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3 1 **Mosaic distribution of cytotypes in a mixed-ploidy plant species: nested**
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5 2 **environmental niches but low geographic overlap**
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27 12 **Short Running Title:** *Cytogeography in *Jasione montana**
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19 **ABSTRACT**

20 Polyploids often have divergent geographic ranges compared to their diploid progenitors, but
21 the causes of such differentiation are poorly understood. The geographic ranges of cytotypes
22 within polyploid complexes may be caused by multiple factors, including historical events,
23 interactions among cytotypes, and divergent environmental tolerances, but the fine-scale
24 geographical arrangement of cytotypes is rarely known for most mixed-ploidy species. In this
25 study, we assessed cytotype diversity and distribution patterns in the *Jasione montana* polyploid
26 complex and examined whether environmental factors can explain the occurrence of
27 tetraploids. Specifically, we reviewed all chromosome counts available in the literature,
28 examined cytotype distributions in a large-scale population survey using flow cytometry (N =
29 278 populations, N = 3396 plants), and used niche modelling to compare cytotype
30 environmental associations. Two cytotypes were detected: diploids, which are widespread
31 across Europe, and tetraploids, restricted to the northwest quadrant of the Iberian Peninsula.
32 The two cytotypes were distributed in a mosaic with areas dominated by diploids intermixed
33 with those dominated by tetraploids, rarely forming mixed-ploidy populations (1.4%). Although
34 having low geographical overlap, the tetraploid niche is fully nested within the diploid niche
35 breadth and occupies only a subset of the environmental envelope of the diploid progenitor,
36 suggesting that polyploidization has not caused niche expansion due to novel environmental
37 preferences. The mosaic diploid-tetraploid contact zones and the lack of mixed-ploidy
38 populations suggest that frequency-dependent selection may play a role in excluding minority
39 cytotypes. Under this scenario, tetraploids would have to disperse to places unoccupied by
40 diploids to successfully establish. The aggregation of tetraploid populations in areas suitable for
41 diploids suggests that tetraploids may also outcompete diploids in certain areas. Collectively,
42 our results indicate that environmental sorting has played a role, at least on a broader scale, in
43 the successful establishment of polyploids in *J. montana*.

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45 **ADDITIONAL KEYWORDS:** diploids, flow cytometry, *Jasione montana*, minority cytotype
46 exclusion, niche modelling, polyploidy, tetraploids.

PDF Proof

47 INTRODUCTION

48 The duplication of whole chromosome sets is a common event in nature (Wood *et al.*, 2009;
49 Husband, Baldwin & Suda, 2013; Marques *et al.*, 2017), giving rise to new polyploids that might
50 establish and spread within diploid/lower-ploidy progenitor **populations**. The recurrent
51 formation of polyploids has been documented multiples times throughout the evolutionary
52 history of particular plant groups (Wood *et al.*, 2009; Otto & Whitton, 2000; Soltis *et al.*, 2010),
53 but also in extant plant populations (e.g., Maceira *et al.*, 1992; Burton & Husband, 2001; Ramsey,
54 2007; Castro *et al.*, 2018). This is likely due to the frequent production of unreduced gametes in
55 nature (Bretagnolle & Thompson, 1995; Ramsey, 2007; Brownfield & Kohler, 2010), although
56 successful polyploid establishment depends on numerous factors (Levin, 1975; Rodriguez,
57 1996). Because polyploids arise frequently and can have significant ecological and evolutionary
58 effects, polyploidy is currently recognized as a major mechanism of sympatric speciation (Otto
59 & Whitton, 2000; Soltis *et al.*, 2010), being considered an important **mechanism of**
60 **diversification** of flowering plants (Soltis & Soltis, 1999). Given its significant contribution, the
61 factors involved in the successful establishment of polyploid lineages have received increased
62 attention in the last decades.

63 Immediately after polyploid formation, the new cytotype is at a numerical disadvantage
64 within the population of its diploid/lower-ploidy progenitor. Theoretical models suggest that the
65 new polyploid can establish within the population of the progenitor only if it has the necessary
66 conditions to increase its numbers; otherwise, it will be excluded from the population due to
67 frequency-dependent selection (Levin, 1975; Rodriguez, 1996; Husband & Schemske, 2000).
68 Polyploid establishment will be favoured by features that increase the probability of successful
69 mating, such as recurring formation of polyploids, spatial clustering, perenniality, increased
70 selfing, and/or increased competitive ability (Fowler & Levin, 1984; Felber, 1991; Rodriguez,
71 1996; Husband & Schemske, 2000; Barringer, 2007). Assortative mating, enforced by various
72 reproductive barriers, may also promote the coexistence of polyploids and their progenitors

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3 73 [e.g., *Chamerion angustifolium* (L.) Holub, Husband & Sabara 2004; *Aster amellus* L., Jersáková
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5 74 *et al.*, 2010; Castro *et al.*, 2011; *Gladiolus communis* L., Castro *et al.*, 2018]. Alternatively,
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7 75 polyploids might disperse elsewhere, thereby escaping minority cytotype exclusion, and
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9 76 establish new populations beyond the environmental limits of the parental individuals (niche
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11 77 shift hypothesis; Levin, 1975, 2004; Husband & Schemske, 2000).

14 78 Different geographical patterns have been documented in diploid-polyploid complexes
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16 79 (namely sympatric, parapatric or allopatric cytotype distributions; e.g., Petit, Bretagnolle &
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18 80 Felber, 1999; Baack, 2004; Kolář *et al.*, 2009; Castro *et al.*, 2012). An association between ploidy
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20 81 level and differences in environmental and spatial boundaries is frequently observed (Levin,
21
22 82 2002), with polyploids often having different geographic ranges from their diploid progenitors
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24 83 (e.g., Balao *et al.*, 2009; Kolář *et al.*, 2009; Sonnleitner *et al.*, 2010; Laport *et al.*, 2013; Casazza
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26 84 *et al.*, 2017). The geographical range occupied by each cytotype is the result of several
27
28 85 potentially interacting forces, including historical patterns of origin or migration, interactions
29
30 86 among cytotypes, and divergence in environmental tolerances (Husband *et al.*, 2013). Historical
31
32 87 processes, including the timing of polyploid emergence, dispersal and colonization patterns, are
33
34 88 among the factors determining geographical patterns (Lowry & Lester, 2006). Recurrent
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36 89 polyploid formation generates primary contact zones and mosaic distributions, while secondary
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38 90 contact zones originate after allopatric divergence and subsequent migration, bringing
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40 91 cytotypes into contact and creating allopatric and parapatric distributions (Petit, Bretagnolle &
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42 92 Felber, 1999). Additionally, changes in cell size and gene expression associated with whole
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44 93 genome duplication may have broad-scale impacts on developmental processes and
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46 94 environmental tolerances that might change the fitness of polyploids (Levin, 1983; Adams &
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48 95 Wendel, 2005). These differences have been linked with increased ecological tolerances, niche
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50 96 partitioning and/or wider geographic ranges (e.g., Levin, 1975; Husband & Schemske, 2000;
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52 97 Buggs & Pannell, 2007; Ramsey, 2011; Hao *et al.*, 2013) and may determine the likelihood of
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54 98 polyploid establishment and subsequent spread. Consequently, knowing the geographic
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3 99 arrangement of diploid-polyploid complexes in nature provides essential information for
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5 100 inferring the processes involved in polyploid establishment, coexistence and divergence (e.g.,
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7 101 Levin, 2002; Petit *et al.*, 1999; Lexer & van Loo, 2006; Castro *et al.*, 2018).

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10 102 Recently, ecological niche modelling (Warren, Glor & Turelli, 2008) and multivariate
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12 103 analyses of niche variables (Broennimann *et al.*, 2012) using cytotype occurrence data and
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14 104 various abiotic factors (e.g., precipitation, temperature, soil characteristics and elevation) have
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16 105 been used to characterize ecological niches and to compare environmental niches between
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18 106 different taxa. This approach has been used to evaluate the niche shift hypothesis in related
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20 107 diploid-polyploid species (e.g., *Houstonia* spp., Glennon, Rissler & Church, 2012; *Leucanthemum*
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22 108 Iberian *taxa*, Oberprieler *et al.*, 2012; *Claytonia perfoliata* Donn ex Willd. complex, McIntyre,
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24 109 2012; *Primula* sect. *Aleuritia* complex, Theodoridis *et al.*, 2013; *Tolmeia* spp., Visger *et al.*, 2016),
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26 110 or in the analysis of different cytotypes within a species (*Houstonia purpurea* L. and *H. longifolia*
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28 111 Gaertn., Glennon *et al.*, 2012; *Heuchera cylindrica* Douglas, Godsoe *et al.*, 2013; *Chamerion*
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30 112 *angustifolium*, Thompson, Husband & Maherali, 2014; *Erysimum mediohispanicum* Polatschek,
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32 113 Muñoz-Pajares *et al.*, 2018). The characterization of the ecological niches enables researchers,
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34 114 not only to identify the potential environmental constraints on the distribution of different
35
36 115 cytotypes, but also to unmask other factors potentially affecting cytogeographic patterns, such
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38 116 as inter-cytotype biotic interactions and historical processes (e.g., Godsoe *et al.*, 2013; Laport *et*
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40 117 *al.*, 2013; Glennon, Ritchie & Segraves, 2014; Laport, Minckley & Ramsey, 2016; Castro, 2018;
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42 118 Mairal *et al.*, in press). Thus, modelling tools have enabled researchers to formulate hypotheses
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44 119 about the successful establishment of certain polyploid complexes, and subsequently, to test
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46 120 these hypotheses experimentally in the field or in controlled conditions, through reciprocal
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48 121 transplants or competition experiments (e.g., *Ranunculus adoneus* A. Gray, Baack & Stanton,
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50 122 2005; *Chamerion angustifolium*, Martin & Husband 2013; *Jasione maritima* (Duby) Merino,
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52 123 Castro, 2018).

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3 124 *Jasione* L. (Campanulaceae) is a small genus distributed in Europe, North Africa and
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5 125 Southwest Asia, with its centre of diversity in the Iberian Peninsula and most of its species
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7 126 showing restricted ranges (Tutin, 1973; Sales & Hedge, 2001a, 2001b; Pérez-Espona *et al.*, 2005).
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10 127 Phylogenetic analyses using ITS suggest a recent origin of the species within the genus, possibly
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12 128 linked with the Pleistocene glaciations (Sales *et al.*, 2004; Pérez-Espona *et al.*, 2005), although
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14 129 the evolutionary history of the genus is still unknown. Polyploidy is often expected to be
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16 130 frequent during glaciation periods (Thompson, 2005; Marques *et al.*, 2017), because of the
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18 131 association between the production of unreduced gametes and temperature fluctuations
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20 132 (Ramsey & Schemske, 1998; Mason *et al.*, 2011). *Jasione* comprises several diploid taxa (e.g., *J.*
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22 133 *foliosa* Cav., *J. corymbosa* Poir.; Silvestre, 1986; Parnell, 1987), but it is also rich in polyploid
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24 134 complexes, including tetraploid species (e.g., *J. sessiliflora* Boiss. & Reut.; Favarger, Galland &
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26 135 Kupfer, 1980) and species with several ploidy levels (e.g., *J. montana*, *J. laevis* Lam., *J. maritima*
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28 136 (Duby) Merino, *J. crispa* (Pourr.) Samp.; Sales & Hedge, 2001a; Rubido-Bará, Horjales Luaces &
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30 137 Villaverde, 2010). Among the latter is the widespread *J. montana* L., which has been formerly
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32 138 described as diploid throughout its distribution range across Europe (e.g., Kovanda, 1968;
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34 139 Bjorkqvist, 1969; Kliphuis & Wieffering 1972; Ubera, 1980), until Leitão & Paiva (1988) reported,
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36 140 for the first-time, tetraploid plants in Central Portugal, and, more recently, Rubido-Bará *et al.*,
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38 141 (2010) described the occurrence of tetraploids in Galicia (Spain). The species exhibits high
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40 142 morphological variability, with diversity of habits, growth form and organ's size (Parnell, 1985,
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42 143 1987; Bokhari & Sales, 2001; Sales *et al.*, 2004). Although some minor morphological differences
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44 144 in plant size, in root thickness and in leaf size have been reported between diploids and
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46 145 tetraploids from Galicia (Rubido-Bará *et al.*, 2010), the morphological traits reveal a high
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48 146 variability and overlap between the two entities, hindering the identification of the cytotypes in
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50 147 the field. This morphological similarity suggests an autopolyploid origin of the tetraploid *J.*
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52 148 *montana*, although allopolyploidization cannot be excluded. While the cytogenetic diversity has
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3 149 been characterized within some populations in Galicia (Spain), the geographical distribution of
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5 150 the tetraploids and the environmental niche preferences of each cytotype are still unknown.
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8 151 The main objective of this study was to explore the diversity and distribution of
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10 152 cytotypes within the *Jasione montana* polyploid complex and identify possible factors involved
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12 153 in the successful establishment and spread of tetraploids. In particular, the goals of the present
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14 154 study were to: (1) delineate the geographical distribution of tetraploids in the Iberian Peninsula;
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16 155 (2) identify minority cytotypes, mixed-ploidy populations and contact zones between cytotypes;
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18 156 and (3) determine whether the cytotypes have different ecological associations that could
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20 157 explain their observed geographical distributions. To accomplish this, we sampled populations
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22 158 throughout the Iberian Peninsula, in particular at detected contact zones, to determine DNA
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24 159 ploidy levels using flow cytometry and assess the distribution patterns of each cytotype. Niche
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26 160 modelling tools were then used to explore the ecological associations of each cytotype in the
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28 161 Iberian Peninsula. We hypothesize that polyploidization results in shifts in environmental
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30 162 preference, which allows tetraploids to establish by colonizing different environmental niches
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32 163 than diploids, thus exhibiting low geographic overlap. The information about cytotype diversity,
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34 164 geographical patterns and environmental associations enabled us to explore the factors
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36 165 involved in the establishment and spread of *J. montana* tetraploid individuals in nature.
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44 167 MATERIALS AND METHODS

