Title: Reproductive modes in populations of late-acting self-incompatible and self-compatible polyploid *Ludwigia grandiflora* subsp. *hexapetala* in western Europe

Short running title: Mixed reproductive modes in *Ludwigia*

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Abstract

Reproductive mode, *i.e.,* the proportion of individuals produced by clonality, selfing and outcrossing in populations, determines how hereditary material is transmitted through generations. It shapes genetic diversity and its structure over time and space, which can be used to infer reproductive modes.

Ludwigia grandiflora subsp. *hexapetala* (*Lgh*) is a partially clonal, polyploid, hermaphroditic, and heteromorphic plant that recently colonized multiple countries worldwide. In western Europe, individuals are either self-incompatible caused by a late-acting self-incompatibility (LSI) system developing long-styled flowers, or self-compatible (SC), with short-styled flowers.

In this study, we genotyped 53 long- and short-styled populations newly colonizing France and northern Spain using SNPs to estimate rates of clonality, selfing and outcrossing. We found that populations reproduced mainly clonally but with a high diversity of genotypes along with rates of sexuality ranging from 10% up to 40%. We also found evidence for local admixture between long- and short-styled populations in a background of genetic structure between floral morphs that was twice the level found within morphs. Long- and short-styled populations showed similar rates of clonality but short-styled populations presented significantly higher rates of selfing, as expected considering their breeding system, and despite the small rates of failure of the LSI system. Within the 53 studied populations, the 13 short-styled populations had fewer effective alleles, lower observed heterozygosity, and higher inbreeding coefficients, linkage disequilibrium and estimates of selfing than what was found in long-styled populations. These results emphasize the necessity to consider the variation of reproductive modes when managing invasive plant species. The overall maintenance of higher genetic diversity with the possibility of maintaining populations clonally in the absence of compatible partners may explain why long-styled individuals seem to be more prevalent in all newly expanding populations worldwide. Beyond *Lgh*, our methodological approach may inspire future studies to assess the reproductive modes in other autopolyploid populations.

Keywords: Partial clonality, Mating system, Late-acting self-incompatibility system, Selfing, Outcrossing, Autopolyploidy, Water primrose

Introduction

Plants reproduce using different mechanisms (i.e., breeding system), including clonality and different types of sexuality (i.e., mating system, including allogamy and selfing; Holsinger 2000). Reproductive mode corresponds to the actual balance between sexual and clonal reproduction, measured by the rate of clonality (De Meeus et al. 2007; Stoeckel et al. 2021a), and, within the proportion of offspring produced by sexuality, the balance between autogamy and geitonogamy (hereafter *selfing*), and allogamy (*outcrossing*), measured by the rate of selfing (Bürkli et al. 2017). Reproductive mode is one of the main drivers of genetic diversity in populations and species (Duminil et al. 2007, Ellegren & Galtier 2016, Glémin et al. 2019). It determines the way hereditary material is transmitted over generations, and thus constrains the range of genetic diversity that can evolve within populations and species (De Meeus et al. 2007, Fehrer 2010, Stoeckel & Masson 2014, Rouger et al. 2016). Its genetic consequences are deep enough to be discernible even in the presence of other affecting factors (Charlesworth 2003). Considering its influence on population genetic diversity and structure, reproductive mode is one of the major biological traits to decipher before interpreting other biological and ecological forces using molecular data (Duminil et al. 2007, Reichel et al. 2016, Bürkli et al. 2017, Orive & Krueger-Hadfield 2021). Given an adequate theoretical framework, population geneticists can use genotypic data to infer reproductive modes in populations (Halkett et al. 2005, Arnaud-Haond et al. 2007, Hardy 2016, Bürkli et al. 2017, Stoeckel et al. 2021a).

Many plant species reproduce using different breeding systems they adapt to different ecological contexts resulting into different reproductive modes (Richards 1997, Charlesworth 2006).

69 Uniparental reproduction, including clonality and selfing, may help plants to spread into new areas
70 (Baker's conjecture: Barrett et al. 2008, Pannell et al. 2015). Clonality consists of a parent producing
71 a new descendant that is a genetic copy of itself with the exception of rare somatic mutations and
72 mitotic recombinations (De Meeus et al. 2007). It results into an average excess of heterozygotes
73 compared to Hardy-Weinberg expectations (*i.e.,* negative F_{IS}), large variance of F_{IS} along the genome
74 and a small excess of linkage disequilibrium, increasing with decreasing population size (Navascues
75 et al. 2010, Stoeckel & Masson 2014, Stoeckel et al. 2021a). When parents can yield multiple
76 descendants by clonality, it may generate repeated genotypes in populations (Arnaud-Haond et al.
77 2007). These effects vary with the rates of clonality and, which in turn, can be used to infer these
78 rates within populations (De Meeus et al. 2006, Becheler et al. 2020, Arnaud-Haond et al. 2020).
79 Hermaphroditic plants have the possibility to sexually reproduce with themselves (selfing). Selfing
80 decreases effective population sizes and gene diversity resulting into high probability of allele fixing
81 (Wright et al. 2008, Roze 2016, Glémin et al. 2019). Selfing also prohibits genetic mixing between
82 different ancestral lineages, which decreases heterozygosity within individuals (David et al. 2007,
83 Hardy 2016) and strongly increases linkage disequilibrium between genes along genomes (Golding
84 & Strobeck 1980, Norborg 2000, Lucek & Willi 2021). Around half of hermaphrodite plants restrict
85 self-fertilization using a variety of molecular mechanisms grouped under the term "self-
86 incompatibility (SI) systems" which favour outcrossing within populations (De Nettancourt 2001,
87 Castric & Vekemans 2004, Gibbs 2014, Steinecke et al. 2022). Outcrossing is overall expected to
88 increase genetic diversity, to limit allele fixation and to reduce linkage disequilibrium when
89 compared to selfing, but only if linked to the genes involved in self-recognition (Glémin et al. 2001,
90 Stoeckel et al. 2006, Navascues et al. 2010). Within the different identified SI systems (Charlesworth
91 et al. 2005, Franklin-Tong 2008), the late-acting SI system (hereafter LSI) is still poorly studied
92 despite being identified in multiple taxonomic groups in angiosperms (De Nettancourt 1997,
93 Gibbs 2014). In LSI systems, self-pollen tubes grow and are only blocked shortly before penetrating

the ovule. Such species may present reduced female fertility due to self-pollen disabling ovules, favouring the clonal regeneration of populations (Vaughton et al. 2010). LSI systems are also characterized by low but recurrent failures of the self-recognition system due to its late mechanism, leading to the production of a low amount of selfed seeds in populations (Seavey & Bawa 1986; Chen et al. 2012, Gibbs 2014). In contrast, gametophytic and sporophytic self-recognition occurring very early in the pistil drastically limit self-pollination and thus selfed seeds (Lawrence 1985, Gibbs 2014). However, we do not know yet if these rare selfed seeds contribute to the dynamics of genetic diversity of LSI populations and species, especially in peripatric conditions or in any situation when compatible partners may lack. Due to these selfed seeds, LSI system may be relatively ineffective at driving genetic diversity within populations, contrary to gametophytic and sporophytic SI systems (Brennan et al. 2002, Stoeckel et al. 2006, Koelling et al. 2011, Busch & Urban 2011). In such situations, the emergence and maintenance of LSI systems appear as new evolutionary puzzles among the reproductive systems.

Water primrose*, Ludwigia grandiflora* subsp. *hexapetala* (Hook. & Arn.) Nesom and Kartesz (2000), hereafter *Lgh*, is an insect pollinated, partially clonal, hermaphroditic and heteromorphic plant supposed to be native from central and south America. This species is one of the most invasive aquatic plants in the world (Thouvenot et al. 2013). *Lgh* is a decaploid species (2n=10x=80 chromosomes), resulting from hybridization of different ancestral diploid species, some of which are represented more than once in the total genome of *Lgh*, which belongs to the genus *Ludwigia* L. section Jussiaea (Hoch et al. 2015, Barloy et al, 2024). Interestingly, *Lgh* includes an autotetraploid set of chromosomes, shared with *L. peploides* subsp. *montevidensis* (2n=2x=16), hereafter *Lpm*, that is clearly distinct from the other ancestral part of the *Lgh* genome (Barloy et al. 2024). *Lpm* is a self-compatible-only diploid species with only one common floral morphology (Estes & Thorp 1974, Grewel et al. 2016). *Lgh* presents heteromorphic flowers corresponding to two floral morphs: L-morph individuals develop long-styled flowers and S-morph individuals develop short-styled

flowers, that cross and result into 100% viable and fertile F1 and F2 descendants (Portillo-Lemus 2021, Portillo-Lemus et al. 2021) while inter-species crosses only result in a low number of chlorotic and unfertile descendants (Barloy et al. 2024).

All tested L-morph flowers expressed an active LSI in western European populations, with only one self-incompatible type detected so far (Portillo-Lemus et al. 2022). During the core flowering season (summer), in experimental greenhouse conditions, L-morph individuals show a stable seemingly insignificant rate of autogamy (around 0.2‰ of the available ovules) that increases at the end of the flowering season, during autumn, to 1‰, which is common in LSI systems (Gibbs 2014). Due to the massive blossoming of this species, growing in very dense populations, these selfed seeds would add up yearly in field populations to a hundred seeds per square meter (Portillo-Lemus et al. 2022). This pattern of low rates of autogamy, that increases at the end of the flowering season, may provide the advantage of reproductive assurance (Goodwillie & Weber 2018). In contrast, all tested S-morph individuals were self-compatible (SC) in western European populations but in their pistils, pollen tubes of the L-morph growed significantly faster and were thus advantaged to fertilize ovules when in competition with self-pollen (Portillo-Lemus et al. 2022). In addition, peripatric *Lgh* populations, including European populations, were previously reported as exclusively clonal with few clonal lines (Dandelot 2004, Okada et al. 2009). Recently established populations in France and northern Spain mostly present only one of the two compatibility modes locally, sometimes with a population of the other type a few to tens of kilometers away, which may result in effective allogamy (Portillo-Lemus et al. 2021, 2022). All these different breeding systems make possible very different reproductive modes in populations, comprising all possible quantitative combinations of mainly clonal, autogamous and allogamous modes.

Here, we assessed the reproductive modes of 53 invasive *Lgh* populations across western Europe. Considering the complex case of *Lgh* in western European populations, we hypothesized that L-morph populations supposed to express a LSI should present typical genetic footprints of dominant

144 outcrossing, or alternatively higher rates of clonality due to the local lack of compatible partners
145 and self-pollen interferences, while S-morph populations supposed to be SC should present higher
146 rates of sexuality prevailed by selfing. To understand the genetic impacts of reproductive modes,
147 we also quantified the covariations of genetic indices and their importance to define the genetic
148 diversity within the 53 genotyped populations, and compared these observations to the theoretical
149 expectations obtained from a Wright-Fisher-like model extended to autotetraploids (Stoeckel et al.
150 2024). Finally, we took advantage from the fact that recent local populations in France and northern
151 Spain still present only one of the two compatibility modes to compare and interpret the genetic
152 differences found within and between L-morph (LSI) and S-morph (SC) populations, with the aim to
153 assess the influence of LSI on genetic diversity, as similarly tackled in sporophytic self-incompatible
154 and SC Brassicaceae species previously (Koelling et al. 2011).
155

156 # Materials and methods

157 **Sampling strategy and floral morphology of populations**

158 We collected 795 stems of *Lgh* from 53 locations (52 locations in France and one in Catalonia, Spain),
159 corresponding to an area that spans 580 kilometres east-to-west and 1,100 kilometres north-to-
160 south (Figure SI1). At each location, we collected 15 stems (hereafter, 'individuals') along a linear
161 transect of 40 meters. Along each transect, we randomly collected three stems at coordinates X_1 =
162 0m; X_2 = 10m; X_3 = 20m; X_4 = 30m and X_5 = 40m within a one meter-square quadrat. The young
163 leaves of each sampled individual were stored after lyophilization until DNA extraction.
164 We visually identified floral morphologies of flowers found along each transect within the sampled
165 *Lgh* populations. In a previous study on seven populations among the 53 studied here (underlined
166 population names in Figure SI1), one hundred and five sampled individuals resulted to identify a
167 binary distribution of floral morphology with formally-identified self-incompatibility types: all L-

