 830 masl., Declive 0°, Sequeira 4442
UMAD s.n.	2n = 14	Portugal: Açores: Flores: Lagoa Negra. Comunidades de <i>Calluna vulgaris</i> , 586 masl. Declive 45°, Exp. W 265°, Sequeira 4475
UMAD s.n.	2n = 14	Portugal: Açores: S. Miguel: ca. Pico do Carvão. Num talude. 670 masl. Declive 80°, Exp. N, Sequeira 4499
<i>Festuca petraea</i> Guthnick ex Seub.		
UMAD s.n.	2n = 14	Portugal: Açores: St. Maria: Maia. Nos taludes expostos à salsugem., 15 masl. Declive 30°, Exp. N, Sequeira 4393
UMAD s.n.	2n = 14	Portugal: Açores: Graciosa: Caldeirinha. Cratera de um pequeno vulcão, com restos de vegetação natural, início de infestação com <i>Hedychium gardneranum</i> , 355 masl. Declive 45°, Exp. NNW, Sequeira 4427
UMAD s.n.	2n = 14	Portugal: Açores: Graciosa: Porto Afonso. Vegetação sob efeito da salsugem, dominada por <i>Critmum maritimum</i> e <i>Festuca petraea</i> , 10 masl. Declive 0°, Exp. WNW 258°, Sequeira 4412
UMAD s.n.	2n = 14	Portugal: Açores: S. Jorge: Urzelina. Ravinas junto ao mar., 20 masl. Declive 10°, Exp. NW 304°, Sequeira 4469
UMAD s.n.	2n = 14	Portugal: Açores: Pico: cais do Pico. Talude com exposição W, como comófito. 50 masl. Declive 90°, Exp. W 278°, Sequeira 4463
UMAD s.n.	2n = 14	Portugal: Açores: Faial: ca. Capelinhos. Sobre detritos vulcânicos arenosos., 215 masl. Declive 0°, Exp. NNW, Sequeira 4429
UMAD s.n.	2n = 14	Portugal: Açores: Flores: St. Cruz. Ravina exposta ao mar e ao efeito do spray marinho. 20 masl. Declive 45°, Exp. ESE, Sequeira 4482
Broad-leaved <i>Festuca</i>		
<i>Festuca donax</i> Lowe		
UMAD s.n.	2n = 14	Portugal: Madeira: Porto Moniz: levada da Ribeira da Janela. Orlas de <i>Clethro arboreae</i> - <i>Ocoteetum foetentis</i> . 167 masl., Sequeira & Catalán, Sequeira 4515
UMAD s.n.	2n = 14	Portugal: Madeira: Seixal: Vêu da Noiva. Bosque de <i>Semele androgyna</i> - <i>Appolonietum barbujaanae</i> . 220 masl. Exp. N, Sequeira 4321