45 168 STUDY SYSTEM

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48 169 *Jasione montana* L. (Campanulaceae) is distributed throughout Europe, from the Mediterranean
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50 170 to approximately 62°N, and from western Asia to North Africa (Tutin, 1973). It grows on rocky
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52 171 ground, heaths and grasslands with thin soil, and prefers acid soils rather than limestone regions
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54 172 (Horwood, 1919). Individuals vary morphologically and may be annual, biennial or perennial;
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56 173 still, no clear association between ploidy level and plant habit has been found. *Jasione montana*
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58 174 plants form a rosette of leaves during the winter and produce erect or ascending stems in the
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3 175 spring, each ending in a capituliform inflorescence of bluish flowers (Parnell, 1980; Sales &
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5 176 Hedge, 2001a). The species comprises diploids ($2n = 2x = 12$ chromosomes) through most of its
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7 177 distribution (e.g., Kovanda, 1968; Ubera, 1980; Luque & Mejias, 1986; Pastor, 1990; Rubido-Bará
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9 178 *et al.*, 2010), while tetraploids ($2n = 4x = 24$ chromosomes) have been reported in Coimbra
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11 179 (Centre of Portugal; Leitão & Paiva, 1988) and in Galicia (Spain; Rubido-Bará *et al.*, 2010). The
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13 180 genome size of the two cytotypes has been estimated to be $2C = 3.24$ pg for diploids, which is
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15 181 roughly half that of tetraploids, $2C = 6.58$ pg (Rubido-Bará *et al.*, 2010).
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21 183 CHROMOSOME COUNTS FROM THE LITERATURE

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23 184 We reviewed the literature on the karyology of *J. montana* (available in Chromosome Counts
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25 185 database, <http://ccdb.tau.ac.il/>, Rice *et al.*, 2015; Floras and published manuscripts), compiled
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27 186 all chromosome counts with reference to the locality of sampling and mapped the distribution
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29 187 of cytotypes. A total of 40 references including 89 identified localities with karyological
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31 188 information were compiled (Table S1) and mapped.
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37 190 FIELD SAMPLING

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39 191 Our sampling was mostly focused on the Iberian Peninsula, particularly the northwest quadrant,
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41 192 where tetraploids have been previously reported (Leitão & Paiva, 1988; Rubido-Bará *et al.*,
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43 193 2010). We also sampled other Mediterranean areas to confirm the diploid dominance reported
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45 194 in the literature. In total, 287 populations were sampled, including 279 in the Iberian Peninsula
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47 195 and eight elsewhere (France, Ireland, Italy and Morocco; Table S2). During spring and summer
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49 196 of 2013-2016, fresh leaves were collected from each population, placed in hermetic plastic bags
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51 197 and stored at 4 °C for flow cytometric analyses. In each population, up to 38 individuals (mean
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53 198 = 12 individuals/population) were randomly sampled, throughout the entire population (Table
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55 199 S2). When the harvesting of fresh material was impractical, mature seeds were collected
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57 200 (procedure followed in 14 populations). Geographic coordinates of each population were
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3 201 recorded, and all populations were mapped in Quantum-GIS version 2.18.3 (Table S2).
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5 202 Herbarium specimens were collected for species confirmation in populations with high
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7 203 morphological variability and vouchers were deposited in SANT herbarium (Table S2).
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12 205 FLOW CYTOMETRIC ANALYSES
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14 206 Fresh leaves were analysed using flow cytometry to estimate genome size and DNA ploidy
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16 207 (hereafter referred as ploidy) of each individual sampled. In brief, 50 mg of sample material and
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18 208 reference standard (*Solanum lycopersicum* 'Stupické', $2C = 1.96$ pg; Doležel, Sgorbati & Lucretti,
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20 209 1992), were co-chopped in 1 ml of Woody Plant Buffer (Loureiro *et al.*, 2007) to obtain a nuclear
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22 210 suspension (Galbraith *et al.*, 1983). Nuclear suspensions were filtered through a 50 μm nylon
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24 211 filter, and 50 $\mu\text{g ml}^{-1}$ of propidium iodide and 50 $\mu\text{g ml}^{-1}$ of RNase (Fluka, Buchs, Switzerland)
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26 212 were added, incubated for 5 min and analysed using a Partec CyFlow Space flow cytometer (532
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28 213 nm green solid-state laser, operating at 30 mW; Partec GmbH., Görlitz, Germany). For each
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30 214 sample, at least 1300 nuclei in both the G_1 peaks of the sample and standard were analysed
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32 215 (Suda *et al.*, 2007). Partec FloMax software v2.4d (Partec GmbH, Münster, Germany) was used
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34 216 to acquire the results as described in Castro *et al.* (2018). Samples were considered acceptable
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36 217 when the coefficient of variation (CV) of the 2C peak of *J. montana* was below 5%; otherwise, a
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38 218 new sample was prepared and analysed until sufficient quality was achieved (Greilhuber,
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40 219 Temsch & Loureiro, 2007).
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46 220 Given the large number of individuals collected in each population, 1-9 randomly
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48 221 selected individuals (mean = 2.4 individuals/population) were analysed individually to estimate
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50 222 their genome size, while the remaining were analysed for ploidy only by pooling 2-6 samples
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52 223 (plus the reference standard). For 14 populations, we analysed ploidy level directly from seeds
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54 224 following the protocol above and the pooled sample method (adapted from Castro *et al.*, 2018).
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57 225 The holoploid genome size ($2C$; *sensu* Greilhuber *et al.*, 2005) of each individual sample
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59 226 was calculated using the following formula: Holoploid genome size (pg) =
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3 227 $\frac{J. montana \text{ G1 peak mean}}{S. lycopersicum \text{ G1 peak mean}} \times S. lycopersicum$ genome size. The monoploid genome size (1Cx; *sensu*
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6 228 Greilhuber *et al.*, 2005) of each individual sample was also calculated by dividing the holoploid
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8 229 genome size (2C) by the ploidy level of each cytotype. Samples were classified as diploid or
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10 230 tetraploid according with Rubido-Bará *et al.* (2010) and with our estimates of genome size and
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12 231 their range of variation: **diploids** range between 2.80 and 3.08 pg/2C, and tetraploids range
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14 232 between 5.63 and 6.06 pg/2C. **The fact that genome size ranges of the two cytotypes are not**
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16 233 **overlapped enabled a confident identification of the ploidy level.** Subsequently, populations
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18 234 were classified according to the ploidy level composition of its individuals, as single-ploidy or
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20 235 mixed-ploidy.
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24 236 Descriptive statistics of holoploid and monoploid genome sizes were calculated for each
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26 237 cytotype based on the individual flow cytometric estimates. Diploid and tetraploid genome sizes
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28 238 (holoploid and monoploid) were compared using GLMs, with Gaussian distribution and identity
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30 239 link function, and with cytotype as a fixed factor and genome size as the response variable. The
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32 240 analyses were performed in R software version 3.0.1 (R Core Development Team 2016), using
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34 241 the packages “car” for Type-III analysis of variance (Fox & Weisberg, 2015), “lme4” for
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36 242 generalized linear models (Bates *et al.*, 2014) and “multcomp” for multiple comparisons after
37
38 243 Type-III analysis of variance (Hothorn *et al.*, 2017).
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45 245 ENVIRONMENTAL NICHE MODELLING

46 246 Abiotic environmental associations with each cytotype were evaluated for the Iberian Peninsula
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48 247 populations. **Nineteen** bioclimatic variables (Bio1-Bio19) plus altitude, latitude and longitude at
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50 248 a 1 km resolution were extracted from the WorldClim database (<http://www.worldclim.org/>).
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52 249 To improve the quality of the niche environmental predictions, six soil-related variables were
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54 250 also obtained at the same resolution: base saturation of the topsoil (bs_top), topsoil cation
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56 251 exchange capacity (cec_top), topsoil organic carbon content (oc_top), slope, dominant surface
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58 252 textural class of the soil topological unit (txsrfd0), and first soil adjective code of the soil
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3 253 topological unit (wrbadj1) (Panagos *et al.*, 2012; European Soil Data Centre). Values for each
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5 254 variable were extracted for all *J. montana* records using the “dismo” package in R (Hijmans *et*
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7 255 *al.*, 2017). Correlations between all variables were assessed. The selection of the variables was
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9 256 based on the variance explained in an exploratory principal component analysis (PCA; data not
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11 257 shown), excluding highly correlated variables (Spearman’s rho correlation > 0.7). In the end, four
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13 258 bioclimatic variables (Bio4 – temperature seasonality, Bio5 – maximum temperature of the
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15 259 warmest month, Bio14 – precipitation of the driest month and Bio15 – precipitation seasonality)
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17 260 and two soil parameters (bs_top – base saturation of the topsoil and txsrfdo - dominant surface
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19 261 textural class of the soil topological unit) were selected (Table S3). Differences between diploid
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21 262 and tetraploid populations with respect to these variables were tested using GLM, with cytotype
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23 263 as fixed factor and each variable as a response variable.
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28 264 The *J. montana* population records (presence/absence of each ploidy) used in this
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30 265 analysis were from multiple sources: 1) our intensive field sampling in the northwest quadrant
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32 266 of the Iberian Peninsula (39.6° to 43.7° in latitude, -6.1° to -9.2° in longitude) and ploidy
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34 267 occurrences (from chromosome counts and flow cytometric analyses) from Rubido-Bará *et al.*
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36 268 (2010) in Galicia; 2) occurrences downloaded from GBIF database (<http://gbif.org>) beyond the
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38 269 area of intensive sampling in the northwest quadrant of the Iberian Peninsula; and 3) randomly
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40 270 selected points from the Iberian Peninsula (background records). Population reports from our
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42 271 sampling and from Rubido-Bará *et al.* (2010) were classified as diploid and tetraploid according
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44 272 with the ploidy level or chromosome numbers available, while GBIF points were classified as
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46 273 diploid according to the literature and our own observations (Tables S1 and S2). Records lying
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48 274 within a distance of 10 km from a given record were excluded from the dataset to avoid
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50 275 oversampling, resulting in a dataset of 462 diploid and 53 tetraploid records. To obtain the
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52 276 background points, we applied a buffer of 20 km to all the points from the presence dataset,
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54 277 and randomly obtained 5000 points beyond this buffer; additionally, a filter of 20 km was used
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56 278 to remove background points that were separated by less than this distance, again to avoid
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3 279 oversampling. This resulted in a final dataset with a total of 739 records (462 diploid, 53
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5 280 tetraploid and 224 background points). For the diploid dataset, diploid populations were
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7 281 recorded as presences and tetraploid populations as absences, and vice-versa for the tetraploid
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9 282 dataset. Background points were used as absences in both analyses.

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12 283 Niche modelling was performed using [maximum entropy \(MaxEnt\)](#) and the R software
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14 284 package “biomod2” (Thuiller *et al.*, 2016). Spatially predictive models were calibrated using the
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16 285 six selected variables and the presence/absence datasets. To reduce uncertainty and produce
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18 286 robust models, the models were replicated 30 times after splitting the data into randomly
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20 287 selected training (70%) and testing (30%) subsets (Phillips, Anderson & Schapire, 2006; Araújo &
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22 288 New, 2007). To guarantee statistical independence of all replicates, each occurrence was used
23
24 289 only once in each run, either as training or as test occurrence (Phillips, 2008). Models were
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26 290 evaluated based on the independent accuracy measure (AUC) of the curve of the receiver
27
28 291 operating characteristic. The models for diploid and tetraploid cytotypes were obtained based
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30 292 on the ensemble forecasting procedure. Only models with $AUC > 0.7$ were considered for the
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32 293 final model. Model evaluation revealed high AUC values (2x: 0.75 ± 0.03 ; 4x: 0.92 ± 0.02) and
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34 294 relatively low omission rates in the final models (2x: 0.29 ± 0.07 and 4x: 0.00 ± 0.02), indicating
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36 295 that the models could predict cytotype occurrences with high accuracy. The final models were
37
38 296 converted into a binary format (threshold of 0.5 as defined by default, package “biomod2”;
39
40 297 Thuiller *et al.*, 2016) to calculate the suitable habitat of each cytotype and assess niche overlap
41
42 298 between diploids and tetraploids.

43 44 45 46 47 48 49 50 300 NICHE EQUIVALENCE AND SIMILARITY TESTS

51
52 301 The statistical framework used to compare niches between cytotype measures the niche overlap
53
54 302 using the metric D of proportional similarity of the distribution (Schoener, 1970). This metric
55
56 303 ranges from 0, representing “no overlap”, to 1, representing a “complete overlap”. We
57
58 304 performed niche equivalency and similarity tests (Warren *et al.*, 2008; Broennimann *et al.*, 2012)

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3 305 and the analyses were run using “ecospat” (Di Cola *et al.*, 2017) and “raster” (Hijmans *et al.*,
4
5 306 2017) R packages using binary projections. Both niche equivalency and similarity tests were
6
7 307 computed to evaluate whether predicted distributions were significantly different between
8
9 308 cytotypes (classification by Smith & Donoghue, 2010; Warren *et al.*, 2008; Broennimann *et al.*,
10
11 309 2012).