168 morph individuals were LSI typed and all S-morph individuals were SC typed (Portillo-Lemus et al
169 2021) while all these individuals succeeded to cross and give viable and fertile plants. Interestingly,
170 these two types of populations spatially distribute in monomorphic populations along different
171 rivers. We supposed for this study the LSI versus SC status of individuals and populations using their
172 floral morphologies: L-morph individuals were supposed to develop a LSI system with only one self-
173 incompatible type found so far and S-morph individuals being SC. To support this conjecture, as
174 done in Portillo-Lemus et al. (2021), we checked the fruitset in each of the 53 sampled and
175 genotyped populations: A low fruitset, or even no fruit at all were found in L-morph individuals and
176 populations, while full fruitsets were found in S-morph individuals and populations, in agreement
177 with our conjecture.
178 In addition to the crossbreeding results in which all L- and S-morph individuals succeeded to cross,
179 giving full fruit set and 100% viable first- and second-generation descendants (Portillo-Lemus et al.
180 2021), we here counted the chromosome numbers on karyotypes of S-morph individuals sampled
181 in two fruitful populations and of L-morph individuals sampled in five fruitless populations to
182 validate that L- and S-morph individuals belong to *Lgh*. Between 50 to 150 kilometers separated two
183 consecutive samples (populations underlined Figure SI1). To prepare the karyotypes, we used the
184 method detailed in Barloy et al (2024) that already karyotyped a S-morph individual sampled near
185 the French Atlantic coast. The same method was used in Bou Manobens et al. (2019) to karyotype
186 a L-morph individual sampled in Catalunya.
187
188 **Definition of the autotetraploid SNP marker set**
189 As no molecular markers suitable for clonal discrimination were yet available for *Lgh* and *Lpm*, we
190 generated an original set of SNP markers via RAD-Seq (Baird et al., 2008) using two pools of 15
191 individuals each, respectively sampled across five *Lgh* and three *Lpm* western European populations.
192 RAD DNA library generation, sequencing and the analysis pipeline to identify *Lgh* SNP markers were

193 carried out as described in Delord et al. (2018). In brief, DNA of *Lgh* and *Lpm* were digested by *Sbfl*
194 restriction enzyme and used to prepare DNA libraries that were then sequenced using an Illumina
195 HiSeq3000 (150bp paired-end reads). A total of 14,233 and 34,287 RAD-Seq-determined SNPs were
196 filtered to yield 340 and 326 SNPs, one per aligned sequence, for *Lpm* and *Lgh*, respectively. Design
197 of primers compatible with Hi-Plex multiplexing was carried out by Melbourne Bioinformatics.
198 Finally, sixty and fifty SNP markers matched the quality criteria to design a Hi-Plex set of SNPs for
199 *Lpm* and *Lgh*, respectively (Hammet et al. 2019). In our study, we only considered polymorphic SNPs
200 that were shared between *Lgh* and *Lpm* to be sure they belong to the tetraploid part of *Lgh* genome
201 derived from *Lpm* (Barloy et al. 2024). We finally kept this set of 36 polymorphic and stable SNP
202 markers to genotype *Lgh* samples and analyze genetic diversity in each sampled population (primer
203 sequences are openly listed in Stoeckel et al. 2023).
204 To verify that this set of SNPs was really tetraploid, we computed the Akaike's information criterion
205 from the maximum likelihood of the best genotype considering the distribution of sequenced allele
206 countings among individuals and markers as a function of the ploidy level, using a similar approach
207 to that proposed by Burnham & Anderson (2002).

208 $$AIC(ploidy) = \sum_{l=1}^{l=36} \sum_{i=1}^{i=795} -2 \log \mathcal{L}\left(genotype_{i,l} \middle| ploidy, data_{i,l}\right) + 2K$$

209 where $data_{i,l}$ is the distribution of sequenced alleles among A, C, G and T within individual i at locus
210 l, and K is the number of possible genotypes given the ploidy and the four possible alleles (A, C, G
211 and T). The likelihood of the possible genotypes $\mathcal{L}\left(genotype_{i,l} \middle| ploidy, data_{i,l}\right)$ follows a
212 multinomial distribution of the sequenced allele countings distributed between the four different
213 possible nucleobases (A, C, G and T).
214

215 **DNA extraction, SNP marker production and genotyping**

216 To genotype the 795 individually-tagged samples with these 36 SNPs, we extracted DNA from 25 to
217 30 mg of dried young leaf tissues. The DNA extractions were performed using the NucleoSpin Plant II
218 from MACHEREY-NAGEL kit, following the manufacturer's recommendations. We used L1 buffer as
219 lysis buffer. All individuals were genotyped from a solution of 20ng/µl of *Lgh* DNA, using a modified
220 version of the Hi-Plex protocol (Hammet et al. 2019; Besnard et al. 2023). Hi-Plex is an amplicon
221 sequencing technique (*sensu* Meek & Larson 2019) in which all SNPs are co-amplified in a multiplex
222 reaction before Illumina or Ion-Torrent sequencing. Here, we used Illumina. Intermediate steps
223 include dual indexing of individual samples used for demultiplexing. Reads were then assigned to
224 loci by aligning them to reference sequences with BWA-MEM 0.7.15-r1140 (Li & Durbin 2010) and
225 alleles were counted with Samtools 1.9 (Danecek et al. 2021).

226

227 **Allele dosage**

228 The posterior probabilities of each single SNP genotype within each individual (hereafter *single SNP*
229 *genotype*) were computed using the likelihood of all possible genotypes considering a multinomial
230 distribution of the number of times each nucleobase was genotyped at one SNP marker within one
231 individual. In order to obtain the most confident dataset possible, we only assigned a genotype
232 when its posterior probability of allele dosage exceeded 70%. When one SNP within one individual
233 presented a posterior probability of allele dosage equal or lower than 70%, we assigned and
234 analysed it afterward as a missing genotype.

235

236 **Genotypic and genetic descriptors**

237 We expected that populations reproducing clonally may yield repeated multi-locus genotypes
238 (MLGs, i.e., the same genotype at all the 36 SNPs found in multiple individuals). By possibly

239 producing these repeated genotypes and by varying the relative distribution of the number of
240 samples of each of these distinct genotypes, rates of clonality impact genotypic richness and
241 evenness in populations (Halkett et al. 2005; Arnaud-Haond et al. 2007).
242 We measured genotypic richness using the R index (Dorken & Eckert 2001, Arnaud-Haond et al.
243 2005), which is defined as:
244 $R = \frac{G-1}{N_g-1}$
245 where G is the number of distinct genotypes (genets) and N_g is the number of genotyped individuals.
246 We also measured genotypic evenness as the parameter β of the Pareto distribution, which
247 describes the slope of the power-law inverse cumulative distribution of the size of MLGs (Arnaud-
248 Haond et al. 2007):
249 $N_{\geq X} = aX^{-\beta}$
250 where $N_{\geq X}$ is the number of ramets in genets containing X or more ramets in the sampled
251 population, and the parameters *α* and *β* are fitted by regression analysis. Low β-values indicate the
252 dominance of one or few clonal lineages. Pareto β is less biased by the sampling effort than clonal
253 richness R (Stoeckel et al. 2021a). In our sample of 15 individuals, we expected a population with
254 only one repeated MLG to present a Pareto β value of 0.03 and a value of 3 in samples with no
255 repeated genotypes. β < 2 indicates a population reproducing with high rates of clonality (greater
256 than 0.8 to 0.9). For a sample size of 15 individuals, 2 < β < 3 indicates intermediate to low rates of
257 clonality (0.6 to 0.8). A β at 3, its maximum value, is indicative of a mainly sexual population, with
258 rates of clonality ranging from zero to 0.6, depending on the other genetic indices.
259 Rates of clonality and selfing confer different effects on the range of within-population genetic
260 polymorphism as well as on probabilities of genetic identities within individuals. We thus also
261 estimated expected and observed heterozygosity H_E and H_O, allelic richness A_E, inbreeding
262 coefficient F_{IS}, and linkage disequilibrium within each sampled population.

263 The inbreeding coefficient F_{IS} (Wright 1931, 1949) accounts for intraindividual genetic variation at
264 one locus as a departure from Hardy-Weinberg assumptions of the genotyped populations. We
265 computed one F_{IS} value per locus per population. We then reported the mean values of F_{IS} (MF_{IS})
266 and the inter-locus variance of F_{IS} ($VarF_{IS}$) for each population. Both MF_{IS} and $VarF_{IS}$ are very
267 informative about the underlying reproductive systems (Stoeckel et al. 2006, David et al. 2007).
268 Clonality makes the MF_{IS} values tend toward -1 along with high interlocus variance (Stoeckel &
269 Masson 2014, Stoeckel et al. 2021a). A moderate amount of sexual reproduction results in MF_{IS}
270 values around 0 (Balloux et al. 2003). $VarF_{IS}$ varies with rates of clonality, from very limited variance
271 expected in sexual populations to high variance in very clonal populations (Stoeckel & Masson 2014,
272 Stoeckel et al. 2021a). Positive MF_{IS} values are expected in populations reproducing using
273 consanguinity and selfing (Castric et al. 2002, David et al. 2007). All sexual reproductive modes,
274 allogamous and autogamous, result in low $VarF_{IS}$ within a population as massive recombination
275 tends to homogenize intra-individual genetic identities along the genomes (Stoeckel et al. 2021a).
276 We measured linkage disequilibrium over all markers using the unbiased multilocus linkage
277 disequilibrium index, $\bar{r}_d$ (Agapow & Burt 2001). This mean correlation coefficient (*r*) of genetic
278 distances (*d*) between unordered alleles at *n* loci ranges from 0 to 1. This metric has the advantage
279 of limiting the dependency of the correlation coefficient on the number of alleles and loci, and it is
280 well suited to measure linkage disequilibrium in partially clonal populations (De Meeus & Balloux
281 2004, De Meeus et al. 2006). In general, LD is only slightly affected by clonality, except when
282 clonality is high (c > 0.9) and/or when genetic drift dominates over mutation rate (*e.g.,* when
283 population sizes are small N<50 compared to u=0.001; Navascues et al. 2010, Stoeckel et al. 2021a).
284 In contrast, inbreeding and selfing are efficient processes for quickly generating strong LD, after only
285 few generations. Finally, we measured genetic differentiation between populations of LSI, between
286 populations of SC and between LSI and SC populations using ρ_{ST}, an index adapted to study
287 autopolyploid populations (Ronfort et al. 1998). All these indices were computed using GENAPOPOP

288 (Stoeckel et al. 2024), a software dedicated to analysing genetic diversity and differentiation in
289 autopolyploid populations genotyped with confident allele dosage and reproducing through all
290 possible rates of clonality and selfing.
291 We also used SPAGEDI (v1.5, Hardy & Vekemans 2002) to estimate rates of selfing within
292 autopolyploid populations genotyped with confident allele dosage. This approach infers rate of
293 selfing (Sg) from identity disequilibrium coefficients (g_{2z} estimator), assuming that populations
294 reproduce by self-fertilization and random mating and are at inbreeding equilibrium (David et al.
295 2007 for diploids, Hardy 2016 for autopolyploids). Identity disequilibrium coefficients are measured
296 as the correlation in heterozygosity of distinct loci within the genome, and present the advantage
297 of being more robust to null alleles and genotyping errors than raw F_{IS} (David et al., 2007). The effect
298 of partial clonality on g_{2z} estimator is not yet defined. We thus considered for this study that clonality
299 would only marginally impact identity disequilibrium coefficients, the possibility to be at the
300 inbreeding equilibrium and the corresponding estimates of selfing. A synthesis of all these
301 hypotheses based on previous knowledge is provided in Table SI1.
302
303 **Ranking populations by clonality and selfing**
304 We proposed and computed a synthesis index ($\Sigma\mathrm{clon}$) calculated from Pareto β and $\mathrm{VarF_{IS}}$ to rank
305 the studied populations from the less to the more clonal. These two population genetic indices are
306 known to vary with rates of clonality being unbiased in samples (Stoeckel et al. 2021a). To avoid
307 issues of scaling as the range of values of these descriptors are different by several orders of
308 magnitude, $\Sigma\mathrm{clon}$ is computed in each population i as the sum of Pareto $\beta(i)$ and $\mathrm{VarFIS}(i)$ values
309 that were previously normalized over the whole dataset, respectively $z_\beta(i)$ and $z_{\mathrm{VarFIS}}(i)$.