12
13
14 310 The “ecospat” R package was used to compare cytotype niches with an ordination
15
16 311 approach using a PCA calibrated with environmental values (Di Cola *et al.*, 2017). The PCA
17
18 312 calculates the occurrence density and environmental factor density along environmental
19
20 313 (principal component) axes for each pixel, maximizing the ecological variance of the areas of the
21
22 314 cytotypes. Then the PCA scores of the two cytotype distributions were projected onto a grid of
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24 315 cells bounded by the maximum and minimum PCA scores, which allows the visual assessment
25
26 316 of the overlap and dynamics of the environmental niches of diploids and tetraploids.
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29
30 317 The niche equivalency test determines whether the environmental niche occupancies of
31
32 318 the cytotypes in their ranges are equivalent. For that, diploid and tetraploid records were pooled
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34 319 into a total database; this total database was divided randomly in two groups, each having a
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36 320 sample size equal to the diploid and tetraploid original databases, to obtain a simulated D value.
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38 321 This process was repeated 100 times to obtain confidence intervals for the evaluation of the null
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40 322 hypothesis i.e., the niche overlap is constant when randomly reallocating the occurrences of
41
42 323 both cytotypes among the two ranges. Thus, if the observed value of D falls within the 95th
43
44 324 percentile of the simulated D values the null hypothesis of niche equivalency cannot be rejected
45
46 325 and the niches of the two cytotypes are equivalent. The niche similarity test assesses whether
47
48 326 the observed overlap of the two cytotypes is greater than the overlap between the range of one
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50 327 cytotype and niches selected at random from the range of the other cytotype, i.e., whether the
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52 328 two cytotypes share a greater portion of their environmental volume that would be expected
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54 329 by chance. As in the equivalency test, the observed D value is compared with the simulated D
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3 330 values after repeating the process 100 times to obtain confidence intervals for null hypothesis
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5 331 evaluation (Broennimann *et al.*, 2012).

6
7 332 All analyses were performed in R software version 3.0.1 (R Development Core Team,
8
9 333 2016). Quantum-GIS was used to observe and build the distribution maps.

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13 14 335 **RESULTS**

15 16 336 FLOW CYTOMETRIC ANALYSES

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19 337 Using flow cytometry, we were able to assign ploidy levels to all analysed plants (Figure 1).
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21 338 Diploids had an average genome size of $2C = 2.92 \pm 0.07$ pg (mean \pm SD, range: 2.80 - 3.08 pg),
22
23 339 while tetraploids had an average genome size of $2C = 5.86 \pm 0.14$ pg (range: 5.63 - 6.06 pg; Table
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25 340 1, Figure 1; Table S4). Holoploid genome size differed significantly between cytotypes ($F_{1,249} =$
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27 341 42233.00, $P < 0.001$), while there was no statistically significant difference in monoploid genome
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29 342 size ($F_{1,249} = 0.40$, $P = 0.525$).

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33 34 344 CYTOTYPE DISTRIBUTION

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37 345 The literature review on the karyology of *J. montana* revealed that diploids are widespread
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39 346 across Europe (white diamonds in Figure 2A, Table S1). This result was reinforced by the
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41 347 additional samples in our study (white circles in Figure 2A).

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44 348 In the Iberian Peninsula, we sampled 279 populations and the great majority of the
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46 349 populations sampled were single-ploidy populations (98.6%, from which 71.3% were pure
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48 350 diploid and 27.3% were pure tetraploid); only four localities (1.4%) harboured both diploid and
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50 351 tetraploid individuals (green circles in Figure 2B; Table S2). Our sampling confirmed that
51
52 352 tetraploids are restricted to the [northwest quadrant of the Iberian Peninsula](#), in particular to
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54 353 Central and Northern regions of Portugal, and to Galicia in Spain (grey circles in Figure 2B; Table
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56 354 S2), as initially suggested by records in the literature (grey diamonds in Figure 2A).

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3 355 In the [northwest quadrant of the Iberian Peninsula](#), the diploid and tetraploid
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5 356 populations appeared intermingled, although most areas are dominated by diploids (Figure 2B).
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7 357 The tetraploid populations are mostly clustered in two regions, one in Central Portugal and
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9 358 another in Galicia (Spain), creating several areas of contact between diploids and tetraploids,
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11 359 [including a few sympatric areas where cytotypes coexist and form mixed-ploidy populations in](#)
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13 360 [Central Portugal](#) (Figure 2B). The composition of the mixed-ploidy populations was variable: two
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15 361 populations were dominated by tetraploids, with only one diploid individual being detected in
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17 362 each population, one population had fairly similar cytotype proportions, and one small
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19 363 population was dominated by diploids, bearing only one tetraploid individual (Table S2). Despite
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21 364 the large sample size ($n = 3396$), especially in the contact zone, no other cytotypes were
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23 365 detected.
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30 367 CYTOTYPE ENVIRONMENTAL CHARACTERISTICS AND NICHE OVERLAP

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32 368 Visual inspection of the distribution models [reveals](#) a high predicted suitability for diploids over
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34 369 most of the Iberian Peninsula, except for the eastern calcareous areas, where the probability of
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36 370 occurrence is very low (Figure 3A). In contrast, the predicted tetraploid distribution was
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38 371 restricted to the [northwest quadrant of the Iberian Peninsula](#), where this cytotype is currently
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40 372 found, and to the eastern coast of Valencia (Figure 3B).
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43 373 The first two axes of the PCA explained a high percentage (74.8%) of ecological (climatic
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45 374 and edaphic) variance (51.3% and 23.5% in Axis 1 and 2, respectively). Maximum temperature
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47 375 of the warmest month (Bio_5) and precipitation in the driest month (Bio_14) had the highest
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49 376 contributions to the first axis, followed by temperature and precipitation seasonality (Bio_4 and
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51 377 Bio_15, respectively). Two soil variables (base saturation of the topsoil, bs_top, and dominant
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53 378 surface textural class, txsrfd) had lower contributions and mostly for the second axis (Figure
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55 379 4A). Based on the first and second principal components, the environmental niche of the
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57 380 tetraploid falls completely within the environmental range of the diploid (Figure 4B-C). However,
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3 381 the reverse is not true; rather, the amplitude of the environmental niche of diploids was much
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5 382 larger than that of the tetraploids (Figure 4B-C), and only 20.7% of the diploid environmental
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7 383 niche overlaps with the tetraploid niche (green area in Figure 4B).

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9 384 **Even though the tetraploid** niche is embedded in the diploid niche envelope (Figure 4B),
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11 385 given the differences in occurrences density in the ecological space between cytotypes, the D
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13 386 metric of niche overlap was low ($D = 0.08$). The niche equivalency test rejected the null
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15 387 hypothesis ($P < 0.05$; Figure 4D) and indicates that the climatic niches of diploids and tetraploids
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17 388 are not equivalent or identical (Glor & Warren, 2011). **However**, the niche similarity test revealed
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19 389 that the observed D value falls within the 95th percentile of the simulated D values, thus failing
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21 390 to reject the null hypothesis of niche similarity. Consequently, cytotype environmental niches
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23 391 were not more similar (or different) from one another than the expected after random sampling
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25 392 ($P > 0.05$, for both diploids growing in the tetraploid potential area, and tetraploids growing in
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27 393 the diploid potential area; Figure 4E-F).

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33 34 395 **DISCUSSION**

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36 396 We found differences in both geographic distribution and **environmental niche breadth** between
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38 397 diploid and tetraploid individuals of *Jasione montana*. Tetraploids are restricted to a relatively
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40 398 small geographic area within the Iberian Peninsula, while diploids are widespread across Europe,
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42 399 a pattern consistent with previous reports (compiled in Table S1 and Figure 2A; Leitão and Paiva,
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44 400 1988; Rubido-Bará *et al.*, 2010). Within the region of overlap, tetraploids and diploids mostly
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46 401 occur as a mosaic of single-ploidy populations. Consistent with their smaller geographic range,
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48 402 the environmental niche of tetraploids is more restricted than, and completely nested within,
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50 403 that of diploids. Contrary to our hypothesis, these observations suggest that whole genome
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52 404 duplication in this species is not associated with the origin of novel environmental requirements;
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54 405 if anything, the tetraploid niche is a narrow subset of the extant diploid niche, a pattern seen in
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56 406 other taxa (Glennon *et al.*, 2014). Below, we discuss the mechanisms underlying these results
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3 407 and their implications for our understanding of polyploid establishment and persistence in
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5 408 plants.

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10 410 PLOIDY LEVELS IN *JASIONE MONTANA*

11 411 Our study confirms the presence of two cytotypes in *J. montana*, diploids and tetraploids.

12 412 Polyploidization is common within the genus *Jasione*. Approximately half of the *Jasione* species

13 413 occurring on the Iberian Peninsula, the centre of diversity for the genus, are polyploid or harbour

14 414 multiple cytotypes (Sales & Hedge, 2001b; Rubido-Bará *et al.*, 2010; Castro, 2018); *J. montana* is

15 415 one such species. [The co-occurrence of diploids and tetraploids](#) is commonly observed in mixed-

16 416 ploidy species, generally (Kolář *et al.*, 2017), and is common among polyploid species in the

17 417 genus *Jasione*. For example, *J. laevis* and *J. maritima* (Sales & Hedge, 2001a; Rubido-Bará *et al.*,

18 418 2010; Castro, 2018) both consist of diploids and tetraploids, whereas *J. crispa* has diploids,

19 419 tetraploids and hexaploids (Sales & Hedge, 2001a). More surprising is the absence of triploid

20 420 hybrids, especially in the contact zone of the Iberian Peninsula. This pattern may reflect the

21 421 spatial separation of cytotypes into single-ploidy populations or the presence of strong triploid

22 422 block (discussed below). The absence of triploids within the four mixed-ploidy populations

23 423 suggests that both factors may be operating in the diploid-tetraploid contact zones of *Jasione*

24 424 *montana*.

25 425

26 426 NESTED AND ASYMMETRICAL GEOGRAPHIC RANGES: HISTORICAL CAUSES

27 427 The nested and asymmetrical geographic ranges of diploids and tetraploids, and the restricted

28 428 geographic range of tetraploids in particular, may reflect the history of formation of tetraploids

29 429 from diploid ancestors in this species. Tetraploids may have arisen and successfully colonized

30 430 the [northwest quadrant of the Iberian Peninsula](#) relatively recently and, thus, have had limited

31 431 time to expand beyond the site of origin, as has been suggested for other mixed-ploidy species

32 432 (Godsoe *et al.*, 2013; Laport *et al.*, 2016). The limited geographic range also suggests that

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3 433 tetraploids have arisen a limited number of times across the entire geographic range. While our
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5 434 sampling was extensive, more intensive screening for rare tetraploids throughout the diploid
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7 435 portion of the range is needed to determine whether tetraploids do in fact arise frequently but
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9 436 are unlikely to establish. Multiple origins of polyploids have been documented in numerous taxa
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11 437 and on a range of spatial scales (e.g., Soltis & Soltis, 1999; Segraves & Thompson, 1999; Sampson
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13 438 & Byrne, 2011). Therefore, we cannot exclude the possibility that the two clusters of tetraploid
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15 439 populations, one in Portugal and the other in Galicia (Spain), represent independent origins. To
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17 440 date, we have little evidence with which to evaluate these historical scenarios in *J. montana*.
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19 441 Phylogenetic analyses of the genus *Jasione* positioned *J. montana* in a clade with other species
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21 442 currently occurring in the northwest quadrant of the Iberian Peninsula (Sales *et al.*, 2004; Pérez-
22
23 443 Espona *et al.*, 2005). However, the internal relationships of the clade remain unresolved and the
24
25 444 time of divergence of tetraploid *J. montana* relative to diploids is not known. Additional research
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27 445 on genetic divergence and phylogeography of tetraploids and diploids may help to shed light on
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29 446 the timing and frequency of whole genome duplication in this species.
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448 ASYMMETRICAL RANGES: ENVIRONMENTAL NICHE SHIFTS?

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38 449 Our results provide insight into the role of environmental differences between cytotypes in
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40 450 determining establishment and geographic distribution of polyploids. Tetraploid plants of *J.*
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42 451 *montana* occupy only a subset of the diploid environmental niche, as depicted in the PCA (Fig
43
44 452 4B, C), and the equivalency test indicates that climatic niches of diploids and tetraploids are not
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46 453 equivalent; still, cytotype environmental niches were not more similar (or different) from one
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48 454 another than expected by chance. Therefore, we conclude that there is no evidence that whole
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50 455 genome duplication, leading to tetraploidy, resulted in a shift in environmental niche beyond
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52 456 that of the diploid niche. Changes in environmental tolerance are often postulated as necessary
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54 457 for polyploid establishment (Husband & Schemske, 2000; Baack & Stanton, 2005; Buggs &
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56 458 Pannell, 2007; Ramsey, 2011) as they promote spatial segregation and within-cytotype mating,
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3 459 allowing rare cytotypes to avoid frequency-dependent selection and exclusion (Levin, 1975;
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5 460 Fowler & Levin, 1984; Felber, 1991; Hao *et al.*, 2013). However, evidence in the literature for
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7 461 niche shifts that occur in association with whole genome duplication is **inconsistent**. Examples
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9 462 of environmental niche divergence between cytotypes **have** been reported in species such as
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11 463 *Chamerion angustifolium* (Thompson *et al.*, 2014), *Centaurea stoebe* (Glennon *et al.*, 2014),
12
13 464 *Tolmiea diplomenziesii* / *menziesii* (Visger *et al.*, 2016), and *Erysimum mediohispanicum* (Muñoz-
14
15 465 Pajares *et al.*, 2018), although it is usually unclear **whether this results directly from the**
16
17 466 **duplication or subsequent evolutionary divergence** (see Maherali *et al.*, 2009 for *C.*
18
19 467 *angustifolium*). **In contrast**, a test of the niche shift hypothesis using 20 different diploid-
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21 468 polyploid species found that most pairs showed significant similarity or no difference in niche
22
23 469 between pairs (Glennon *et al.*, 2014). Collectively, these results suggest that polyploids
24
25 470 frequently occupy similar environmental conditions to their diploid progenitors and that niche
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27 471 differentiation, measured as climatic variables, may not be a necessary requirement for
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29 472 polyploid establishment and persistence in nature (Glennon *et al.*, 2014).
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36 474 ASYMMETRICAL GEOGRAPHIC RANGES: ENVIRONMENTAL SORTING

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39 475 While evidence for a **significant** environmental niche shift in tetraploids is lacking, environmental
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41 476 sorting may still explain, in part, the differences in geographical range between cytotypes of *J.*
42
43 477 *montana*. The smaller geographical range of tetraploids is congruent **with their narrower**
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45 478 **environmental niche compared to diploids**. This correlation suggests a possible causal
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47 479 relationship; specifically, that the narrower niche of tetraploids is limiting the expansion and
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49 480 establishment on a larger geographical scale. Additionally, polyploids with smaller niche ranges
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51 481 than their diploid relatives may be well adapted, or even specialized, to a given set of
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53 482 environmental conditions (Parisod & Broennimann, 2016). Thus, the restricted and spatially
54
55 483 clustered distribution of the tetraploids could reveal areas harbouring a combination of
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57 484 environmental conditions where the tetraploid niche is at its optimum, allowing them to
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3 485 outcompete the diploids. In the case of *J. montana*, the tetraploid distribution coincides with
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5 486 the boundaries between four main biogeographic sectors (the Galicia and North Portugal sector,
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7 487 the North Lusitania Sierra sector, the Montemuro and Estrela Sierras sector and the north part
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9 488 of the Divisorio Portuguese sector; Rivas-Martínez *et al.*, 2017) and is characterized by a
10
11 489 subatlantic-submediterranean climate and with similar floristic compositions (Rivas-Martinez *et*
12
13 490 *al.*, 2017). This biogeographic region of contact between different sectors constitutes the
14
15 491 interface between the temperate and Mediterranean biomes and harbours several species
16
17 492 adapted to this transition (Amigo *et al.*, 2017). Niche relationships between closely related
18
19 493 species or cytotypes in which the niche of one taxon/cytotype is much narrower than, and
20
21 494 nested within, the niche of the other, have been interpreted as indicative of environmental
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23 495 specialization (Knouft *et al.*, 2006; Vamosi *et al.*, 2014; Parisod & Broennimann, 2016). To
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25 496 confirm this hypothesis, reciprocal transplant experiments are necessary to establish that
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27 497 tetraploids are, indeed, unable to thrive in diploid environments outside of their current
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29 498 geographic range.
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36 500 WHAT ALLOWS TETRAPLOIDS TO PERSIST WITH DIPLOIDS ON THE IBERIAN PENINSULA?

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39 501 If tetraploid *J. montana* is restricted in distribution to a small portion of the diploid range, what
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41 502 then allowed tetraploids to persist and coexist with diploids? Theoretical models predict that, in
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43 503 randomly mating populations, rare cytotypes will experience a frequency-dependent mating
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45 504 disadvantage, due to increased inter-cytotype mating, which is generally ineffectual. As a result,
46
47 505 dominant cytotypes will preclude establishment of newly arising cytotypes (Levin, 1975;
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49 506 Rodriguez, 1996; Husband & Schemske, 2000) unless new cytotypes can disperse to unoccupied
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51 507 locations (e.g., Godsoe *et al.*, 2013; Thompson *et al.*, 2014; Visger *et al.*, 2016; Muñoz-Pajares *et*
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53 508 *al.*, 2018), resulting in a mosaic of single-ploidy populations on the landscape, each excluding
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55 509 the other from their respective populations through minority cytotype exclusion (Petit *et al.*,
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57 510 1999; e.g., Baack, 2004; Kolář *et al.*, 2009; Castro *et al.*, 2012). In *J. montana*, the diploids and
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3 511 tetraploids indeed form a complex mosaic of single-ploidy populations in the [northwest](#)
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5 512 [quadrant of the Iberian Peninsula](#), with areas dominated by [diploids intermingled](#) with areas
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7 513 dominated by tetraploids. [Furthermore, the rarity of mixed populations suggests that](#)
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9 514 [reproductive barriers between the two cytotypes might be insufficient to prevent inter-cytotype](#)
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11 515 [mating, resulting in minority cytotype disadvantage \(triploid block; Levin, 1975; Husband, 2000\).](#)
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13 516 [Indeed, preliminary pollination experiments revealed that both cytotypes are unable to produce](#)
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15 517 [viable offspring after inter-cytotype crosses \(M. Castro, unpublished data\).](#) Minority cytotype
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17 518 exclusion has been tested experimentally only by Husband (2000) in *Chamerion angustifolium*
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19 519 [and by Baack \(2005\) in *Ranunculus adoneus*](#), but it has been referred as an important mechanism
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21 520 driving cytotype distribution patterns in numerous diploid-polyploid contact zones (e.g., Levin,
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23 521 2002; Španiel *et al.*, 2008; Castro *et al.*, 2011).

27 522 Our results suggest that, to establish successfully, tetraploids of *J. montana* must
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29 523 disperse and colonize unoccupied areas in the landscape and, thus, avoid the minority cytotype
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31 524 exclusion (e.g., Godsoe *et al.*, 2013; Thompson *et al.*, 2014; Visger *et al.*, 2016; Muñoz-Pajares
32
33 525 *et al.*, 2018). Interestingly, there is a tendency for tetraploid *J. montana* to occupy ruderal and
34
35 526 more disturbed habitats than diploids, which can occupy both less disturbed areas as well as
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37 527 some ruderal habitats (M. Castro & M. Serrano, Field observations). This pattern has been
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39 528 observed in other polyploid complexes, in which tetraploids spread into ruderal habitats forming
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41 529 mosaic contact zones even without differences in climatic niche (e.g., Španiel *et al.*, 2008; Kolář
42
43 530 *et al.*, 2016). Our observations remain to be confirmed but highlight the hypothesis that cytotype
44
45 531 coexistence in the contact zone can be enabled by microhabitat differences between cytotypes
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47 532 within the existing climatic niche. Also, the aggregation of tetraploid populations in regions
48
49 533 suitable for diploids may indicate an ability to outcompete diploids in specific environments,
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51 534 thereby excluding them from population. Fitness advantages such as increased competitive
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53 535 ability (Maceira, Jacquard & Lumaret, 1993; Laport *et al.*, 2013) and asymmetric assortative
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55 536 mating (Husband & Sabara, 2004; Buggs & Pannell, 2006, 2007; Laport *et al.*, 2016), among other
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3 537 factors, play crucial roles in the success of polyploid lineages. In *J. montana*, some morphological
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5 538 differences between cytotypes have been observed (Rubido-Bará *et al.*, 2010) that might be
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7 539 associated with plant fitness and competitive ability, such as increased plant size and tendency
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9 540 for perenniality in the tetraploids (M. Serrano, field observations). Nevertheless, further studies
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11 541 are needed to unravel possible fitness differences that could explain cytotype interactions at
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14 542 contact zones.
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18 544 **CONCLUDING REMARKS**

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21 545 Our results indicate that polyploidization in *J. montana* has not caused expansion to novel
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23 546 environmental conditions. Under this scenario, tetraploids will be subjected to strong
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25 547 frequency-dependent selection and will be excluded by diploids unless they can disperse to sites
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27 548 unoccupied by diploids, where they in turn may exclude diploid colonists. The mosaic of diploid
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29 549 and tetraploid populations and a lack of mixed-ploidy populations is consistent with a frequency-
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31 550 dependent selection influencing distributions within the mixed-ploidy zones, although the
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33 551 influence of inter-cytotype competition cannot be ruled out. The interplay between both
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35 552 mechanisms is unclear and future molecular and experimental studies will be important for fully
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37 553 understanding the role of environmental sorting and evolutionary history in governing the
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39 554 distribution of cytotypes within the contact zones of *J. montana*.
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47
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53
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3 562 by the “Programa Operacional Regional do Centro 2014-2020 (Centro2020) - CENTRO-01-0145-
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5 563 FEDER-000007”.

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10 565 **SUPPORTING INFORMATION**

11
12 566 The following additional information is available:

13
14 567 **Table S1** – Chromosome counts of *Jasione montana* available in the bibliography.

15
16 568 **Table S2** – Geographic information of *Jasione montana* populations sampled in this study.

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18 569 **Table S3** – Selected environmental variables for *Jasione montana* cytotypic niche modelling.

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21 570 **Table S4** – Genome size estimates in *Jasione montana*.

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PDF Proof

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870 **FIGURE CAPTIONS**

871 **Figure 1** – Flow cytometric histogram of relative propidium iodide fluorescence intensity (PI
872 fluorescence) of nuclei isolated from fresh leaves of *Solanum lycopersicum* ‘Stupické’ (S.I.;
873 reference standard with $2C = 1.96$ pg) and of *Jasione montana* diploid (2x) and tetraploid (4x)
874 plants. For each peak, the mean relative fluorescence (Mean FL), DNA index (DI, Mean FL of *J.*
875 *montana* peak / Mean FL of the reference standard) and coefficient of variation of each peak
876 (CV, in %) are provided.

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878 **Figure 2** – *Jasione montana* records. A) bibliographic information from chromosome counts and
879 ploidy levels in Europe (diamonds) and additional estimates provided in this study (circles); B)
880 detail of the Iberian Peninsula, including estimates from this study (big circles) and GBIF
881 occurrences (small circles). Diamonds – bibliographic records (BR); big circles – populations
882 screened in this study; small circles – GBIF occurrences; Ploidy levels: white – diploids (2x), grey
883 – tetraploids (4x), black – diploid-tetraploid populations.

884

885 **Figure 3** – Predictive suitable niche for each cytotype: A) diploids and B) tetraploids of *Jasione*
886 *montana* in the Iberian Peninsula. With colors represent habitats with low suitability and black
887 colors habitats with high suitability.

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889 **Figure 4** – Ecological niche models for *Jasione montana* cytotypes at the Iberian Peninsula: A)
890 contribution of climatic and soil variables to the first two axes of the principal component
891 analyses (PCA) and the percentage of variance explained by each axis; B) environmental niche
892 of diploids and tetraploids, respectively, based on the PCA of selected variables; **coloured areas**
893 **represent the following: dark grey – suitable habitats for diploids, green – denotes both the**
894 **suitable habitats for tetraploids and the overlapping areas between diploid and tetraploid**
895 **environmental niches; the continuous line corresponds to the whole climatic space, while the**

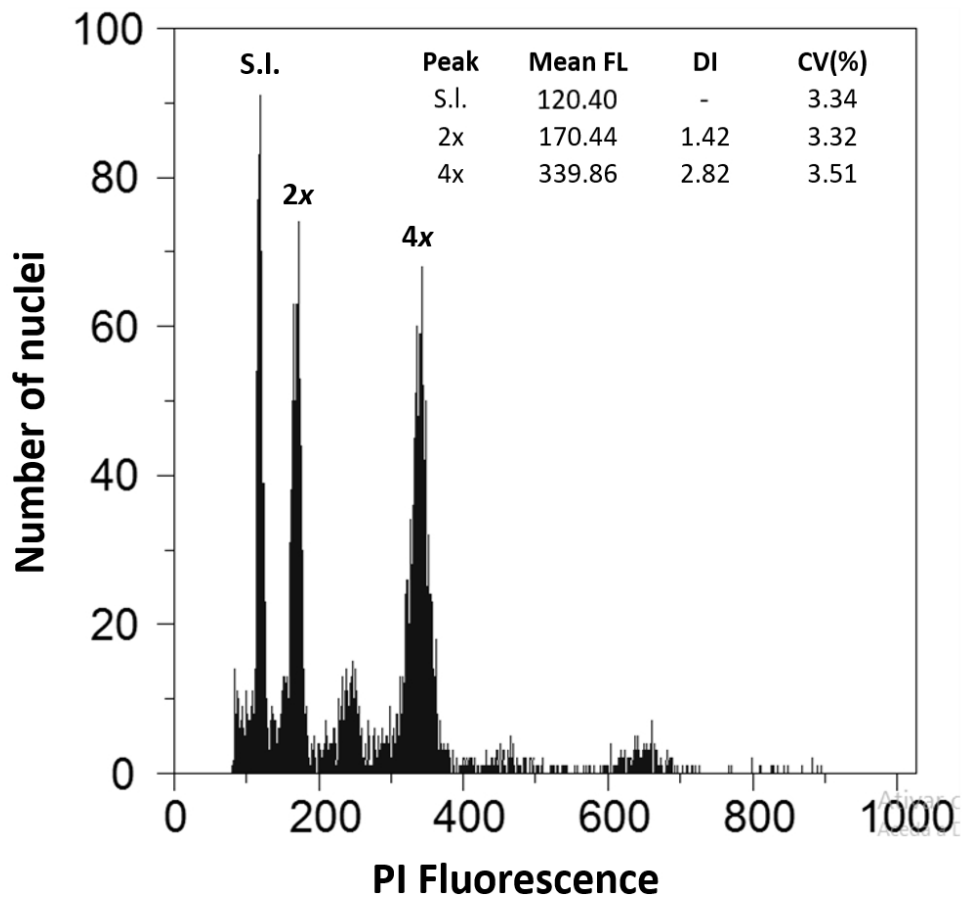
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3 896 dashed line indicates the 75th percentile; C) magnitude of environmental niches of each cytotype
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5 897 (white and dark grey represent the occupation of niches in PCA1 and PCA2, respectively); D)
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7 898 equivalency test results; E) similarity test results of diploids growing in the tetraploid
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9 899 environmental niche; and F) similarity tests of tetraploids growing in the diploid environmental
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11 900 niche. In D, E and F, dark grey diamonds represent the observed *D* values, while grey bars
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13 901 correspond to the frequency of calculated *D* value resulting from a similarity test process.
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902 **TABLES**

903 **Table 1** – Genome size estimates in *Jasione montana* according with each cytotype. Ploidy level
 904 and mean, standard deviation of the mean (SD), coefficient of variation (CV, in %), minimum and
 905 maximum values of holoploid genome size (2C, in pg) are given. Mean and standard deviation
 906 of the mean (SD) of estimated monoploid genome size (1Cx, in pg) and the total number of
 907 populations and individuals analysed are also presented for each cytotype. Two ploidy levels
 908 were observed: diploids (2x) and tetraploids (4x). Different letters correspond to statistically
 909 significant differences at $P < 0.05$, n.s. denote non-significant differences at $P > 0.05$.