310 $$z_\beta(i) = 1 - \frac{\beta(i) - min(\mathrm{B})}{max(\mathrm{B}) - min(\mathrm{B})}$$

311 $$z_{\mathrm{VarFIS}}(i) = \frac{\mathrm{VarFIS}(i) - min(\mathrm{VF})}{max(\mathrm{VF}) - min(\mathrm{VF})}$$

$$\Sigma\mathrm{clon}(i) = {}^{1}/_{2}\left[z_{\beta}(i) + z_{\mathrm{VarFIS}}(i)\right]$$

where $\beta(i)$ and $\mathrm{VarFIS}(i)$ are the measured Pareto β and variance of F_{IS} over loci in the population i, and where $\mathrm{B} = \{\beta(1), \beta(2), \ldots, \beta(55)\}$ and $VF = \{\mathrm{VarFIS}(1), \mathrm{VarFIS}(2), \ldots, \mathrm{VarFIS}(55)\}$ are the respective sets of Pareto β and $\mathrm{VarF_{IS}}$ over loci in the 53 populations used to obtain their minimum (min) and maximum (max) values.

Using the same approach as for $\Sigma\mathrm{clon}$, we computed a synthesis index ($\Sigma\mathrm{self}$) calculated from $\bar{r}_d$, $\mathrm{MF_{IS}}$ and Sg to rank the studied populations from the less to the more selfed.

$$\Sigma\mathrm{self}(i) = {}^{1}/_{3}\left[z_{\bar{r}_d}(i) + z_{\mathrm{MFIS}}(i) + z_{\mathrm{Sg}}(i)\right]$$

Statistical data analysis

First, to better understand the correlation between population genetic indices and estimates of reproductive modes from a large field dataset and to compare with the theoretical correlations obtained from simulations (Stoeckel et al. 2024), we computed a Principal Component Analysis (PCA) to comprehend the covariations of genetic indices, the correlations and the redundancies between the 17 genetic diversity indices (described above: G, R, D, Pareto β, $\bar{r}_d$, Ae, varAe, He, varHe, Ho, varHo, $\mathrm{MF_{IS}}$, $\mathrm{varF_{IS}}$, PIDsib, PIDu, Sg, SE.Sg) measured among the 53 sampled populations, and their link with reproductive modes including self-compatibility. We reported the amount of variation retained by the first two principal components and the correlation circle on which we plotted predictions of $\Sigma\mathrm{clon}$ and $\Sigma\mathrm{self}$ as supplementary variables. We also reported the score plot to visualize how L-morph (LSI) and S-morph (SC) populations distribute along the principal components of population genetic diversity. To avoid scaling issues, all descriptors were normalized before analysis.

We then analyzed the relationship between genetic diversity indices and their relationships with $\Sigma\mathrm{clon}$ and $\Sigma\mathrm{self}$ measured in the 53 sampled populations using Kendal partial rank-order correlation tests. We reported the corresponding matrices of correlation.

336 To detect differences in distribution of population genetic indices, $\Sigma\mathrm{clon}$ and $\Sigma\mathrm{self}$, among floral
337 morphs, we computed non-parametric Kruskal-Wallis tests that do not make any assumptions about
338 the type of distribution and about homogeneity of variances between the tested distributions.
339 When needed, we used post-hoc pairwise tests for multiple comparisons of mean rank sums
340 (Nemenyi's test).
341 All statistical tests were computed using PYTHON 3.11, SCIPY.STATS 1.9.3 (Virtanen et al. 2020) and
342 SCIKIT-POSTHOCS (Terpilowski 2019), except the PCA that was performed using R V4.2.2 and the library
343 FACTOMINER (Lê et al. 2008).
344

345 # Results

346 Among the 53 populations, we found 40 populations with only L-morph individuals and 13
347 populations with only S-morph individuals (Table SI2, Figure SI1). Karyotypes of L-morph plants from
348 two populations and of S-morph plants from five populations all presented the same number of
349 chromosomes (2n=80, Figure SI2) confirming that L and S-morph individuals in France and L-morph
350 individuals in northern Spain (Bou Manobens et al. 2019) belong to *L. grandiflora* subsp. *hexapetala*
351 (2n=10X=80, Barloy et al. 2024). Akaike's information criterion on the distribution of allele counting
352 over all our data supported tetraploidy as the best ploidy level for this set of 36 SNPs (Figure SI3),
353 as expected from Barloy et al (2024).

354

355 **Allele dosage and missing genotypes**

356 Within the 795 individuals genotyped at 36 SNPs (resulting in a total of 28,620 single-SNP
357 genotypes), 99.97% (28,612) of SNPs were genotyped with posterior probability of allele dosage
358 superior to 70% (Table SI3). In total, 785 individuals were genotyped with a full set of 36 SNPs with
359 posterior probabilities of allele dosage higher than 70%. Ten individuals distributed in nine

360 populations showed one of their SNP markers with posterior probabilities of allele dosage equal or
361 lower than 70%, that we assigned therefore as missing genotypes.

362

363 **Statistical power of the developed SNP marker set**

364 Over the 36 polymorphic SNPs, we found an effective number of alleles (A_E) of 1.36 per SNP over all
365 populations (Table SI2). Among populations, mean A_E values over the 36 SNPs were homogenous,
366 ranging from 1.22 to 1.55 (median=1.34). We, however, found large standard deviation of A_E
367 between SNPs within populations, ranging from 1.6 to 2.3 (median=2.1). Some SNPs were
368 apparently fixed in some populations while polymorphic at the scale of the whole dataset. When
369 not fixed, gene diversity H_E in polymorphic SNPs ranged from 0.14 to 0.33 (median=0.2).
370 These 36 SNPs in the autotetraploid part of *Lgh* would theoretically allow $4^{36}=4.7\times10^{21}$ different
371 possible MLGs considering the four possible nucleobases and $2^{36}=6.9\times10^{10}$ different possible MLGs
372 assuming two possible nucleobases per locus. Considering allele frequencies in the sampled
373 populations, the probabilities of identities under panmixia ranged from 8.5×10^{-12} to 5.2×10^{-5}
374 (median=2.1×10^{-7}) and the unbiased probabilities of identity between sibs $P_{ID\text{-}SIB}$ ranged from $4.2\times10^{-}$
375 ^{6} to 8.3×10^{-3} (median=5.6×10^{-4}; Table SI2). We then considered that the SNP set we used to genotype
376 the 795 sampled individuals via Hi-Plex method showed sufficient statistical power to distinguish
377 between true MLGs, and that individuals with identical MLGs were true repeated genotypes
378 (ramets) of a clonal lineage (a genet).

379

380 **Genetic and genotypic diversity**

381 Across populations, we identified a total of 462 distinct MLGs (genets) within the 795 sampled
382 individuals genotyped. Among them, we found 404 genets (88%) with a single ramet and only 58
383 genets (12%) with more than two ramets (Table SI4). Forty-eight genets had two to seven ramets

384 distributed over one to six populations (median=2), seven genets with 10 to 33 ramets distributed
385 over two to 17 populations (median=9), and one large genet of 99 ramets distributed over 24
386 populations (Figure 1).
387 Within populations, we found from three to 15 different genets (median=12) per population among
388 the 15 sampled individuals (Table SI4), implying the clear occurrence of repeated genotypes but also
389 a wide diversity of genets within most populations (Figure 1). Accordingly, genotypic richness (R)
390 ranged from 0.14 to 1 (median=0.79). The genotypic evenness, Pareto β, ranged from 0.056 to 3
391 (median=1.478, Table SI2).

392 Observed heterozygosity H_O was also high in most populations, slightly above expected
393 heterozygosity, ranging from 0.13 to 0.38 (median=0.26). The mean standard inbreeding
394 coefficients (MF_{IS}) averaged over all genotyped loci within populations were negative in all 53
395 populations and ranged from -0.33 to -0.126 with a very negative median of -0.274 (Table SI2).
396 Variances of F_{IS} between loci within population were very high, ranging from 1.75 to 36.60
397 (median=27.57). All these measures argued for reproductive modes implying high rates of clonality.
398 Estimates of selfing (S_g) were overall low with a handful of high values, ranging from 0 to 0.61
399 (median=0.06). Fifteen populations (28%) were estimated with no selfing. Nineteen populations
400 (36%) showed non-zero estimates under 0.1, 13 populations (25%) between 0.1 and 0.29, and six
401 populations (11%) with estimates between 0.42 and 0.61.
402 Finally, linkage disequilibria within populations between genotyped SNPs were overall low, with r_d
403 values ranging from 0 to 0.51 with a median value of 0.12. Forty-two populations (79%) were under
404 0.25, and only eight populations (21%) showed linkage disequilibrium between 0.25 and 0.51.
405

406 **Analyses of covariations between population genetic indices**

407 The first two components of the principal component analysis on the values of genetic diversity
408 found in the 53 genotyped populations accounted for 70.6% of the total variance between

409 populations (Figure SI4). Non-parametric Kendall partial rank-order correlations between genetic
410 indices (Table SI5) and correlations on the first two principal components from the 17 population
411 genetic indices measured showed three non-collinear groups of associated genetic indices (Figure
412 SI4.A) that are very similar to the theoretical groups of population genetic indices expected to covary
413 with different rates of clonality, selfing and outcrossing in autopolyploids (Stoeckel et al. 2024). A
414 first cluster regrouped G, D*, R, Pareto β and $VarF_{IS}$, indices that are known to be sensitive to
415 clonality (Stoeckel et al. 2021a) but also VarHe, VarHo, and VarAe. This cluster largely explains the
416 first dimension (47.7% of the total variance) of the PCA and was collinear to Σ_{CLON} (also see Figure
417 SI4.B). As expected under partial clonality, $VarF_{IS}$ was negatively correlated to genotypic diversity
418 indices (R and β; Figure SI5). The second cluster regrouped $P_{ID\text{-}SIB}$, $P_{ID\text{-}u}$, H_E, A_E and H_O, indices that
419 are linked to the general genetic diversity of populations (Figure SI4.A). The third cluster regrouped
420 S_g, SE.Sg, MF_{IS} and r_D, indices that are usually used to identify, rank and estimate rates of selfing
421 versus outcrossing in sexual populations (Castric et al. 2002, Bürkly et al. 2017). The second
422 dimension of the PCA (22.9% of the total variance) was mostly correlated to Ho, Sg, He and r_D which
423 was collinear to Σ_{SELF} (Figure SI4.C). The clusters of genetic indices were corroborated by the
424 correlation between Σ_{CLON} and genetic indices (Figure SI5) and Σ_{SELF} and genetic indices (Figure SI6).
425 The correlation across populations between Σ_{CLON} and Σ_{SELF} was negative and highly significant (r_s = -
426 0.66; $p < 0.001$; Figure 2). This correlation within LSI populations was negative and highly significant
427 (r_s = -0.65; $p < 0.001$) while it was no significant in SC populations (r_s = -0.32; p =0.289).
428

429 **Differences in genetic diversity and structure between L- and S-morph populations**

430 The number of ramets per genet was similar in L-morph (LSI) and S-morph (SC) populations (H=1.85,
431 p=0.173, Figure 1 & SI7), as were their genotypic richness (R, H=1.23, p=0.267) and evenness
432 (Pareto's β, H=1.60, p=0.206; Table SI2). The distributions of other population genetic indices

433 related to clonality were also not significantly different between in L-morph (LSI) and S-morph (SC)
434 populations ($VarF_{IS}$: H=2.94, p=0.086; P_{ID-SIB}: H=1.98, p=0.160 and Σ_{CLON} H=2.28, p=0.131; Figure 3).
435 Mean observed heterozygosity (Ho, H=5.27, p=0.022) and their variances (VarHo, H=13.85,
436 p<0.001), mean effective number of alleles per locus (Ae, H=5.13, p=0.024) and its variance over
437 locus (VarAe, H=9.49, p=0.002), MF_{IS} (H=11.85, p<0.001), linkage disequilibrium (r_D, H=5.55,
438 p=0.018), estimate of selfing rates (Sg, H=12.39, p<0.001) and its standard error over loci (SESg,
439 H=9.58, p=0.002) significantly differed between in L-morph (LSI) and S-morph (SC) populations. S-
440 morph (SC) populations, compared to L-morph (LSI), showed lower mean observed heterozygosity
441 (medians, L-morph: 0.262, S-morph: 0.210) and less variance between loci (medians, L-morph:
442 2.400, S-morph: 1.859), lower effective number of alleles (medians, L-morph: 1.341, S-morph:
443 1.283) and less variance between loci (medians, L-morph: 4.419, S-morph: 3.580), higher linkage
444 disequilibrium (medians, L-morph: 0.113, S-morph: 0.152), higher estimates of selfing rate
445 (medians, L-morph: 0.045, S-morph: 0.175) even if with higher standard error between loci
446 (medians, L-morph: 0.058, S-morph: 0.156) and higher Σ_{SELF} (medians, L-morph: 0.222, S-morph:
447 0.495).
448 Genetic differentiation (ρ_{ST}) among pairs of S-morph (SC) populations (median value of ρ_{ST}=0.27)
449 was comparable to the genetic differentiation found among pairs of L-morph (LSI) populations
450 (median value of ρ_{ST}=0.26; H=319.37, post-hoc p=0.322; Figure 4). The median of ρ_{ST} between pairs
451 of L-morph (LSI) and S-morph (SC) populations reached 0.65. The distribution of inter-morph ρ_{ST}
452 values differed both from the distribution of pairs of intra-L-morph (LSI) ρ_{ST} values (post-hoc
453 p<0.001) and from the distribution of pairs of intra-S-morph (SC) ρ_{ST} values (post-hoc p<0.001). The
454 minimum spanning tree of genetic distances between individuals computed with *GenAPoPop*
455 showed quite clustered distributions of L-morph (LSI) and S-morph (SC) individuals but also with
456 clear evidence of admixtures between their lineages (Figure 5).
457

458 # Discussion

459 We used population genetics on autotetraploid SNPs with allele dosage to assess the rate of
460 clonality, of selfing and of outcrossing (i.e., reproductive mode) of 53 populations of *Ludwigia*
461 *hexapetala* subsp. *grandiflora* (*Lgh*) recently colonizing western European watersheds.
462 To achieve this goal, we developed reproducible codominant molecular markers (SNPs) that enabled
463 allele dosage-based genotyping of sampled individuals. We then used tailored computational
464 analyses to perform confident population genetic analyses in autopolyploids. These two
465 methodological steps allowed solving the remaining challenges to perform population genetic
466 analyses in autopolyploids (Dufresne et al. 2014), including *Lgh*. The resulting framework can be
467 applied to any autopolyploid species.
468 *Lgh* develops two floral morphs respectively associated with a Late-acting self-incompatible system
469 (L-morph) and a self-compatible system (S-morph, Portillo-Lemus et al. 2021, 2022). Interestingly,
470 the sampled populations in western Europe showed either L- or S-morph resulting in two groups of
471 sampled populations: one group of 40 L-morph (LSI) populations and one group of 13 S-morph (SC)
472 populations. They live in similar ecological conditions (Portillo-Lemus et al. 2021) which thereby
473 provides a rare opportunity to characterize the genetic consequences of an LSI system compared to
474 a group of SC populations in the same species and ecological context.
475 **F**ive L-morph and two S-morph water primrose populations separated by 50 to 150 km from one to
476 the other in France we karyotyped, all had 80 chromosomes corresponding unequivocally to the
477 species *Ludwigia grandiflora* subsp. *hexapetala* and thus didn't find any individual with 48
478 chromosomes corresponding to *Ludwigia grandiflora* subsp. *grandiflora*. These results agree with
479 previous observations of Dandelot et al (2005) and Barloy et al. (2024) in France, Bou Manobens et
480 al. (2019) in northern Spain and Armitage et al. (2013) in Great Britain.
481

482 **Most of the genetic variance explained by clonality and selfing among *Lgh* populations**

483 The correlations of population genetics indices on the 53 genotyped *Lgh* populations (Figure SI4)
484 were congruent with previous theoretical predictions on the variations of genetic indices with
485 different rates of clonality and selfing for autopolyploid populations (Stoeckel et al. 2024). Identical
486 covariations were already predicted by Wright-Fisher-like models adapted for diploid (Stoeckel et
487 al. 2021a) and haplodiplontic life-cycles (Stoeckel et al. 2021b), and validated respectively using
488 multiple field populations of marine phanerogams for diploids (Arnaud-Haond et al. 2020) and of
489 brown, green, red algae and mosses for haplodiplontics (Krueger-Hadfield et al. 2021). All these
490 predictions and observations argue for the primary importance of reproductive mode, especially of
491 high clonality, to drive variation in genetic diversity within populations (Duminil et al. 2007). They
492 support the methodological importance of accurate assessment of rates of clonality and of selfing
493 before starting to interpret genetic diversity.

494

495 **Dominant clonality within the western European *Lgh* populations**

496 Previous field and lab observations of peripatric *Lgh* populations reported massive production of
497 dispersing vegetative propagules, rapid expansions of patches, and an important capacity for
498 spontaneous cutting-planting (Dandelot et al. 2005, Thouvenot et al. 2013, Grewell et al. 2016,
499 Skaer-Thomason et al. 2018a,b). Okada et al. (2009) genotyped around 800 individuals sampled in
500 27 *Lgh* populations in Californian wetlands using a set of eight AFLP markers and reported an
501 extremely reduced clonal diversity: 95% of the samples had the same genotype and 18 populations
502 over 27 (67%) supported a single AFLP-genotype. All over the UK, only two haplotypes on 14
503 sampled stems were found using chloroplast sequences (Armitage et al. 2013). These haplotypes
504 were even shared with some *Lgh* samples invading California. With no measure of the probability
505 of identity to assess the marker set, these results could be due to the lack of resolution of the
506 markers used resulting in an artificially elevated measure of clonality (Waits et al. 2001, Villate et al.

507 2010). In any case, all these studies concluded that invasive populations of *Lgh* in Europe and in USA
508 reproduce by exclusive clonality, with a very narrow base of ancestral clones or being monoclonal
509 (Dandelot et al. 2005, Okada et al. 2009, Thouvenot et al. 2013, Grewell et al. 2016).
510 In our study, we chose to develop and use SNPs rather than AFLP markers. The latter can be
511 challenging in terms of precisely sizing fragments, leading to suboptimal reproducibility, particularly
512 across different platforms (Fry et al. 2009). The 36 SNPs we developed are easily reproducible,
513 accurate enough to distinguish between offspring of sibling mating as evidenced by the probabilities
514 of identity we obtained, and less expensive.
515 With these SNPs, we found typical genetic signatures of high clonality, including clear occurrence of
516 replicated genotypes and mean negative F_{IS} values with high interlocus variances, in all the 53
517 western European populations we genotyped. Globally, gene diversities measured in these invasive
518 *Lgh* populations were in the higher range of values commonly found for SNPs (Fischer et al. 2017,
519 Schmidt et al. 2021). Such levels of gene diversity are in line with strong rates of clonality that buffer
520 the loss of alleles due to genetic drift (Reichel et al. 2016, Stoeckel et al. 2021b). Among the 58 MLGs
521 with replicates, seven MLGs were found in a single population and 51 MLGs were found in multiple
522 sites. Clonal propagules of *Lgh* are known to disperse carried by river current and by birds (zoochory:
523 seedlings have the ability to stick to feathers; Grewel et al. 2016). Clonal reproduction by rhizomes
524 at the local scale and by the dispersal of clonal propagules would thus be the main source of
525 population growth and invasive spread of *Lgh* in western Europe.
526 Our results also report a previously-underestimated genotype diversity in the invasive populations
527 in western Europe. We found 462 distinct MLGs (genets) out of 795 individuals within these
528 populations and the large majority of these MLGs (404, thus 88%) were only sampled once in the 53
529 populations. Our results are still congruent with Dandelot (2004) unpublished measures obtained in
530 three Mediterranean populations using inter-simple sequence repeats (ISSRs). This data reports
531 three and seven genotypes over 11 sampling units within two *Lgh* populations and two genotypes

532 over nine sampling units in a third population, all sampled in the south-east of France. These results
533 could reveal contrasted situations between *Lgh* populations introduced in the USA and continental
534 Europe.
535
536 **Between 10 and 40% of sexual reproduction in *Lgh* populations in western Europe**
537 Beyond the qualitative indication of dominant clonality, we then aimed at estimating the rates of
538 sexuality in each of these 53 populations. All showed small to medium values of linkage
539 disequilibrium between SNPs. Such values are expected in highly but non-exclusive clonal
540 populations with large population sizes (Navascues et al. 2010, Stoeckel et al. 2021a). Thirty-two
541 populations showed Pareto β values of their distributions of clonal sizes under 2, which are only
542 found in theoretical populations with rates of clonality higher than 0.8 (Stoeckel et al. 2024). All 53
543 populations showed mean negative F_{IS} with high interlocus variance, with values observed in
544 theoretical populations with rates of clonality higher than 0.6 but under 0.9 (Balloux et al. 2003,
545 Stoeckel & Masson 2014, Reichel et al. 2016, Stoeckel et al. 2024). All these values of population
546 genetic indices were consistent with the interpretation that *Lgh* populations in western Europe must
547 reproduce with effective rates of clonality between 60% and 90%, thus with 10% to 40% sexuality
548 (De Meeus et al. 2006, Arnaud-Haond et al. 2020, Stoeckel et al. 2024). These estimates were also
549 supported by the local evidence of recombination between clonal lineages, and even between L-
550 morph (LSI) and S-morph (SC) lineages at the scale of a watershed (Figure 5). The diversity of
551 genotypes detected in western Europe is thus indicative of rare but significant local sexual events
552 rather than of a large clonal diversity that would have maintain and propagate by exclusive clonality
553 since its introduction. Our results newly advocate that sexual reproduction should not, therefore,
554 be overlooked in these invasive populations, especially in management plans.
555
556 **Genetic consequences of LSI compared to self-compatible populations**

557 The late-acting SI system remains one of the less studied breeding systems among the mechanisms
558 favouring outcrossing in plants (Gibbs 2014). Its efficiency to favour outcrossing and its
559 consequences on genetic diversity within populations, especially considering its low but common
560 failures, were not yet deciphered and not yet compared to SC populations in the same ecological
561 conditions, as already previously explored for gametophytic and sporophyte SI systems (Busch 2005,
562 Koelling et al. 2011).
563 The maintenance of SI systems is one of the most intriguing evolutionary puzzles (Porcher &
564 Lande 2005). Indeed, SI systems are fated to breakdown because SC individuals present the
565 advantage of reproductive assurance when compatible partners are limited, especially in peripatric
566 conditions (Eckert et al. 2006). This advantage is even absolute when no compatible partners are
567 available within pollination range, as we commonly found in *Lgh* populations in western Europe
568 (Figure SI1). Conversely, outcrossing imposed by SI systems decreases the probability of expressing
569 deleterious mutations in descendants as compared to selfing with the same genetic background
570 (Rice 2004, Navascues et al. 2010).
571 We found that the 40 populations with L-morph individuals (LSI) had a higher number of effective
572 alleles, higher gene diversity, higher observed heterozygosity and less linkage disequilibrium than
573 the 13 populations with S-morph individuals (SC; Figure 3).
574 These genetic differences are very unlikely to directly result from the consequences of the LSI
575 system hitchhiked to the whole genome in L-morph individuals and populations. Indeed, it would
576 imply either that all the 36 SNPs would be physically linked to the genes under negative frequency-
577 dependent selection coding for the LSI or that *Lgh* outcrossed for many generations in small
578 population sizes (Glémin et al. 2001, Navascues et al. 2010). *Lgh* in western Europe develops
579 including far more than thousands of stems per local population (Portillo-Lemus et al. 2021) and the
580 linkage disequilibrium values also argued for large effective population sizes. We found similar
581 estimated rates of sexuality and of clonality between L-morph and S-morph populations, using Σ_{CLON}