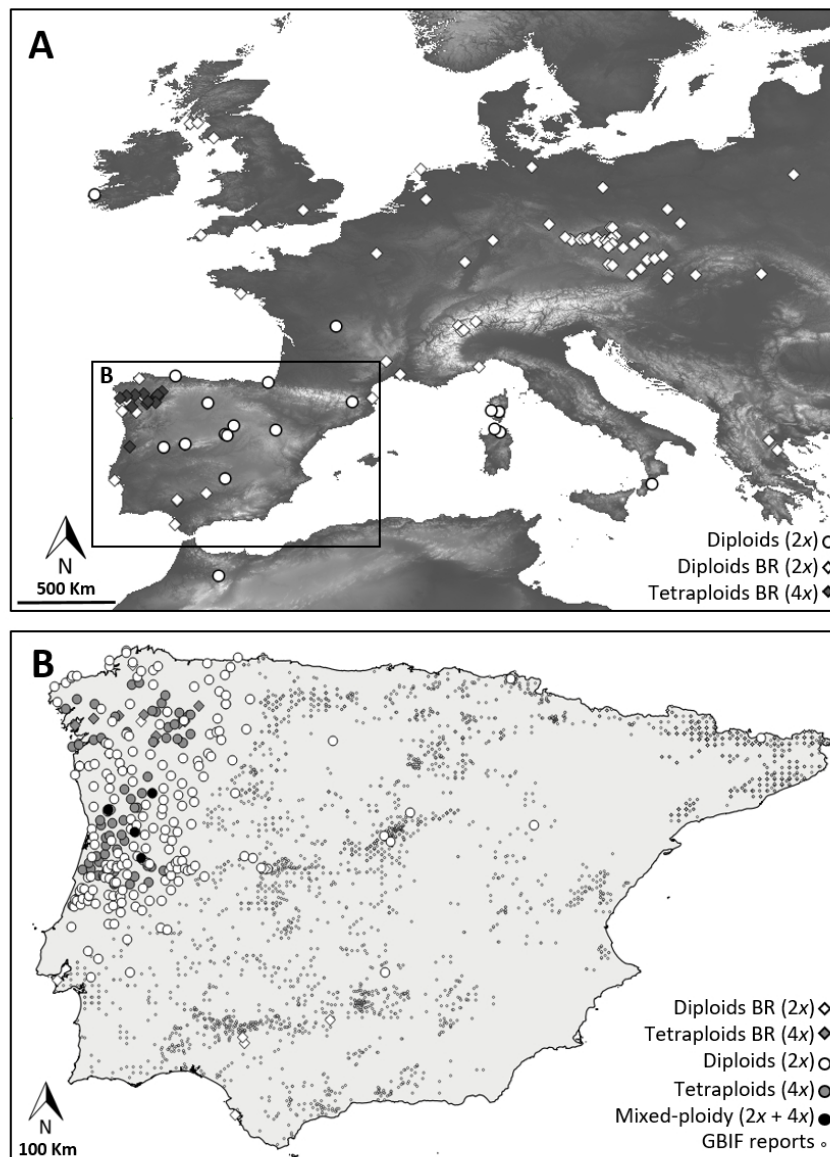
Ploidy level	Holoploid genome size (2C, pg)					Monoploid genome size (1Cx, pg)		Populations (individuals)
	Mean	SD	CV (%)	Min	Max	Mean	SD	
2x	2.92 ^a	0.07	2.9%	2.80	3.08	1.46 ^{n.s.}	0.04	84 (205)
4x	5.86 ^b	0.14	3.3%	5.63	6.06	1.46 ^{n.s.}	0.04	24 (46)

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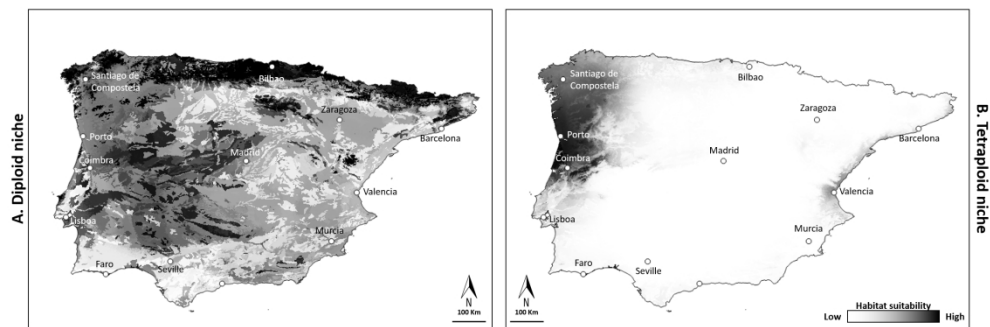
Flow cytometric histogram of relative propidium iodide fluorescence intensity (PI fluorescence) of nuclei isolated from fresh leaves of *Solanum lycopersicum* 'Stupické' (S.I.; reference standard with $2C = 1.96$ pg) and of *Jasione montana* diploid (2x) and tetraploid (4x) plants. For each peak, the mean relative fluorescence (Mean FL), DNA index (DI, Mean FL of *J. montana* peak / Mean FL of the reference standard) and coefficient of variation of each peak (CV, in %) are provided.

173x167mm (150 x 150 DPI)



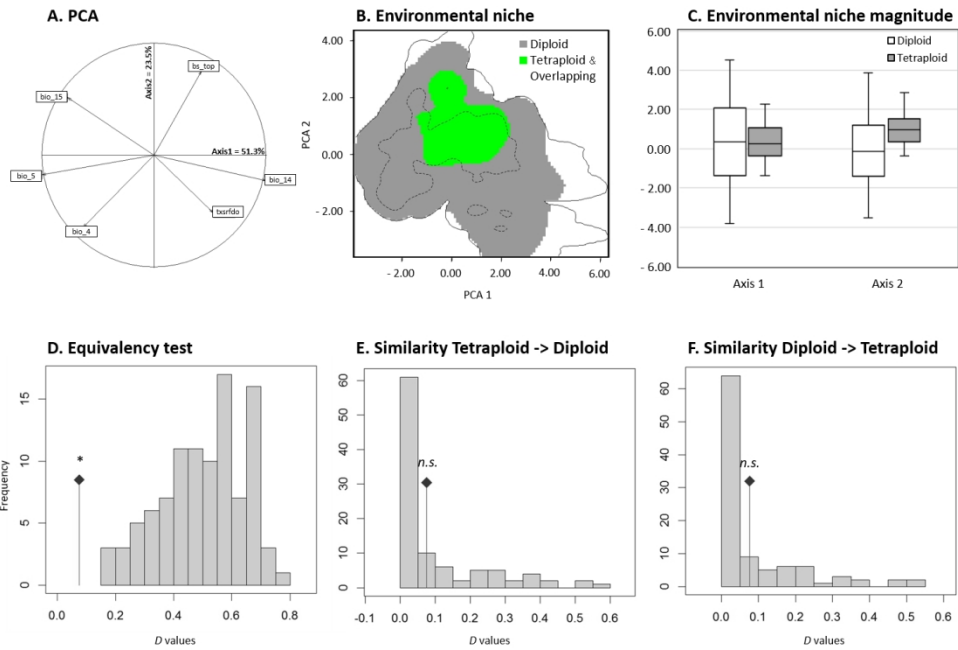
45 *Jasione montana* records. A) bibliographic information from chromosome counts and ploidy levels in Europe
46 (diamonds) and additional estimates provided in this study (circles); B) detail of the Iberian Peninsula,
47 including estimates from this study (big circles) and GBIF occurrences (small circles). Diamonds –
48 bibliographic records (BR); big circles – populations screened in this study; small circles – GBIF occurrences;
49 Ploidy levels: white – diploids (2x), grey – tetraploids (4x), black – diploid-tetraploid populations.

50 148x199mm (150 x 150 DPI)



Predictive suitable niche for each cytotype: A) diploids and B) tetraploids of *Jasion montana* in the Iberian Peninsula. With colors represent habitats with low suitability and black colors habitats with high suitability.

418x152mm (150 x 150 DPI)



Ecological niche models for *Jasionne montana* cytotypes at the Iberian Peninsula: A) contribution of climatic and soil variables to the first two axes of the principal component analyses (PCA) and the percentage of variance explained by each axis; B) environmental niche of diploids and tetraploids, respectively, based on the PCA of selected variables; coloured areas represent the following: dark grey – suitable habitats for diploids, green – denotes both the suitable habitats for tetraploids and the overlapping areas between diploid and tetraploid environmental niches; the continuous line corresponds to the whole climatic space, while the dashed line indicates the 75th percentile; C) magnitude of environmental niches of each cytotypic (white and dark grey represent the occupation of niches in PCA1 and PCA2, respectively); D) equivalency test results; E) similarity test results of diploids growing in the tetraploid environmental niche; and F) similarity tests of tetraploids growing in the diploid environmental niche. In D, E and F, dark grey diamonds represent the observed *D* values, while grey bars correspond to the frequency of calculated *D* value resulting from a similarity test process.

265x176mm (150 x 150 DPI)

1 **SUPPORTING INFORMATION**

2 **Table S1** – Chromosome counts of *Jasione montana* available in the bibliography. Information
 3 about the original name (currently a synonym of *J. montana*), under which chromosome counts
 4 were made, is provided when applicable. Number of chromosomes (n, gametophytic; 2n,
 5 sporophytic), ploidy level (2x, diploid) and reference are given. References highlighted in bold
 6 provide geographic information about the material used for the chromosome counts.

Species	n	2n	Ploidy	References
<i>Jasione montana</i> L.		12	2x	Anchev, 1976
<i>Jasione montana</i> L. (as <i>Jasione blepharodon</i> Boiss. & Reut.)		12	2x	Bjorkqvist, 1969
<i>Jasione montana</i> L.		12	2x	Brullo <i>et al.</i> , 1977
<i>Jasione montana</i> L.	6	12	2x	Contandriopoulos, 1966
<i>Jasione montana</i> L.	6		2x	Delay, 1969
<i>Jasione montana</i> L.		12	2x	Dobes, Kiehn & Vitek, 1996
<i>Jasione montana</i> L.		12	2x	Gadella, 1966
<i>Jasione montana</i> L.	6	12	2x	Gadella & Kliphuis, 1966
<i>Jasione montana</i> L.		12	2x	Gadella & Kliphuis, 1968
<i>Jasione montana</i> L.		12	2x	Gadella & Kliphuis, 1970
<i>Jasione montana</i> L.		12	2x	Kliphuis & Wieffering, 1972
<i>Jasione montana</i> L.		12	2x	Kovanda, 1968
<i>Jasione montana</i> L.		12	2x	Kovanda, 1983
<i>Jasione montana</i> L.		12	2x	Králik & Hrozičik, 2000
<i>Jasione montana</i> L.		12	2x	Lago Canzobre & Castroviejo, 1992
<i>Jasione montana</i> L.		12	2x	Lövkvist, 1999
<i>Jasione montana</i> L.	6		2x	Luque & Mejias, 1986
<i>Jasione montana</i> L.		12	2x	Majovsky, 1970
<i>Jasione montana</i> L. (as <i>Jasione echinata</i> Boiss. & Reut.)		12	2x	Ottonello, Romano & Alliata, 1986
<i>Jasione montana</i> L.		12	2x	Parfenov & Dmitrieva, 1985
<i>Jasione montana</i> L.		12	2x	Parfenov & Dmitrieva, 1988
<i>Jasione montana</i> L.	6		2x	Parnell, 1982
<i>Jasione montana</i>		12	2x	Parnell, 1986
<i>Jasione montana</i> L. (as <i>Jasione echinata</i> Boiss. & Reut. Nyman)	6	12	2x	Pastor, 1990
<i>Jasione montana</i> L.		12	2x	Poddubnaja-Arnoldi, 1933
<i>Jasione montana</i> L.		12	2x	Podlech, 1963
<i>Jasione montana</i> L.	6	12	2x	Pogan, Wcislo & Jankun, 1980
<i>Jasione montana</i> L.		12	2x	Rohweder, 1937
<i>Jasione montana</i> L.		12	2x	Rosén, 1932
<i>Jasione montana</i> L.		12	2x	Sugiura, 1940

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3	<i>Jasione montana</i> L.		12	2x Sugiura, 1942
4				
5	<i>Jasione montana</i> L.	6		2x Ubera, 1980
6	<i>Jasione montana</i> L.		12	2x Uhríková & Králik, 2000
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8	<i>Jasione montana</i> L.		12	2x Van Den Brand, 1979.
9	<i>Jasione montana</i> L.	6	12	2x Van Loon, 1979
10				
11	<i>Jasione montana</i> L.		12	2x Wcislo, 1983
12	<i>Jasione montana</i> L.		12	2x Wisskirchen & Haeupler, 1998
13				
14	<i>Jasione montana</i> L.		12	2x Wulff, 1937
15	<i>Jasione montana</i> L.		24	4x Leitão & Paiva, 1988
16				
17	<i>Jasione montana</i> L.	6, 12		2x and 4x Rubido-Bará, Horjales Luaces & Villaverde, 2010.
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Table S2 – Geographic information of the *Jasione montana* populations sampled in this study. For each population, an ID code, estimated ploidy level, sample size (n) and information on the location and GPS coordinates (angular) are presented. Two ploidy levels were observed: diploids (2x) and tetraploids (4x). Populations were divided in two groups according with their ploidy composition: single-ploidy populations and mixed-ploidy populations. The five locations marked with an asterisk (*) constitute unpublished chromosome counts by Miguel Serrano. The superscript notation (^s) denote populations where ploidy level was assessed directly from seeds.