582 and all indices sensible to clonality (R, Pareto β and VarFis; Figure 3). We however found significant
583 difference in estimates of selfing between L-morph (LSI) and S-morph (SC) populations, and in Σ_{SELF}
584 and in all indices sensible to selfing (Ae, linkage disequilibrium, Ho, mean Fis) revealing typical
585 signatures of higher selfing rates in S-morph populations. Hardy (2016) method estimated a median
586 selfing rate (Sg) of 0.18 in S-morph populations versus 0.05 in L-morph populations. Consequently,
587 the genetic differences we found between S-morph and L-morph populations may thus rather be
588 due to the effects of selfing impacting S-morph (SC) populations than due to outcrossing protecting
589 the loss of genetic diversity or rates of clonality in L-morph (LSI) populations.
590
591 **Advantages of dominant clonality with preferential allogamy in invasive *Lgh* populations**
592 Uniparental clonal reproduction (including clonality and selfing) may help the demographical
593 maintenance of plants spreading out of their native range with limited or even without compatible
594 or less related sexual partner at pollination distance (Baker's conjecture: Barrett et al. 2008, Pannell
595 et al. 2015). If they rather reproduce using selfing, their descendants increase the probability to
596 express inbreeding depression and to lose heterozygosity. Some invasive populations develop with
597 low genetic diversity (He et al. 2024), questioning on the biological and environmental factors that
598 may explain their success (i.e., the genetic paradox of invasions, Allendorf & Lundquist 2003). But
599 many other plants spread out of their native ranges with substantial genetic diversity and using
600 reproductive modes that favour outcrossing (Roman & Darling 2007, Forsman 2014), like *Lgh*
601 populations in western Europe, mostly when developing in harsh and stressful conditions (Fox &
602 Reed, 2011) or when the costs of inbreeding depression expressed by selfing are superior to the
603 benefits of reproductive assurance (Layman et al. 2017).
604 Peripatric populations of *Lgh* in western Europe seem to solve all these problems and paradox by
605 mixing clonality with preferential allogamy: clonality allows the local maintenance and spreading of
606 population without losing heterozygosity and genetic diversity, and subtle but significant sexuality

607 with preferential allogamy, favoured by LSI and faster growth of crossed-pollen tubes, enables
608 recombination between lineages favouring the emergence of locally adapted genomes with
609 potential higher vigour and fertility (heterosis: Darwin 1876, Lippman & Zamir 2007, Birchleret al.
610 2010).
611 This reproductive mode, i.e. dominant clonality with preferential allogamy, is common in plants
612 (Vallejo-Marin et al. 2010). The micro-physiological mechanism(s) slowing down the growth of self-
613 pollen tubes, rather than blocking them, in simultaneous monoecious and hermaphrodite SC species
614 and resulting to favour allogamy when compatible pollen is available may also be common in plant,
615 although potentially overlooked (Glover 2007, Nasrallah 2017). In such peripatric conditions, selfing
616 would thus only present the limited or transient advantage to produce seeds that can maintain in
617 local seed banks and with different dispersal properties compared to clonal propagules. The limited
618 benefit of selfing in this species may explain why, based on photos collected on the web, L-morph
619 (LSI) individuals seem more frequent in peripatric than in native populations, and why in western
620 Europe, around 76% of the populations are L-morph (LSI) against 24% of S-morph (SC; Portillo et al.
621 2021). Our results thus call for measuring the true proportions of LSI and SC in invasive versus native
622 worldwide *Lgh* populations with estimation of their rates of clonality and of selfing.
623
624 **Unusual selfing syndrome in *Lgh* populations**
625 Highly selfed populations of different plant species tend to share similar morphology and functions
626 resulting in a set of traits called *selfing syndrome*, including reduced flower size (Darwin 1876,
627 Tsuchimatsu & Fujii 2022). Selfing syndrome, including reduced flower size, seems to evolve rapidly,
628 as observed for example in five generations in *Mimulus guttatus* (Bodbyl-Roels & Kelly 2011), in four
629 generations in *Silene latifolia* (Delph et al. 2004), in three generations in *Phlox drummondii* (Lendvai
630 & Levin 2003) and after only two generations in *Eichhornia paniculata* (Worley & Barrett 2000).

631 On the contrary, S-morph (SC) individuals in *Lgh* develop larger flowers than allogamous L-morphs
632 (Portillo-Lemus et al. 2021). However, S-morph (SC) individuals and populations dominantly
633 reproduced using clonality and produced selfed offspring only when lacking crossed pollen. Both
634 clonality and faster growth of crossed-pollen tubes may delay or even compromise the emergence
635 of the first steps of a selfing syndrome. These two traits may call for further studies on the
636 emergence of selfing syndrome in partially clonal and selfed populations.
637
638 **Conclusion**
639 We found that peripatric populations of *Lgh* in western Europe reproduced using dominant clonality
640 with limited but stable significant sexuality with preferential outcrossing, in nearly all populations,
641 within a large clonal diversity. *Lgh* is one of the most invasive aquatic plants in the world, and
642 considerable efforts are made to limit its deleterious effects in the newly colonized ecosystems
643 (Thouvenot et al. 2013, Grewell et al. 2016, Portillo-Lemus et al. 2021). The rare sexual events,
644 allogamous when possible, occurring in invasive peripatric populations of *Lgh* may favour the
645 emergence of new genotypes, more adapted to local conditions. Managers should thus chiefly
646 concentrate their actions on the most sexual populations, and on the contact zones between S- and
647 L-morph populations in France. We also found variations of the rates of clonality and of selfing
648 among *Lgh* populations. Considering the importance of reproductive modes on the dynamics and
649 evolution of populations (Duminil et al. 2007, Ellegren& Galtier 2016, Glémin et al. 2019), our results
650 advocate that management actions should consider the local effective reproductive modes to
651 control *Lgh* population by population. Finally, knowing the reproductive modes of populations, the
652 distribution of clones and the self-compatibility across western European populations now allow
653 deciphering and interpreting their population structure, identifying their origin and routes, and
654 predicting their possible short-term dynamics.

655 **Data Accessibility Statement**

656 The data that support the findings of this study are openly available on Zenodo
657 (https://doi.org/10.5281/zenodo.12760022).

658

659 **Benefits Generated**

660 Benefits from this research accrue from the sharing of our data and results on public databases as
661 described above.

662

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678

679 **Conflict of interest**

680 The authors declare that they have no financial conflicts of interest based on the content of this
681 article.
682
683 **Author contributions**
684 DB and SS laid the foundation of this work, conceived the study and were responsible for funding
685 applications. LPL, MH and DB collected samples and performed field observations. LPL and DB read
686 karyotypes and counted chromosomes. EP and DB conceived and supervised RAD4SNP and Hi-Plex
687 methodology, BJP and DJP contributed to define the SNP set. LPL, MH and ALB performed RAD4SNP
688 approach to develop the SNPs. LPL and MH extracted DNA and genotyped the samples, SMC and
689 RCV sequenced the data. GL performed the bioinformatic analyses and SS computed the Bayesian
690 allele dosages. DB, LPL and SS performed early data explorations at the end of LPL PhD thesis that
691 was supervised by DB and SS. RB and SS performed computations and analyses, tracked and
692 managed the bibliography, produced tables, figures and interpretation. DB, RB and SS wrote the
693 manuscript. All authors read and approved the final manuscript.
694

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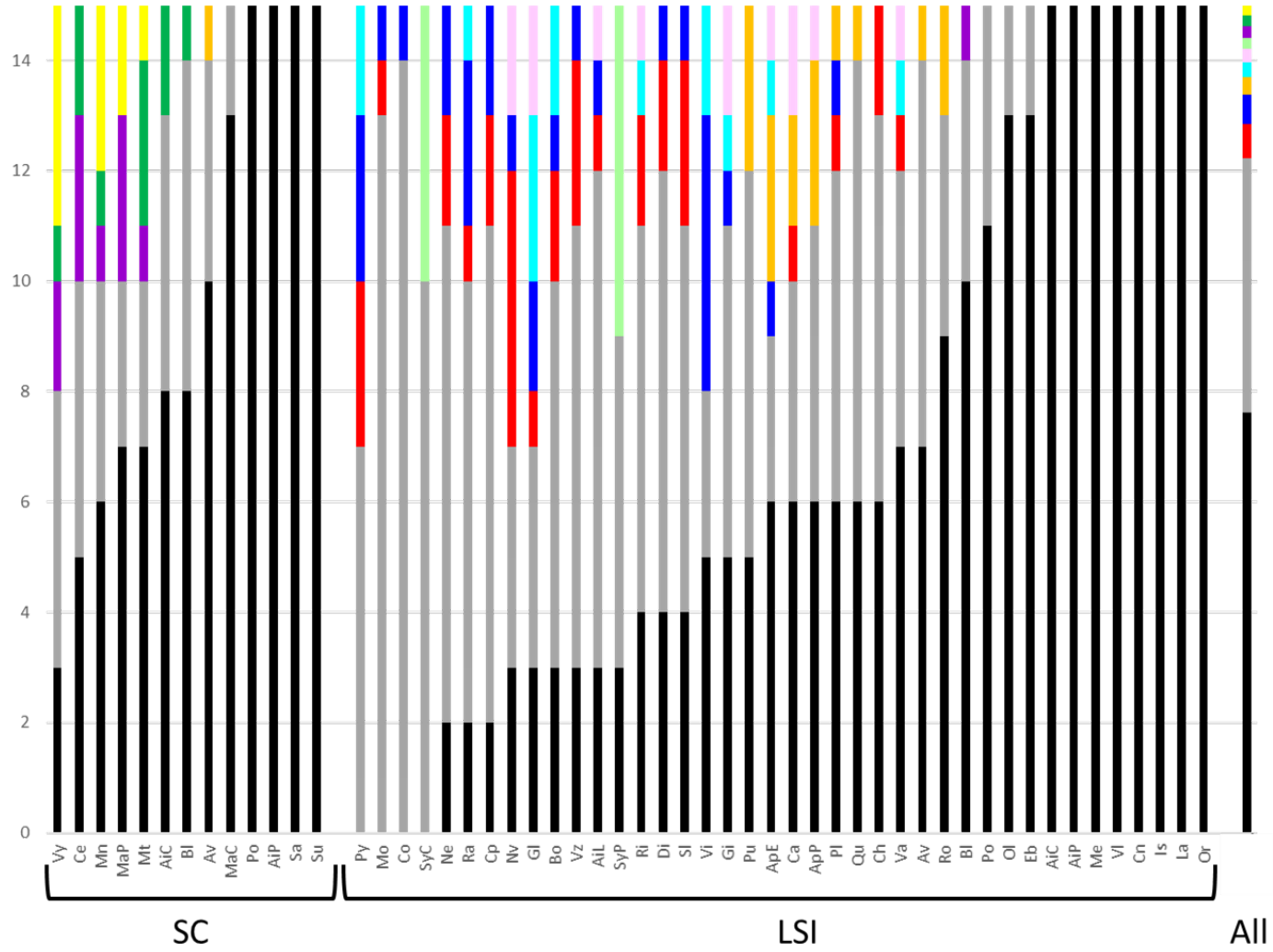

Figure 1: Distributions of Multi-Locus Genotypes (MLGs, we consider as genet) among LSI and SC *Lgh* populations in western Europe. The y-axis represents the distribution of the genotyped samples (ramets) with the different following categories: In black, the number of unique MLGs (MLGs that were found only once over all populations); In grey, the number of MLGs with less than 7 ramets over all populations; In color, MLGs with more than 10 ramets found in multiple populations (one color per different genet, identifying the same genet across all populations). Proportions of each types of MLGs over all the populations (795 genotyped individuals) are plotted in the last right bar (All). More than half the samples are unique genotypes (black bar).