ID code	Ploidy level (n)	Location	Herbarium voucher	Geographic coordinates	
				Latitude	Longitude
Single-ploidy populations					
MS03-01 ^s	2x (15)	Jbel Tazekka, Taza, Morocco	SANT62794	34.08691	-4.18341
MS16-015	2x*	Pazzano, Calabria, Italy	SANT61450	38.46803	16.41768
AA002	2x (6)	Pegões, Setúbal, Portugal		38.65242	-8.61802
MC357	2x (5)	Arraiolos, Évora, Portugal		38.72755	-7.98722
MS05-01 ^s	2x (15)	Aldea del Rey, Ciudad Real, Spain	SANT60479	38.73722	-3.87583
MC356	2x (7)	Coruche, Santarém, Portugal		38.94409	-8.49414
MC353	2x (17)	Muge, Salvaterra de Magos, Portugal		39.06865	-8.66193
MC358	2x (3)	Domingão, Ponte de Sor, Portugal		39.24543	-8.02592
SC122	2x (4)	Jardim, Marvão, Portugal	SANT68767	39.39334	-7.38971
SC121	2x (5)	Quinta Mão do Novilheiro, Castelo de Vide, Portugal	SANT68768	39.42844	-7.48734
DT017	2x (3)	Chãos, Alcobertas, Portugal		39.42958	-8.92472
MC341	2x (19)	Rio de Moinhos, Abrantes, Portugal		39.47490	-8.23640
MC342	2x (6)	Constância, Santarém, Portugal		39.48597	-8.33026
MC340	2x (25)	Penedo, Oleiros, Portugal		39.66357	-8.12899
MC221	2x (30)	Peral, Proença-a-Nova, Portugal		39.69370	-7.79880
MC346	2x (26)	Pias, Ferreira do Zêzere, Portugal		39.71898	-8.32942
SC191	2x (19)	Vale de Urso, Proença-a-Nova, Portugal	SANT70515	39.74483	-7.89155
MC309	2x (23)	Albergaria, Marinha Grande, Portugal		39.74603	-8.88242
LM014	2x (6)	Dornes, Ferreira do Zêzere, Portugal		39.76843	-8.26169
MC362	2x (13)	Janardo, Leiria, Portugal		39.78695	-8.77387
MC350	2x (29)	Vale da Meda, Ourém, Portugal		39.79381	-8.53367
SC192	2x (16)	Sertã, Portugal	SANT70473	39.80863	-8.11590
SC190	2x (3)	Silveira dos Figos, Castelo Branco, Portugal	SANT70477	39.83898	-7.67476
MC360	2x (1)	Vermoil, Pombal, Portugal		39.84320	-8.66282

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4	SC189	2x (3)	Castelo Branco, Portugal	SANT70476	39.84571	-7.47026
5	LM009	2x (25)	Souto de Carpalhosa, Leiria, Portugal		39.84595	-8.83335
6						
7	MC368	2x (16)	Granja, Bajouca, Portugal		39.85191	-8.87618
8	MC361	2x (9)	Valeirão, Pombal, Portugal		39.87102	-8.75234
9						
10	EM001	2x (24)	Pedrogão Pequeno, Sertã, Portugal		39.89622	-8.13783
11						
12	SC193	2x (14)	Poço Negro, Figueiró-dos-Vinhos, Portugal		39.91965	-8.25304
13						
14	LM005	2x (6)	Casal Fernão João, Pombal, Portugal	SANT70512	39.91980	-8.64870
15	LM003	2x (5)	Casal Fernão João, Pombal, Portugal	SANT70513	39.92007	-8.65293
16						
17	MC330	2x (20)	Pedrogão Grande, Portugal		39.92688	-8.15120
18	MC336	2x (22)	Cancinos, Oleiros, Portugal		39.92747	-7.92447
19						
20	MC329	2x (18)	Fato, Figueiró dos Vinho, Portugal		39.92843	-8.32886
21						
22	MC371	2x (18)	Matos do Carriço, Pombal, Portugal		39.98608	-8.79657
23						
24	SC188	2x (9)	São Miguel da Acha, Idanha-a-Nova, Portugal	SANT70485	40.00324	-7.33108
25	MC354	2x (1)	Redinha, Pombal, Portugal		40.00532	-8.58961
26						
27	MC334	2x (12)	Alvares, Góis, Portugal		40.00980	-8.10677
28						
29	MC298	2x (11)	Casal da Rola, Pombal, Portugal		40.02076	-8.71501
30	SC184	2x (11)	Bogas de Baixo, Oleiros, Portugal	SANT70531	40.03460	-7.77846
31						
32	MC369	2x (5)	Torneira, Pombal, Portugal		40.03966	-8.75807
33						
34	SC182	2x (18)	Vilarinho, Lousão, Portugal	SANT70479	40.12720	-8.20791
35	AM002	2x (4)	Vale, Lousã, Portugal		40.13018	-8.23468
36						
37	AM001	2x (6)	Vale, Lousã, Portugal		40.13082	-8.23518
38	SC187	2x (16)	Penamacor, Portugal	SANT70506	40.13903	-7.20258
39						
40	SC186	2x (15)	Fatela, Fundão, Portugal	SANT70478	40.16101	-7.42409
41						
42	MC271	2x (10)	Caneiro, Miranda do Corvo, Portugal		40.17904	-8.31525
43						
44	MC292	2x (12)	Torre do Mondego, Coimbra, Portugal		40.19314	-8.38946
45	SC179	2x (2)	Madorno, Montemor-o-Velho, Portugal	SANT70480	40.19499	-8.65544
46						
47	MS01-01^s	2x (15)	San Martín de Trevejo, Cáceres, Spain	SANT62914	40.20992	-6.78700
48						
49	MC307	2x (9)	Santo António dos Olivais, Coimbra, Portugal		40.21064	-8.40051
50						
51	SC180	2x (10)	Meães, Montemor-o-Velho, Portugal	SANT70481	40.21384	-8.60850
52	AA001	2x (4)	Salgueiro, Fundão, Portugal		40.22853	-7.33197
53						
54	MC272	2x (6)	Quinta Grande, Coimbra, Portugal		40.23437	-8.42012
55	MC306	2x (10)	Secarias, Arganil, Portugal		40.24692	-8.03352
56						
57	SC225	2x (10)	Caria, Belmonte, Portugal		40.29917	-7.35661
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59	SC230	2x (10)	Malcata, Sabugal, Portugal		40.29997	-7.05845
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3	SC231	2x (7)	Quadrazais, Sabugal, Portugal		40.31300	-7.00049
4	SC229	2x (7)	Sabugal, Portugal		40.33050	-7.09721
5	SC227	2x (4)	Azenha, Sabugal, Portugal		40.33134	-7.22465
6	MS07-02^s	2x (15)	Candelario, Salamanca, Spain	SANT76423	40.33659	-5.76651
7	MC281	2x (9)	Cácemes, Mortágua, Portugal		40.33928	-8.34688
8	MS15-062	2x (6)	Montemayor del Río, Salamanca, Spain	SANT71386	40.34574	-5.88048
9	MC287	2x (12)	Almacinha, Mortágua, Portugal		40.35560	-8.19451
10	SC241	2x (3)	Catraia do Buraco, Belmonte, Portugal		40.37214	-7.32858
11	SC232	2x (3)	Alfaiates, sabugal, Portugal		40.37808	-6.94432
12	MC283	2x (17)	Freixo, Mortágua, Portugal		40.38542	-8.19956
13	MC282	2x (14)	Barracão, Mortágua, Portugal		40.38915	-8.28630
14	MC269	2x (9)	Várzea, Mealhada, Portugal		40.39001	-8.38288
15	MC285	2x (12)	Santa Comba Dão, Portugal		40.39751	-8.12613
16	SC127	2x (3)	Senhora do Espinheiro, Seia, Portugal	SANT68766	40.41127	-7.67038
17	SC169	2x (27)	Póvoa da Forcada, Carregal do Sal, Portugal	SANT70482	40.41677	-8.03776
18	JCO001	2x (20)	Quintãzinha do Mouratão, Guarda, Portugal		40.51386	-7.21647
19	SC239	2x (3)	Quinta do Souto, Belmonte, Portugal		40.42413	-7.25116
20	SC238	2x (4)	A-de-Moura, Guarda, Portugal		40.45907	-7.2212
21	SC236	2x (7)	Adão, Guarda, Portugal		40.46016	-7.14943
22	SC208	2x (11)	Vila Verde, Seia, Portugal		40.46337	-7.77766
23	MC273	2x (23)	Santo Amaro, Tondela, Portugal		40.50142	-8.07658
24	MS15-065	2x (6)	Villanueva del Conde, Salamanca, Spain	SANT71411	40.50603	-6.01425
25	SC176	2x (19)	Palhaça, Oliveira do Bairro, Portugal	SANT70475	40.51136	-8.59473
26	SC170	2x (21)	Nelas, Portugal	SANT70483	40.53123	-7.86441
27	MS15-069	2x (6)	Peña de Francia, Salamanca, Spain	SANT71409	40.53424	-6.14993
28	MG001	2x (11)	Caramulo, Tondela, Portugal		40.57402	-8.17512
29	MC248	2x (17)	Granja, Guarda, Portugal	SANT70492	40.60071	-7.10506
30	SC210	2x (4)	Lajeosa do Mondego, Guarda, Portugal		40.63036	-7.35552
31	MC249	2x (28)	Germil, Penalva do Castelo, Portugal	SANT70494	40.65076	-7.73303
32	SC171	2x (28)	Abraveses, Viseu, Portugal	SANT70484	40.68324	-7.92715
33	MC296	2x (2)	Vale, Estarreja Portugal		40.70659	-8.55917
34	MS16-020	2x*	Soto del Real, Madrid, Spain		40.75042	-3.79266
35	MC291	2x (14)	Parada, Sever do Vouga, Portugal		40.77002	-8.29246
36	FM001	2x (10)	Falachos, Trancoso, Portugal	SANT70514	40.77475	-7.31173
37	MC247	2x (26)	Palhais, Trancoso, Portugal	SANT70491	40.81355	-7.43169

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4	MC295	2x (6)	Torre, Ovar, Portugal		40.82751	-8.57485
5	MS01-02 ^s	2x (15)	Rascafria, Madrid, Spain	SANT62736	40.84123	-3.89195
6						
7	MC253	2x (25)	Vila Nova de Paiva, Portugal		40.86050	-7.74368
8	EA001	2x (11)	Folgosa, Castro Daire, Portugal	SANT70496	40.89789	-7.89528
9						
10	MC246A	2x (9)	Devesas, Mêda, Portugal		40.90534	-7.22070
11						
12	MS04-01 ^s	2x (15)	Luras, Sardenha, Italy	SANT61449	40.93919	9.19006
13	MC255	2x (23)	Beselga, Penedono, Portugal		40.94033	-7.41715
14						
15	MC294	2x (16)	Gamoal, Santa Maria da Feira, Portugal		40.95436	-8.59781
16	MC275A	2x (1)	Carvalhosa, Castro Daire, Portugal		40.95601	-7.97051
17						
18	MC254	2x (22)	Moimenta da Beira, Portugal		40.96929	-7.60611
19						
20	MC265	2x (20)	Bigorne, Lamego, Portugal	SANT70501	41.00491	-7.88631
21						
22	MS09-01	2x*	Gallocanta, Zaragoza, Spain		41.01189	-1.48764
23	SC211	2x (16)	Almendra, Vila Nova de foz Côa, Portugal		41.02128	-6.99439
24						
25	MS04-02	2x*	Li Cossi, Trinitá d'Agultu, Italy	SANT61452	41.04651	8.93621
26	MC256	2x (20)	Sebadelhe, Vila Nova de Foz Côa, Portugal		41.05878	-7.28996
27						
28	MC276	2x (18)	Fornelos, Resende, Portugal		41.10547	-7.97133
29						
30	MC264	2x (15)	Varais Lamego, Portugal	SANT70974	41.14550	-7.77917
31						
32	MC257	2x (14)	Ervedosa do Douro, São João da Pesqueira, Portugal	SANT70511	41.17167	-7.47849
33						
34	MS01-03 ^s	2x (5)	Cerezo de Arriba, Segóvia, Spain	SANT62744	41.20521	-3.47356
35	SC220	2x (16)	Cancelas, Paredes, Portugal		41.22045	-8.35026
36						
37	SC221	2x (16)	Gondalães, Paredes, Portugal		41.22737	-8.33442
38	SC222	2x (14)	Vales, Vizela, Portugal		41.24451	-8.29639
39						
40	SC219	2x (21)	Grifão, Paredes, Portugal		41.24545	-8.34648
41						
42	SC218	2x (15)	Sobroso, Paredes, Portugal		41.25119	-8.32535
43	SC194	2x (9)	Via Pouca, Vizela, Portugal		41.25188	-8.29536
44						
45	MC258	2x (9)	Alijo, Portugal	SANT70504	41.26786	-7.45585
46	MC394	2x (8)	Fontiela, Trofa, Portugal		41.28733	-8.56441
47						
48	MC259	2x (9)	Vila Flor, Portugal	SANT70502	41.30274	-7.15472
49						
50	MC263	2x (19)	Bairro da Carvalha, Vil Real, Portugal	SANT70933	41.31980	-7.73189
51						
52	MC245	2x (31)	Alfândega da Fé, Vinhais, Portugal	SANT70488	41.36845	-6.95750
53	MC262	2x (15)	Seixo, Murça, Portugal	SANT70510	41.40044	-7.44749
54						
55	MC388	2x (17)	Infantas, Guimarães, Portugal		41.42072	-8.24202
56	MC260	2x (15)	Golfeiras, Mirandela, Portugal	SANT70503	41.48038	-7.20335
57						
58	MC244	2x (30)	Bairro de Santa Luzia, Miranda do Douro, Portugal	SANT70497	41.49838	-6.29006
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MC243	2x (29)	Macedo de Cavaleiros, Portugal	SANT70486	41.52176	-6.97683
MC385	2x (9)	Vargens, Valpaços, Portugal		41.57491	-7.44362
MC239	2x (29)	Rita, Povia de Lenhoso, Portugal	SANT70490	41.58439	-8.32088
MC393	2x (10)	Rugem, Barcelos, Portugal		41.61484	-8.56782
MC384	2x (10)	Torre de Dona Chama, Mirandela, Portugal		41.65511	-7.14501
SC099	2x (16)	Outeiro, Montalegre, Portugal	SANT76617	41.68602	-7.94060
MC391	2x (12)	Bouças, Terras do Bouro, Portugal		41.72944	-8.30498
MC382	2x (19)	Mosca, Baçal, Portugal		41.76454	-6.80383
MC381	2x (17)	Bolideira, Chaves, Portugal		41.77525	-7.32473
SC120	2x (28)	Praia de Afife, Viana do Castelo, Portugal	SANT69671	41.78630	-8.87042
MC392	2x (12)	Salvador, Ponte da Barca, Portugal		41.80221	-8.35737
SC104	2x (18)	Torneiros, Ourense, Spain	SANT69680	41.85503	-8.11290
SC100	2x (21)	Pitões das Júnias, Montalegre, Portugal	SANT69683	41.86905	-7.95285
MS08-01^s	2x (15)	Zicavo, Córsega, France		41.87789	9.15295
MC237	2x (30)	Coussourado, Paredes de Coura, Portugal	SANT70489	41.92381	-8.63805
MS08-02^s	2x (1)	Campo dell'Oro, Córsega, France		41.92438	8.782612
SC119	2x (13)	As Eiras, Pontevedra, Spain	SANT69674	41.92591	-8.78854
SC097	2x (9)	Parâmio, Braçal, Portugal	SANT68764	41.92869	-6.88478
MC380	2x (4)	Pazos, Ourense, Spain		41.93630	-7.46737
SC151	2x (5)	Porreiras, Paredes de Coura, Portugal		41.94870	-8.55439
SC105	2x (27)	Guxinde, Ourense, Spain	SANT68769	41.97209	-8.15188
SC098	2x (7)	Castrelos, Zamora, Spain	SANT69682	42.00266	-6.89702
MS15-049	2x (7)	Mombuey, Zamora, Spain	SANT71438	42.01321	-6.34481
MS15-051	2x (8)	A, Gudiña, Ourense, Spain	SANT71431	42.04722	-7.13633
MS15-046	2x (6)	Riego de Loma, Zamora, Spain	SANT71436	42.07390	-6.68260
SC107	2x (11)	Fiães, Melgaço, Portugal	SANT76434	42.09481	-8.19062
MC379	2x (13)	Trandeiras, Ourense, Spain		42.10841	-7.66274
SC108	2x (22)	San Bieito, Pontevedra, Spain	SANT76619	42.11550	-8.31584
MS14-061	2x (2)	Lagoa de Samabria, Zamora, Spain	SANT70541	42.11619	-6.72132
MC378	2x (17)	Mandrás, Ourense, Spain		42.14466	-7.94348
SC109	2x (12)	Vixiáns, Pontevedra, Spain	SANT69681	42.26487	-8.33807
SC110	2x (13)	O Piñeiro da Igrexa, Pontevedra, Spain	SANT69679	42.27738	-8.37261
SC111	2x (30)	O Piñeiro da Igrexa, Pontevedra, Spain	SANT76439	42.28235	-8.38272
MS11-01^s	2x (15)	Carrión de los Condes, Paléncia, Spain		42.31130	-4.72234
MS14-126	2x (1)	Saceda, León, Spain	SANT70551	42.32858	-6.52247

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4	MS02-01 ^s	2x (15)	Queralbs, Girona, Spain	SANT60186	42.35393	2.16396
5	MS14-052	2x (4)	O Rego, Ourense, Spain	SANT70534	42.38071	-8.00801
6						
7	MS14-050	2x (2)	Casanova, Ourense, Spain		42.41356	-8.04025
8	MS14-125	2x (1)	Pombriego, León, Spain	SANT70552	42.41944	-6.69567
9						
10	SC162	2x (17)	Montes de Valdueza, León, Spain	SANT70517	42.44958	-6.59802
11						
12	SC164	2x (6)	San Clemente de Valdueza, León, Spain	SANT70554	42.46647	-6.54864
13	SC283	2x (2)	Pradorrey, León, Spain		42.48694	-6.11456
14						
15	SC284	2x (3)	Catro Camiños, Lugo, Spain		42.55973	-7.51190
16						
17	MS14-110	2x (7)	Alto do Cadám, A Noveliza, Pontevedra, Spain	SANT76600	42.60561	-8.26591
18	MS15-120	2x (5)	Moreda do Courel, Lugo, Spain	SANT72398	42.62507	-7.11880
19						
20	SC271	2x (11)	Fresnedo, León, Spain	SANT76432	42.65532	-6.57830
21						
22	MS15-122	2x (5)	Pedrachantada, Lugo, Spain	SANT72404	42.75522	-7.38493
23						
24	SC267	2x (4)	Pedrachantada, Lugo, Spain		42.75539	-7.38570
25	MS14-129	2x (1)	Pico Sacro, A Coruña, Spain		42.80715	-8.44611
26						
27	SC272	2x (10)	Palacios del Sil, León, Spain	SANT76436	42.87388	-6.45031
28	SC273	2x (2)	Caboalles de Abajo, León, Spain	SANT76620	42.87388	-6.45031
29						
30	MS14-121	2x (6)	O Ieboreiro, A Coruña, Spain	SANT76601	42.88406	-7.98270
31						
32	SC075	2x (12)	Gures, A Coruña, Spain	SANT69213	42.91249	-9.14875
33						
34	SC276	2x (16)	Villasecino, León, Spain	SANT76433	42.96125	-6.05127
35	SC274	2x (5)	Brañas de Arriba, Asturias, Spain	SANT76427	43.01310	-6.44518
36						
37	SC275	2x (10)	Pontarás, Asturias, Spain	SANT76614	43.08285	-6.54761
38	MS13-004	2x (1)	Cabo Vilán, LA Coruña, Spain	SANT68615	43.15536	-9.20540
39	MS14-118	2x (1)	Vegadécima, Asturias, Spain	SANT76602	43.15547	-6.95791
40						
41	SC265	2x (3)	Toiral, Lugo, Spain		43.17893	-7.61298
42						
43	MS14-048	2x (6)	Soesto, La Coruña, Spain	SANT70546	43.20609	-9.01620
44	MS14-117	2x (4)	Lago, Asturias, Spain	SANT76603	43.25826	-6.71970
45						
46	MS04-03 ^s	2x (15)	Oiartzun, Gipuzkoa, Spain	SANT61509	43.27755	-1.85519
47	MS15-074	2x (3)	Praia da Barda, A Coruña, Spain	SANT76604	43.28271	-8.92655
48						
49	SC261	2x (27)	As Toxeiras de Riba, Lugo, Spain	SANT76612	43.30417	-7.86336
50						
51	SC245	2x (2)	Beo, La Coruña, Spain		43.32059	-8.84373
52						
53	MS14-045	2x (28)	Faro de Punta Nariga, A Coruña, Spain	SANT70544	43.32071	-8.91042
54	MS14-116	2x (4)	La Estrella, Asturias, Spain	SANT76605	43.32573	-6.46229
55						
56	SC259	2x (19)	O Reguengo, Lugo, Spain	SANT76438	43.39240	-7.80027
57	MS14-112	2x (5)	Vilariño, Lugo, Spain	SANT76606	43.41257	-7.60451
58						
59	MS14-115	2x (3)	Castañedo, Asturias, Spain	SANT76608	43.44945	-6.37391
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3	MS14-113	2x (2)	Serra de Xistral, Lugo, Spain	SANT76607	43.46848	-7.54026
4	SC250	2x (3)	Cariño, A Coruña, Spain	SANT76428	43.47066	-8.31559
5	MS14-114	2x (5)	Coaña, Asturias, Spain	SANT76621	43.50813	-6.74835
6	SC257	2x (7)	A Agraxoiba, A Coruña, Spain	SANT76430	43.52127	-7.94350
7	SC253	2x (6)	Cabo Prior, A Coruña, Spain	SANT76422	43.55296	-8.31155
8	MS10-001^s	2x (15)	Cabo Vidio, Asturias, Spain	SANT64247	43.59356	-6.24420
9	SC256	2x (8)	A Areosa, A Coruña, Spain	SANT73435	43.68170	-8.04729
10	SC254	2x (14)	Punta Candieiro, A Coruña, Spain	SANT76437	43.70746	-8.05096
11	MS16-017	2x*	Ambazac, Limoges, France		45.95951	1.39128
12	M. Sheeby n.n.	2x (15)	Anascaul Lake, Kerry, Ireland	ABD n.n.	52.17875	-10.06399
13	MC367	4x (6)	Amieira, Marinha Grande, Portugal	SANT	39.76900	-8.91144
14	SC038	4x (37)	Amor, Leiria, Portugal	SANT68779	39.79586	-8.85416
15	SC042	4x (34)	Corucho, Bajouca, Portugal	SANT68762	39.86877	-8.83948
16	SC183	4x (13)	Moradias, Pampilhosa da Serra, Portugal	SANT70526	40.09120	-7.99071
17	MC323	4x (8)	Nossa Senhora da Piedade, Lousã, Portugal		40.09878	-8.23438
18	SC185	4x (13)	São Martinho, Fundão, Portugal	SANT70522	40.12071	-7.68483
19	JM002	4x (26)	Parrozelos, Arganil, Portugal	SANT70507	40.20324	-7.90134
20	JC002	4x (20)	Mata da Margaraça, Arganil, Portugal		40.21075	-7.92777
21	JC001	4x (21)	Relva Velha, Arganil, Portugal	SANT70509	40.21613	-7.90580
22	JM001	4x (11)	Mata da Margaraça, Arganil, Portugal	SANT70505	40.21738	-7.92158
23	SC181	4x (5)	São João do Campo, Coimbra, Portugal	SANT70527	40.23508	-8.50015
24	MC290	4x (7)	Caneiro, Penacova, Portugal		40.23770	-8.31345
25	SC168	4x (28)	Ponte, Penacova, Portugal	SANT70524	40.27446	-8.27590
26	MC268	4x (16)	Mata, Montemor-o-Velho, Portugal		40.28449	-8.67892
27	SC299	4x (5)	Gavinhos, Coimbra, Portugal		40.28935	-8.31675
28	MC289	4x (6)	Miro, Penacova Portugal		40.29552	-8.24626
29	MC280	4x (26)	Casalito, Penacova, Portugal		40.29764	-8.31378
30	AA004	4x (7)	Terlamonte, Covilhã, Portugal		40.29845	-7.44582
31	SC285	4x (5)	Casal do Céu, Figueira da Foz, Portugal		40.29999	-8.78180
32	MC267	4x (5)	Casal do João, Cantanhede, Portugal		40.32617	-8.75311
33	SC178	4x (20)	Lemedo, Cantanhede, Portugal	SANT70528	40.33496	-8.61014
34	MC286	4x (1)	Almacinha, Mortágua, Portugal		40.36062	-8.19430
35	SC131	4x (3)	Lagoa Seca, Serra da Estrela, Portugal	SANT68773	40.37139	-7.63578
36	SC145	4x (5)	Ponte Jugais, Seia, Portugal	SANT68777	40.38427	-7.70510
37	JC011	4x (32)	Seia, Portugal		40.41750	-7.70591

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4	MC266	4x (6)	Cabeços, Vagos, Portugal		40.42994	-8.65257
5	MC270	4x (14)	S. Lourenço do Bairro, Anadia, Portugal		40.44003	-8.47327
6						
7	MC297	4x (2)	Bunheira, Oliveira do Bairro, Portugal		40.52468	-8.51850
8						
9	SC175	4x (5)	Bolfiar, Águeda, Portugal	SANT70529	40.56382	-8.39382
10	SC174	4x (30)	Arca, Oliveira de Frades, Portugal	SANT70520	40.60769	-8.21392
11						
12	SC173	4x (21)	Agros, Vouzela, Portugal	SANT70521	40.63570	-8.19801
13						
14	MC141	4x (29)	Ponte de S. Tiago, Sever do Vouga, Portugal		40.70100	-8.37497
15	SC172	4x (17)	Vilar, São Pedro do Sul, Portugal	SANT70525	40.77453	-8.07215
16						
17	MC301	4x (14)	Paredes, Vale de Cambra, Portugal		40.81652	-8.37489
18	DT001	4x (6)	Paço de Mato, Vale de Cambra, Portugal	SANT70530	40.85100	-8.31014
19						
20	LM004	4x (7)	Drave, Arouca, Portugal		40.85963	-8.11861
21						
22	MC252	4x (13)	Ribolhos, Castro Daire, Portugal		40.88682	-7.92844
23	MC275	4x (31)	Carvalhosa, Castro Daire, Portugal		40.95807	-7.96297
24						
25	MC279	4x (32)	Canedo, Santa Maria da Feira, Portugal		41.01160	-8.45774
26	MC278	4x (32)	Santa Cecília, Castelo de Paiva, Portugal		41.04545	-8.27445
27						
28	MC277	4x (11)	Cidadelhe, Cinfães, Portugal		41.07827	-8.08915
29						
30	SC223	4x (19)	Boialvo, Gondomar, Portugal		41.08075	-8.47271
31						
32	SC091	4x (18)	Fragas, Amarante, Portugal	SANT68763	41.26528	-7.90186
33	SC152	4x (5)	Cotorinho, Vila Real, Portugal		41.27313	-7.87249
34						
35	SC089	4x (17)	Candemil, Amarante, Portugal	SANT76616	41.27966	-7.91785
36	MC387	4x (13)	Veade, Celorico de Basto, Portugal		41.41861	-7.97825
37						
38	MC241	4x (29)	Portela de Santa Eulália, Ribeira de Pena, Portugal	SANT70523	41.50069	-7.79296
39						
40	MC389	4x (11)	Figueiró do Monte, Fafe, Portugal		41.55806	-8.07242
41						
42	MC390	4x (11)	Pombal, Vieira do Minho, Portugal		41.57658	-8.11106
43						
44	MC377	4x (16)	Barracão, Montalegre, Portugal		41.76378	-7.70449
45	MS09-02	4x (1)	Illa do Faro, Galiza, Spain	SANT60536	42.21562	-8.90836
46						
47	SC115	4x (10)	Limens, Pontevedra, Spain	SANT69676	42.25865	-8.81608
48						
49	MS14-060	4x (1)	As Ermidas, Ourense, Spain	SANT70537	42.28400	-7.12801
50	MS14-053	4x (2)	Vale de Marcelle, Ourense, Spain	SANT70533	42.30219	-7.61265
51						
52	MS15-113	4x (7)	Soutomaior, Pontevedra, Spain	SANT72405	42.33273	-8.56529
53	MS14-057	4x (1)	Mendoia, Ourense, Spain	SANT70532	42.33795	-7.22352
54						
55	MS14-055	4x (3)	Piñeira, Ourense, Spain	SANT70536	42.36158	-7.40242
56						
57	SC112	4x (19)	Forzans, Pontevedra, Spain	SANT76440	42.36261	-8.45016
58	MS14-059	4x (2)	O Barco, Ourense, Spain	SANT70538	42.42409	-7.01482
59						
60	MS15-043	4x (6)	Fontaíñas, Ourense, Spain	SANT71426	42.45200	-8.02619