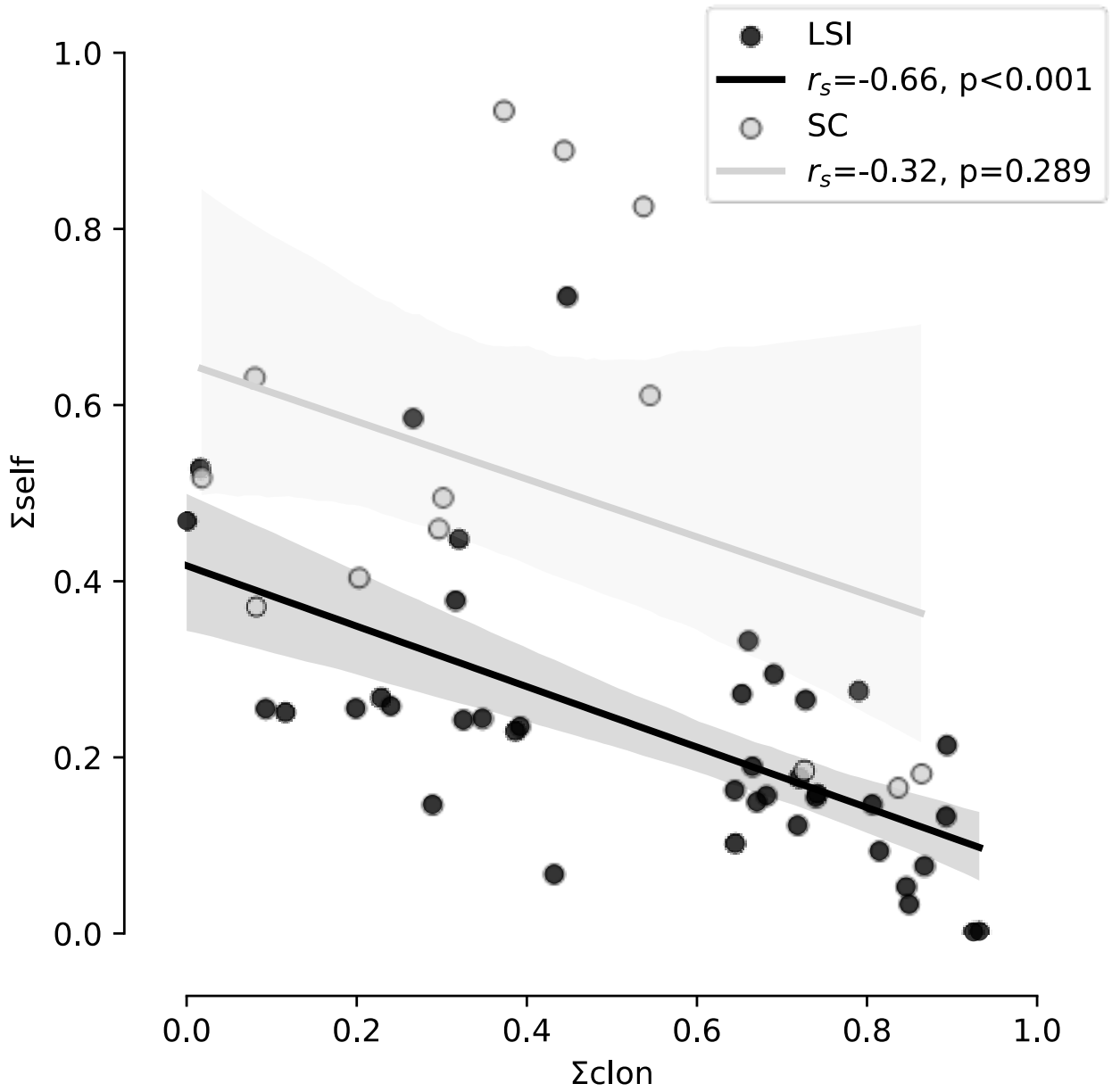

Figure 2: Correlations between Σ_{CLON} and Σ_{SELF} found in 13 SC populations (light grey points and regression line) and 40 LSI populations (black points and regression line). 95% confidence intervals are given for LSI and SC populations in dark grey and light grey, respectively. Spearman rank-order correlation coefficients (r_s) and probabilities that Σ_{CLON} and Σ_{SELF} would not be correlated (p) are reported for LSI and SC populations.

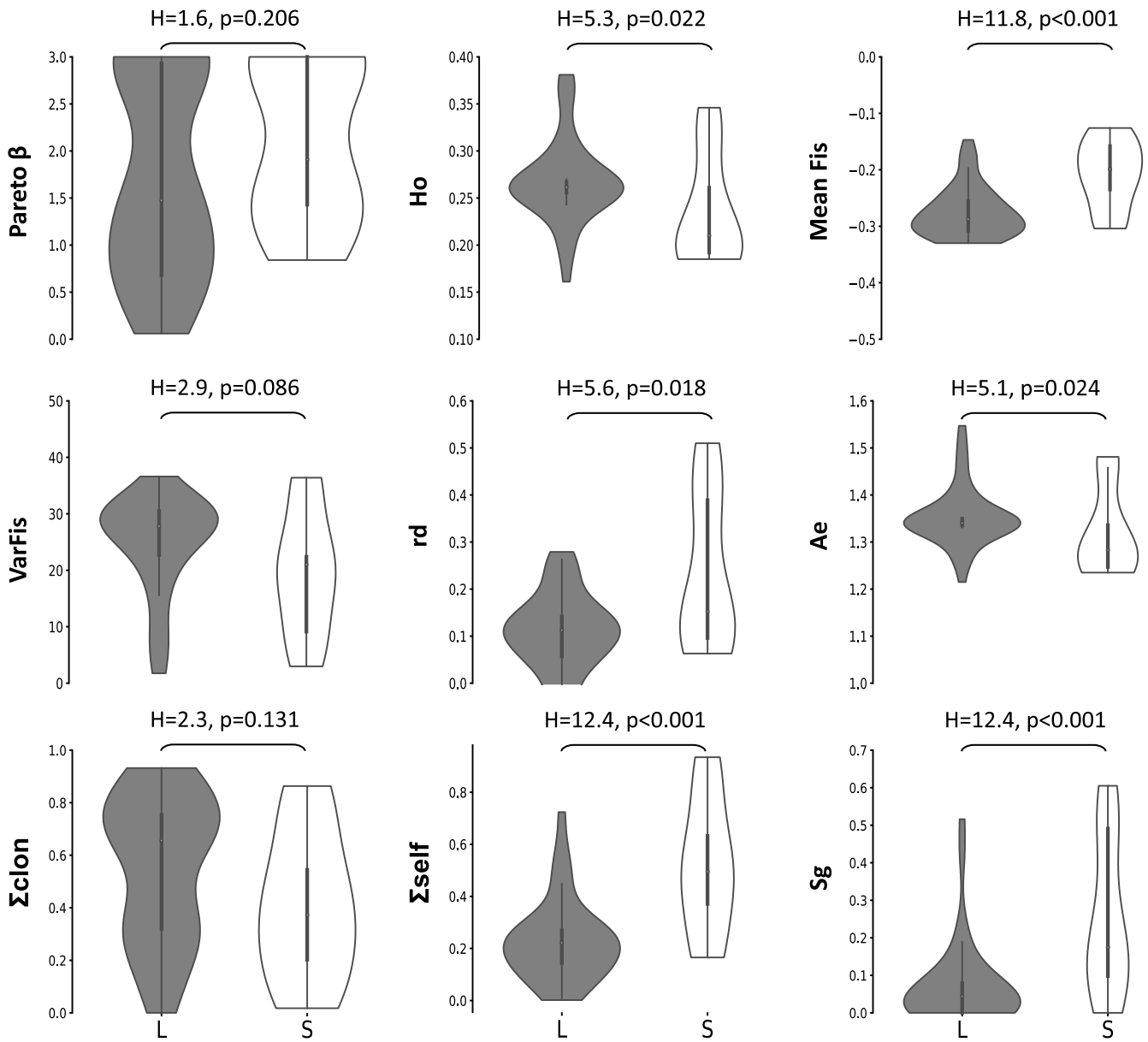

Figure 3: Violin plots comparing distributions of genetic indices in LSI (L, grey) versus SC (S, white) populations. Violin plots are cut for their minimum and maximum values. Kruskall-Wallis tests are reported as H-statistics as well as the probability that LSI and SC population would present the same distribution of genetic indices.

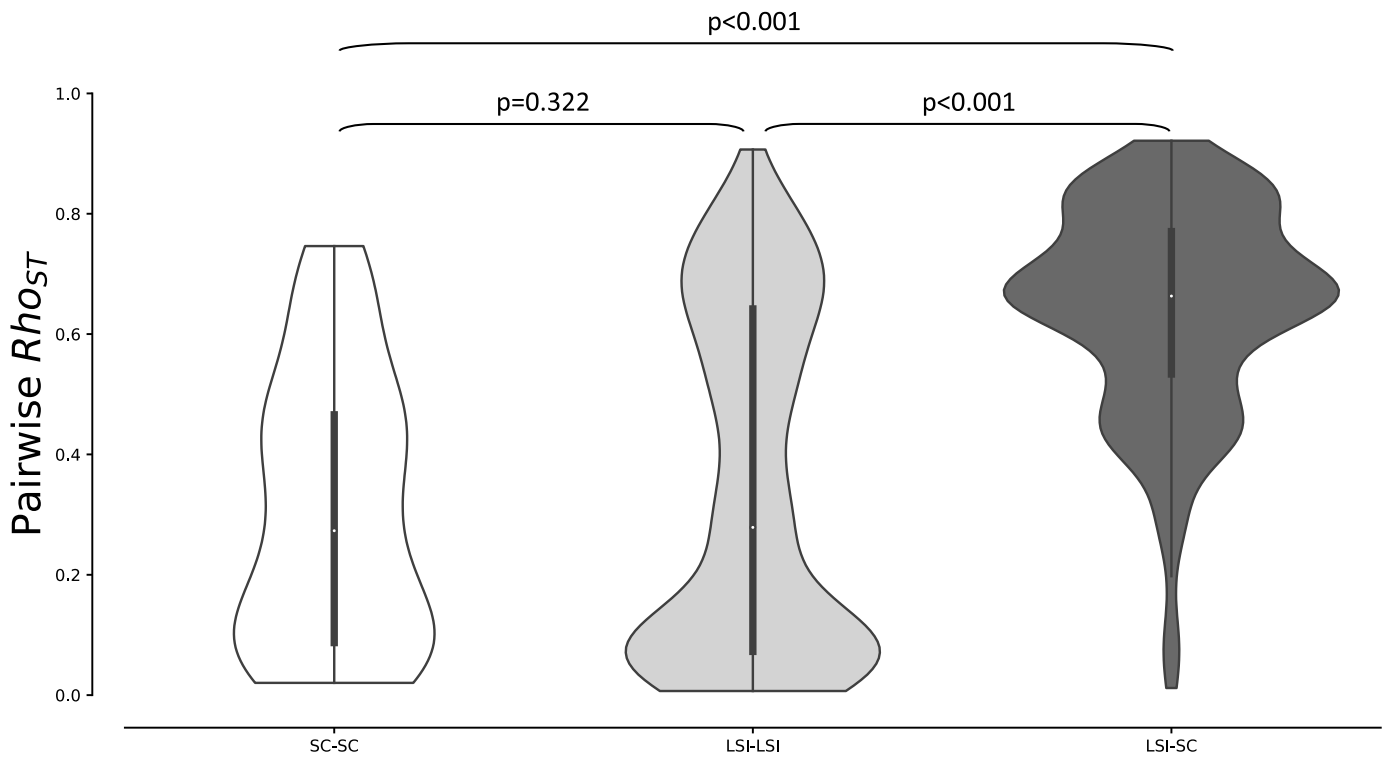

Figure 4: Distributions of pairwise rho_{st} within pairs of SC populations (white violin plot), pairs of LSI populations (light grey violin plot) and between pairs of LSI and SC populations (dark grey violin plot). Probabilities that pair of non-parametric distributions are equal are reported.

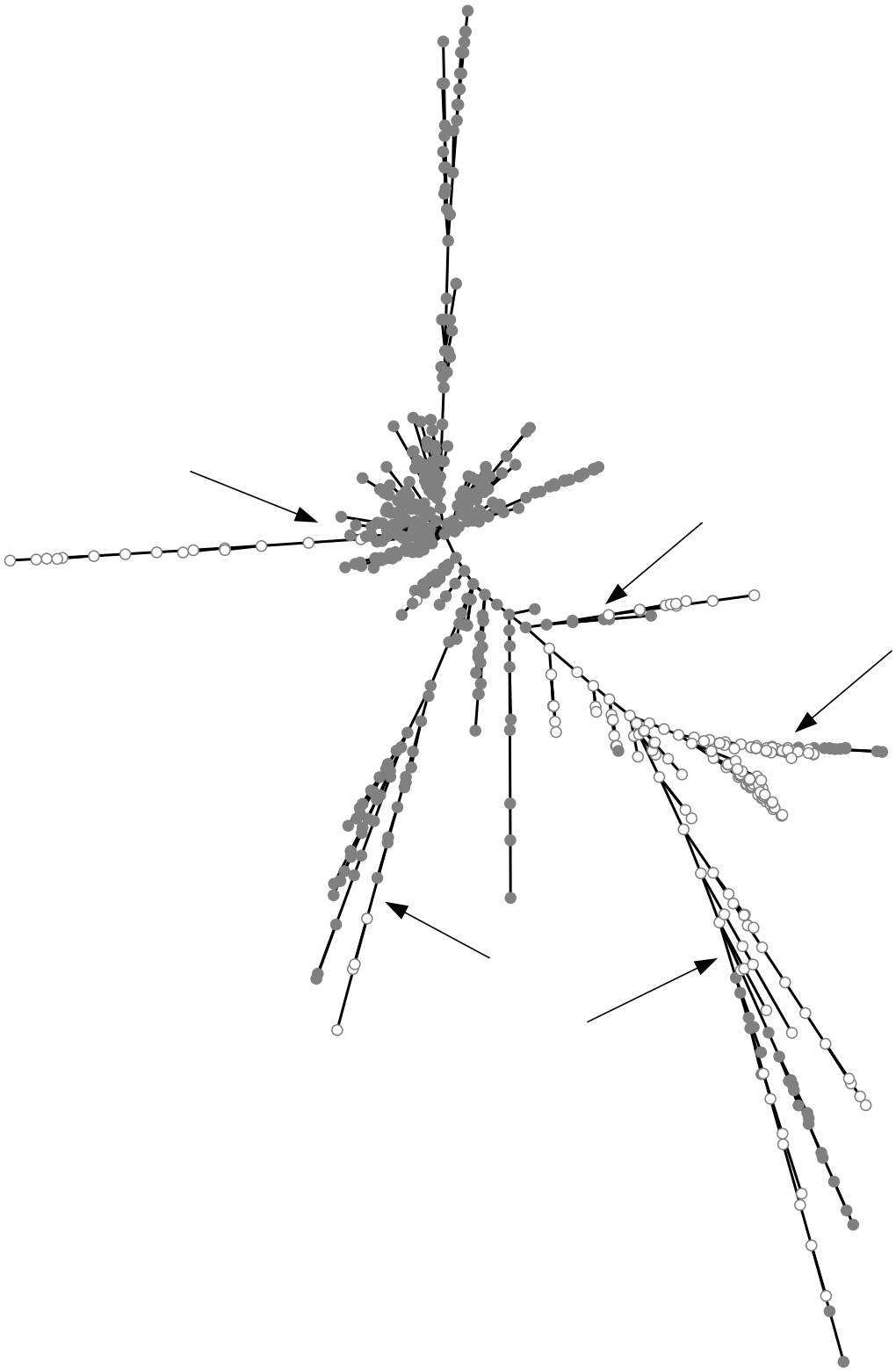

Figure 5: Minimum spanning tree of the genetic distances (number of different alleles) between L-morph (LSI, grey points) and S-morph (SC, white points) individuals. Arrows indicate some of the evidences of admixture between LSI and SC lineages.

Supplementary Information (SI)

Situation of *Lgh* in USA and Mediterranean populations in previous studies (Dandelot 2004, Okada et al. 2009, Armitage et al. 2013):
- nearly monoclonal populations arguing for the ecological advantage of clonality (fragmentation and rhizome propagation) in these invasive populations.

But
- using weakly polymorphic markers,
- before the demonstration of the heterostyly and of the Late-acting self-incompatibility system in *Lgh*

Situation of *Lgh* in western Europe, studied here:
- Spatial context: The other compatible type populations are away from hundreds of meters to several kilometers (Portillo-Lemus et al. 2021)
- Clonality through fragmentation and rhizomes, as in other populations (Portillo-Lemus 2021).
- Massive blossoming with a lot of pollinators harvesting flowers (Portillo-Lemus 2021).
- Faster pollen tube elongation of pollen from the other compatible type (Portillo-Lemus et al. 2022)

Our working hypotheses about the main, realized reproductive modes in population:

- **LSI populations:** A very limited production of seeds (Portillo-Lemus et al. 2021)

Hypothesis 1: **preferential clonality** due to lack of compatible partners to produce seeds → Baker's conjecture on reproductive modes (Pannell et al. 2015).

Hypothesis 2: The rare seeds really contribute to the populations → biological feature of seeds or recombination would have advantage over clones. If so, are they mainly:

Selfed seeds → reproductive assurance and advantage of recombination over clonality in a uniparental context?

Outcrossed seeds → advantage of outcrossed seeds due to heterosis and/or recombination despite the lack of locally-available compatible partners → selection of long pollination ranges

- **SC populations:** Production of a lot of seeds in natural populations

Hypothesis 1: despite a huge production of seeds, **preferential clonality** due to the physiological and/or ecological advantage of clones over seed germination process?

Hypothesis 2: the mass of seeds does contribute to the populations or some evolutionary advantage of recombination in offspring compared to clonal fragmentation and rhizome propagation to adapt local conditions? If so, are these seeds mainly:

Selfed seeds → within uniparental reproduction, biological feature of seeds or genetic recombination would provide advantage over clones?

Outcrossed seeds → Faster pollen tube elongation of pollen from the other type gives real advantage or advantage to allogamy to adapt new local conditions?

Supplementary information1: Synthesis of the questionings of our study to estimate reproductive mode of 53 populations of invasive Lgh using genetic diversity on the autotetraploid part of its genome.

Genotypic or genetic index	Range of values	Main driver	Behavior	references
β*	ϵ [0; +∞]	Rate of clonality	↘ when c ↗ $\beta \rightarrow 0$ when $c \rightarrow 1$	Halkett et al. 2005, Arnaud-Haond et al. 2007, 2020, Stoeckel et al. 2021
$varF_{IS}$	ϵ [0; +∞]	Rate of clonality	↗ when c ↗ $VarF_{IS} \rightarrow 0$ when $c \rightarrow 0$	Stoeckel & Masson 2014, Stoeckel et al. 2021
r_d	ϵ [0; 1]	Rate of selfing	slightly ↗ when c ↗ ↗ when s ↗ $r_d \rightarrow 1$ when $s \rightarrow 1$	Weir and Cockerham 1973, Navascues et al. 2010, Halkett et al. 2005
H_O	ϵ [0; 1]	Rate of selfing	↘ when s ↗ $H_O \rightarrow 0$ when $s \rightarrow 1$	David et al. 2007, Halkett et al. 2005
F_{IS}	ϵ [-1; 1]	Both rates	↘ when c ↗ $FIS \rightarrow -1$ when $c \rightarrow 1$ $FIS \rightarrow +1$ when $s \rightarrow 1$	Stoeckel & Masson 2014, Balloux et al. 2003, Stoeckel et al. 2021
H_E	ϵ [0; 1]	Rate of selfing	↘ when s ↗ $H_E \rightarrow 0$ when $s \rightarrow 1$	Glémin et al. 2001, Ritland & Ganders 1987
$P_{ID\text{-}SIB}$	ϵ [0; +∞]	Both rates	↘ when c ↗ ↘ when s ↗	Waits et al. 2001
S_g**	ϵ [0; 1]	Rate of selfing	↗ when s ↗	David et al. 2007, Hardy et al. 2016

* similar behavior of the clonal richness R
** inferred values of s, according to the method of Hardy (2016)

Table SI1: Hypotheses about the influence of clonality and selfing on the expected variations of genetic indices.