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3	MS15-118	4x (5)	A Frieira, Lugo, Spain	SANT72397	42.52176	-7.36829
4	RC001	4x (5)	Vilamor, Lugo, Spain	SANT72406	42.56537	-7.23140
5	MS15-119	4x (4)	Parada dos Montes, Lugo, Spain	SANT72399	42.57203	-7.27245
6	MS14-049	4x (9)	Taboada, Pontevedra, Spain	SANT70535	42.68757	-8.22030
7	SC269	4x (7)	Pedrafita do Cebreiro, Lugo, Spain	SANT76431	42.71282	-7.00560
8	SC268	4x (6)	Fillobal, Lugo, Spain	SANT76615	42.73892	-7.18958
9	MS15-123	4x (4)	Paradela, Lugo, Spain	SANT72400	42.76807	-7.56978
10	MS15-124B	4x (3)	Paradela, Lugo, Spain	SANT72403	42.77391	-7.62728
11	MS15-124A	4x (4)	Paradela, Lugo, Spain	SANT72402	42.77436	-7.62736
12	SC072A	4x (7)	Ventim, A Coruña, Spain	SANT69208	42.80037	-9.02645
13	MS09-03	4x (2)	Santiago de Compostela, A Coruña, Spain	SANT60533	42.87518	-8.55576
14	SC266	4x (10)	Sabarei de Abaixo, Lugo, Spain	SANT76613	42.89582	-7.47154
15	SC195	4x (6)	Parada, A Coruña, Spain		42.96313	-8.84410
16	MS14-119	4x (2)	Gondar, Lugo, Spain	SANT76609	43.02258	-7.41816
17	MS14-111	4x (5)	O Porto Velho, Lugo, Spain	SANT76610	43.18596	-7.95939
18	SC263	4x (7)	San Salvador, Lugo, Spain	SANT76611	43.20826	-7.83605
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32	Mixed-ploidy populations					
33	SC203	2x (1), 4x (15)	Vila Rosa, Seia, Portugal		40.50062	-7.81098
34	MC274	2x (1), 4x (25)	Fareja, Castro Daire, Portugal		40.89768	-7.91486
35	MC235	2x (19), 4x (14)	Sobroso, Paredes, Portugal	SANT70500	41.25087	-8.33041
36	MC386	2x (8), 4x (1)	Nozedo, Vila Pouca de Aguiar, Portugal		41.50717	-7.62201

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3 97 **Table S3** – Selected environmental variables using the sampled diploid and tetraploid
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5 98 populations of *Jasione montana*. For each cytotype, mean and standard error of the mean (Mean
6
7 99 \pm SE) are given. Different letters represent statistically significant differences between cytotypes
8
9 100 at $P < 0.05$, n.s. denote non-significant differences at $P > 0.05$.

Variables	CODE	Diploid	Tetraploid
		Mean \pm SE, n = 462	Mean \pm SE, n = 53
Temperature seasonality	Bio_4	5451.46 \pm 41.39 ^a	4530.78 \pm 88.31 ^b
Maximum temperature of warmest month	Bio_5	278.04 \pm 1.72 ^a	256.70 \pm 2.51 ^b
Precipitation of driest month	Bio_14	19.56 \pm 0.74 ^{n.s.}	18.82 \pm 1.21 ^{n.s.}
Precipitation seasonality	Bio_15	41.57 \pm 0.64 ^a	49.44 \pm 0.82 ^b
Base saturation of the tops	bs_top	1.56 \pm 0.03 ^a	1.90 \pm 0.04 ^b
Dominant surface textural class of the STU	txsrfdo	1.89 \pm 0.03 ^{n.s.}	1.85 \pm 0.05 ^{n.s.}

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Table S4 – Genome size estimates in *Jasione montana*. In each population, mean, standard deviation of the mean (SD), coefficient of variation (CV, in %) and minimum (Min) and maximum (Max) values of holoploid genome size (2C, in pg) are given for diploids and tetraploids. Information about the number of individuals analysed in each population (N) and mean monoploid genome size (1Cx, in pg) are also presented. In bold the mixed-ploidy populations are highlighted.

ID code	Holoploid genome size (2C)						Monoploid genome size (1Cx)
	Mean	SD	CV (%)	Min	Max	N	
Diploids	2.92	0.07	2.2	2.80	3.08	213	1.46
DT017	2.91	0.02	0.7	2.89	2.93	3	1.45
JCO001	2.96	0.01	0.3	2.95	2.97	3	1.48
LM009	2.86	0.03	1.1	2.83	2.88	2	1.43
LM014	2.89	0.02	0.5	2.88	2.90	2	1.45
MC246A	2.89	0.03	1.1	2.84	2.92	7	1.44
MC247	2.81	-	-	-	-	1	1.40
MC248	2.81	0.00	0.2	2.81	2.82	2	1.41
MC249	2.88	0.08	2.7	2.80	3.00	7	1.44
MC253	2.91	0.04	1.2	2.88	2.96	4	1.45
MC254	2.85	0.05	1.6	2.81	2.96	9	1.42
MC263	2.83	-	-	-	-	1	1.42
MC274	2.97	-	-	-	-	1	1.49
MC275A	2.90	-	-	-	-	1	1.45
MC276	2.91	0.03	1.1	2.88	2.93	3	1.46
MC283	2.94	-	-	-	-	1	1.47
MC298	2.85	-	-	-	-	1	1.42
MC306	2.95	-	-	-	-	1	1.47
MC307	2.87	-	-	-	-	1	1.43
MC309	2.88	0.04	1.6	2.85	2.93	3	1.44
MC329	2.88	0.06	2.0	2.84	2.95	3	1.44
MC330	2.97	0.01	0.3	2.96	2.98	3	1.48
MC334	3.00	0.02	0.7	2.98	3.02	3	1.50
MC235	2.91	0.07	2.5	2.81	3.00	8	1.45
MC336	2.90	0.02	0.7	2.88	2.92	3	1.45
MC340	2.87	0.00	0.0	2.87	2.87	2	1.44
MC341	2.91	0.09	3.2	2.84	3.01	3	1.45
MC346	2.89	0.06	2.1	2.84	2.96	3	1.45
MC350	2.95	0.01	0.3	2.94	2.95	3	1.47
MC354	2.85	-	-	-	-	1	1.43
MC360	2.87	-	-	-	-	1	1.43
MC378	3.00	-	-	-	-	1	1.50
MC378	2.89	-	-	-	-	1	1.44
MC386	2.92	0.04	1.5	2.89	2.95	2	1.46
MS13-004	3.05	-	-	-	-	1	1.53
MS14-118	2.87	-	-	-	-	1	1.44

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3	MS14-121	2.92	0.04	1.3	2.87	2.95	3	1.46
4	MS14-125	2.85	-	-	-	-	1	1.42
5	MS14-126	2.88	-	-	-	-	1	1.44
6	MS14-129	2.99	-	-	-	-	1	1.49
7	MS15-046	2.82	0.01	0.4	2.81	2.83	3	1.41
8	MS15-049	2.82	0.03	1.0	2.80	2.84	2	1.41
9	MS15-051	2.86	0.08	2.9	2.81	2.96	3	1.43
10	MS15-062	2.90	0.07	2.5	2.84	2.98	3	1.45
11	MS15-065	2.88	0.06	2.0	2.84	2.94	3	1.44
12	MS15-069	2.85	0.05	1.7	2.81	2.90	3	1.42
13	MS15-074	3.06	0.01	0.5	3.05	3.07	2	1.53
14	SC120	2.87	0.03	1.2	2.83	2.89	3	1.43
15	SC164	2.84	0.03	1.0	2.82	2.87	3	1.42
16	SC169	2.91	-	-	-	-	1	1.45
17	SC203	2.83	-	-	-	-	1	1.41
18	SC208	2.90	-	-	-	-	1	1.45
19	SC210	2.91	-	-	-	-	1	1.45
20	SC211	2.99	-	-	-	-	1	1.49
21	SC221	2.97	0.04	1.2	2.93	3.01	3	1.49
22	SC225	2.87	0.04	1.5	2.84	2.90	2	1.44
23	SC227	2.98	-	-	-	-	1	1.49
24	SC229	3.03	-	-	-	-	1	1.52
25	SC230	2.95	-	-	-	-	1	1.48
26	SC231	2.92	-	-	-	-	1	1.46
27	SC232	3.00	-	-	-	-	1	1.50
28	SC236	2.91	-	-	-	-	1	1.46
29	SC238	3.02	-	-	-	-	1	1.51
30	SC239	2.91	0.01	0.4	2.90	2.92	2	1.46
31	SC241	2.96	-	-	-	-	1	1.48
32	SC245	3.02	0.02	0.7	3.01	3.04	2	1.51
33	SC250	2.99	0.03	1.1	2.96	3.02	3	1.50
34	SC253	2.99	0.02	0.7	2.96	3.03	6	1.49
35	SC254	3.04	0.03	0.9	3.01	3.08	7	1.52
36	SC256	3.04	0.03	1.1	3.00	3.08	4	1.52
37	SC257	3.05	0.03	1.1	3.01	3.07	3	1.52
38	SC259	3.02	0.03	1.1	3.00	3.04	2	1.51
39	SC261	3.00	0.02	0.5	2.99	3.02	3	1.50
40	SC265	2.94	0.02	0.7	2.93	2.96	2	1.47
41	SC267	2.93	0.03	1.1	2.90	2.97	4	1.46
42	SC271	2.90	0.04	1.4	2.86	2.95	5	1.45
43	SC272	2.92	0.01	0.5	2.90	2.93	5	1.46
44	SC273	2.98	0.02	0.5	2.97	2.99	2	1.49
45	SC274	2.94	0.03	0.9	2.92	2.97	3	1.47
46	SC275	2.96	0.01	0.3	2.95	2.97	3	1.48
47	SC276	2.88	0.04	1.4	2.82	2.92	5	1.44
48	SC283	2.90	0.02	0.7	2.89	2.92	2	1.45
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3	SC284	2.93	0.03	0.9	2.91	2.96	3	1.46
4	SC75	3.03	0.03	0.8	3.02	3.06	3	1.52
5	SC97	2.83	0.01	0.4	2.82	2.83	2	1.41
6	SC98	2.82	-	-	-	-	1	1.41
7								
8	<i>Tetraploids</i>	5.86	0.12	2.1	5.63	6.06	47	1.47
9								
10	AA004	5.97	0.06	1.0	5.93	6.01	2	1.49
11	JC011	6.29	-	-	-	-	1	1.51
12	MC274	6.10	0.07	1.1	6.03	6.16	1	1.48
13	MC286	6.23	0.10	1.6	6.12	6.31	1	1.49
14	MC323	5.91	-	-	-	-	3	1.46
15	MC235	5.95	-	-	-	-	1	1.49
16	MC386	5.97	-	-	-	-	1	1.51
17	MS14-057	6.24	-	-	-	-	1	1.49
18	MS14-060	5.85	0.07	1.2	5.78	5.93	1	1.43
19	MS15-043	6.22	-	-	-	-	3	1.48
20	MS15-113	6.02	-	-	-	-	1	1.51
21	MS15-118	5.97	-	-	-	-	3	1.41
22	MS15-119	5.72	-	-	-	-	3	1.43
23	MS15-123	6.08	0.20	3.4	5.80	6.29	2	1.44
24	MS15-124A	6.02	-	-	-	-	1	1.46
25	MS15-124B	5.65	0.03	0.6	5.63	5.69	2	1.42
26	RC001	5.70	0.07	1.2	5.63	5.74	3	1.46
27	SC131	5.78	0.09	1.5	5.71	5.84	3	1.49
28	SC203	5.82	-	-	-	-	5	1.43
29	SC230	5.68	0.02	0.4	5.67	5.70	1	1.49
30	SC263	5.86	0.06	1.1	5.78	5.90	3	1.48
31	SC266	5.97	0.07	1.2	5.89	6.03	2	1.49
32	SC269	5.72	0.09	1.5	5.65	5.87	1	1.52
33	SC285	6.19	-	-	-	-	1	1.51
34	SC299	6.29	-	-	-	-	1	1.47
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