			Measured population genetic indices																	Relative rankings of reproduction modes										
Population	**Mating**	**Morph**	**G**	**R**	**D***	**Pareto's β**	**HO**	**var(ho)**	**HE**	**var(he)**	**AE**	**var(Ae)**	**FIS**	**var(FIS)**	**rd**	**PIDu**	**PIDsib**	**Sg**	**SE(Sg)**	**rk_cl**	**sn_cl**	**rk_self**	**sn_self**	**z_β (i)**	**z_varFis (i)**	**z_rD (i)**	**z_MFis (i)**	**z_Sg (i)**	**Σclon**	**Σself**
AiC	LSI	L	15	1.00	3.71	3.00	0.31	1.84	0.25	1.14	1.40	3.90	-0.241	15.60	0.128	5.70E-09	1.01E-04	0.00	0.00	8	0.199	26	0.157	0.000	0.398	0.331	0.436	0.000	0.199	0.256
AiL	LSI	L	11	0.71	3.17	0.68	0.27	2.40	0.21	1.37	1.34	4.19	-0.308	25.88	0.103	1.29E-07	4.51E-04	0.04	0.05	42	0.740	18	0.125	0.787	0.692	0.287	0.108	0.069	0.740	0.155
AiP	LSI	L	15	1.00	3.71	3.00	0.38	1.01	0.33	0.56	1.55	2.73	-0.147	2.84	0.233	8.47E-12	4.16E-06	0.10	0.20	2	0.016	45	0.311	0.000	0.031	0.515	0.897	0.172	0.016	0.528
ApE	LSI	L	12	0.79	3.40	1.48	0.26	2.56	0.20	1.52	1.35	4.88	-0.295	28.64	0.120	1.48E-07	4.84E-04	0.00	0.01	30	0.644	16	0.124	0.517	0.772	0.317	0.172	0.000	0.644	0.163
ApP	LSI	L	12	0.79	3.40	1.48	0.26	2.59	0.21	1.53	1.35	5.06	-0.273	29.24	0.175	1.62E-07	5.20E-04	0.08	0.08	32	0.653	34	0.199	0.517	0.789	0.413	0.279	0.124	0.653	0.272
Av	LSI	L	13	0.86	3.52	1.91	0.30	1.95	0.24	1.10	1.39	3.93	-0.235	7.37	0.279	6.65E-09	1.18E-04	0.42	0.26	14	0.266	49	0.424	0.371	0.161	0.595	0.466	0.694	0.266	0.585
Bl	LSI	L	13	0.86	3.52	1.91	0.21	1.66	0.18	1.08	1.28	3.37	-0.177	20.00	0.263	1.33E-06	1.50E-03	0.52	0.20	27	0.447	51	0.486	0.371	0.524	0.567	0.750	0.853	0.447	0.723
Bo	LSI	L	10	0.64	3.15	0.95	0.27	2.51	0.20	1.46	1.34	4.50	-0.308	27.57	0.088	1.67E-07	4.96E-04	0.00	0.00	38	0.718	9	0.099	0.695	0.741	0.261	0.108	0.000	0.718	0.123
Ca	LSI	L	12	0.79	3.43	1.32	0.26	2.46	0.20	1.48	1.34	4.77	-0.29	30.00	0.168	1.94E-07	5.83E-04	0.17	0.10	37	0.690	38	0.228	0.570	0.811	0.401	0.196	0.288	0.690	0.295
Ch	LSI	L	12	0.79	3.43	1.32	0.25	2.38	0.20	1.43	1.34	4.42	-0.243	27.91	0.133	3.20E-07	6.87E-04	0.14	0.11	33	0.660	37	0.217	0.570	0.751	0.340	0.426	0.231	0.660	0.333
Cn	LSI	L	15	1.00	3.71	3.00	0.37	1.59	0.29	0.93	1.47	3.32	-0.267	9.85	0.144	2.13E-10	2.06E-05	0.05	0.10	7	0.116	30	0.174	0.000	0.232	0.359	0.309	0.086	0.116	0.251
Co	LSI	L	3	0.14	1.49	0.06	0.26	2.57	0.20	1.45	1.33	4.42	-0.33	31.86	-0.057	2.43E-07	6.02E-04	0.00	0.00	55	0.932	2	0.002	1.000	0.864	0.007	0.000	0.000	0.932	0.002
Cp	LSI	L	10	0.64	3.21	1.39	0.27	2.46	0.20	1.42	1.34	4.33	-0.309	27.60	0.036	1.69E-07	5.08E-04	0.02	0.03	31	0.645	8	0.077	0.548	0.742	0.170	0.103	0.035	0.645	0.103
Di	LSI	L	11	0.71	3.25	0.95	0.26	2.40	0.20	1.44	1.33	4.43	-0.286	28.25	0.206	2.48E-07	6.05E-04	0.07	0.08	41	0.728	36	0.208	0.695	0.760	0.468	0.216	0.114	0.728	0.266
Eb	LSI	L	15	0.93	3.62	2.91	0.24	2.40	0.20	1.52	1.34	5.31	-0.24	24.88	0.106	2.98E-07	6.49E-04	0.00	0.06	21	0.348	23	0.145	0.032	0.664	0.292	0.441	0.000	0.348	0.245
Gi	LSI	L	14	0.93	3.62	2.91	0.26	2.56	0.20	1.50	1.34	4.63	-0.307	30.77	-0.010	2.21E-07	5.59E-04	0.00	0.00	25	0.432	5	0.042	0.032	0.833	0.089	0.113	0.000	0.432	0.067
Gl	LSI	L	11	0.71	3.30	1.43	0.26	2.50	0.20	1.47	1.34	4.50	-0.304	30.63	0.079	2.26E-07	5.75E-04	0.06	0.06	36	0.682	15	0.12	0.535	0.829	0.245	0.127	0.098	0.682	0.157
Is	LSI	L	15	1.00	3.71	3.00	0.36	1.53	0.28	0.89	1.46	3.18	-0.26	8.22	0.119	3.01E-10	2.51E-05	0.07	0.13	6	0.093	29	0.169	0.000	0.186	0.315	0.343	0.107	0.093	0.255
La	LSI	L	15	1.00	3.71	3.00	0.16	1.99	0.13	1.19	1.22	3.95	-0.261	29.07	0.090	5.22E-05	8.31E-03	0.06	0.21	24	0.392	24	0.15	0.000	0.784	0.264	0.338	0.102	0.392	0.235
Me	LSI	L	15	1.00	3.71	3.00	0.37	1.18	0.31	0.75	1.51	3.26	-0.197	1.75	0.140	5.14E-11	9.83E-06	0.24	0.18	1	0.000	42	0.289	0.000	0.000	0.352	0.652	0.402	0.000	0.469
Mo	LSI	L	4	0.21	1.72	0.09	0.26	2.56	0.20	1.45	1.33	4.42	-0.329	31.79	-0.061	2.43E-07	6.02E-04	0.00	0.00	54	0.925	1	0.001	0.988	0.862	0.000	0.005	0.000	0.925	0.002
Ne	LSI	L	5	0.29	2.20	0.23	0.26	2.53	0.20	1.46	1.34	4.43	-0.318	31.31	0.251	2.40E-07	5.97E-04	0.02	0.03	53	0.894	33	0.198	0.940	0.848	0.546	0.059	0.036	0.894	0.214
Nv	LSI	L	9	0.57	2.99	0.68	0.26	2.51	0.20	1.46	1.34	4.41	-0.313	31.05	0.052	2.38E-07	5.95E-04	0.00	0.00	46	0.814	7	0.075	0.788	0.841	0.198	0.083	0.000	0.814	0.094
Ol	LSI	L	15	1.00	3.71	3.00	0.30	2.26	0.23	1.33	1.38	4.13	-0.292	21.90	0.050	2.37E-08	2.00E-04	0.04	0.06	15	0.289	10	0.1	0.000	0.578	0.194	0.186	0.060	0.289	0.147
Or	LSI	L	15	1.00	3.71	3.00	0.19	1.86	0.16	1.32	1.28	4.84	-0.177	23.79	0.059	3.62E-06	2.30E-03	0.11	0.18	18	0.316	32	0.195	0.000	0.633	0.210	0.750	0.175	0.316	0.378
Pl	LSI	L	14	0.93	3.62	2.91	0.26	2.43	0.20	1.43	1.34	4.46	-0.272	27.60	0.115	2.29E-07	6.19E-04	0.06	0.06	23	0.387	27	0.158	0.032	0.742	0.308	0.284	0.098	0.387	0.230
Po	LSI	L	14	0.93	3.62	2.91	0.32	2.03	0.25	1.18	1.41	3.84	-0.278	16.59	0.137	5.49E-09	9.98E-05	0.12	0.07	12	0.229	31	0.194	0.032	0.426	0.347	0.255	0.202	0.229	0.268
Pu	LSI	L	12	0.79	3.40	1.48	0.26	2.55	0.20	1.51	1.34	4.91	-0.3	30.46	0.067	1.80E-07	5.53E-04	0.05	0.04	35	0.670	13	0.11	0.517	0.824	0.224	0.147	0.078	0.670	0.150
Py	LSI	L	5	0.29	2.46	0.51	0.27	2.59	0.20	1.49	1.34	4.56	-0.321	31.42	-0.031	2.09E-07	5.50E-04	0.00	0.00	49	0.849	3	0.023	0.847	0.851	0.053	0.044	0.003	0.849	0.033
Qu	LSI	L	12	0.79	3.34	0.95	0.26	2.46	0.20	1.46	1.34	4.69	-0.275	27.73	0.088	1.85E-07	5.56E-04	0.00	0.04	39	0.720	14	0.116	0.695	0.746	0.261	0.270	0.000	0.720	0.177
Ra	LSI	L	7	0.43	2.58	0.39	0.26	2.58	0.20	1.48	1.34	4.56	-0.319	31.32	0.040	2.15E-07	5.58E-04	0.00	0.00	51	0.867	6	0.065	0.886	0.849	0.177	0.054	0.000	0.867	0.077
Ri	LSI	L	10	0.64	3.08	0.67	0.26	2.47	0.20	1.48	1.34	4.48	-0.296	30.29	0.091	2.21E-07	5.70E-04	0.01	0.01	45	0.806	12	0.109	0.793	0.819	0.266	0.167	0.008	0.806	0.147
Ro	LSI	L	14	0.93	3.62	2.91	0.25	2.18	0.21	1.36	1.35	4.39	-0.225	22.97	0.234	1.39E-07	4.98E-04	0.19	0.18	19	0.320	44	0.307	0.032	0.609	0.517	0.515	0.312	0.320	0.448
Sl	LSI	L	13	0.86	3.49	1.47	0.25	2.40	0.20	1.44	1.33	4.42	-0.289	29.94	0.139	2.87E-07	6.51E-04	0.01	0.06	34	0.665	22	0.143	0.521	0.809	0.350	0.201	0.018	0.665	0.190
SyC	LSI	L	4	0.21	2.34	0.96	0.21	2.40	0.16	1.37	1.27	4.12	-0.32	36.60	-0.001	3.88E-06	2.34E-03	0.00	0.00	48	0.846	4	0.041	0.692	1.000	0.105	0.049	0.005	0.846	0.053
SyP	LSI	L	8	0.50	2.81	0.51	0.21	2.32	0.16	1.33	1.27	3.97	-0.31	34.47	0.050	4.22E-06	2.47E-03	0.07	0.07	52	0.893	11	0.102	0.847	0.939	0.194	0.098	0.107	0.893	0.133
Va	LSI	L	15	1.00	3.71	3.00	0.26	2.31	0.21	1.41	1.34	4.41	-0.269	24.42	0.112	1.54E-07	4.80E-04	0.08	0.09	20	0.325	28	0.165	0.000	0.650	0.303	0.299	0.126	0.325	0.243
Vi	LSI	L	9	0.57	2.99	0.68	0.26	2.47	0.20	1.48	1.35	4.59	-0.274	29.36	0.170	2.26E-07	5.69E-04	0.09	0.09	44	0.790	35	0.202	0.788	0.792	0.405	0.275	0.147	0.790	0.275
Vl	LSI	L	15	1.00	3.71	3.00	0.29	2.23	0.24	1.43	1.41	4.97	-0.234	18.48	0.113	1.30E-08	1.38E-04	0.00	0.01	13	0.240	25	0.152	0.000	0.480	0.305	0.471	0.000	0.240	0.258
Vz	LSI	L	9	0.57	2.95	0.67	0.26	2.38	0.20	1.37	1.34	4.23	-0.305	25.78	0.141	1.90E-07	5.44E-04	0.00	0.00	43	0.741	19	0.131	0.793	0.690	0.354	0.123	0.000	0.741	0.159
AiCS	SC	S	12	0.79	3.40	1.48	0.19	1.86	0.16	1.13	1.25	3.47	-0.199	21.68	0.143	8.43E-06	3.55E-03	0.51	0.16	29	0.544	48	0.399	0.517	0.572	0.357	0.642	0.835	0.544	0.611
AiPS	SC	S	15	1.00	3.71	3.00	0.33	1.39	0.29	1.01	1.48	3.69	-0.126	7.32	0.321	2.48E-10	2.12E-05	0.14	0.23	4	0.080	47	0.388	0.000	0.160	0.669	1.000	0.226	0.080	0.632
AvS	SC	S	14	0.93	3.62	2.91	0.21	1.95	0.17	1.21	1.28	3.66	-0.221	21.67	0.389	1.54E-06	1.64E-03	0.10	0.18	17	0.302	46	0.361	0.032	0.571	0.788	0.534	0.162	0.302	0.495
Bl	SC	S	13	0.86	3.52	1.91	0.20	1.75	0.17	1.08	1.27	3.40	-0.158	19.74	0.468	2.50E-06	2.01E-03	0.54	0.19	26	0.444	53	0.627	0.371	0.516	0.926	0.843	0.899	0.444	0.890
Ce	SC	S	10	0.64	3.18	0.84	0.19	2.06	0.15	1.24	1.24	3.58	-0.286	36.39	0.127	1.37E-05	4.49E-03	0.00	0.00	50	0.864	20	0.133	0.733	0.994	0.329	0.216	0.000	0.864	0.182
MaC	SC	S	14	0.93	3.62	2.91	0.35	1.77	0.28	1.15	1.48	4.45	-0.222	6.34	0.163	4.57E-10	2.58E-05	0.12	0.09	5	0.082	39	0.236	0.032	0.132	0.392	0.529	0.192	0.082	0.371
MaP	SC	S	11	0.71	3.30	1.43	0.25	1.44	0.22	0.87	1.33	2.62	-0.139	9.13	0.434	4.24E-08	3.12E-04	0.61	0.15	22	0.373	54	0.643	0.535	0.212	0.867	0.936	1.000	0.373	0.934
Mn	SC	S	11	0.71	3.30	1.43	0.19	2.07	0.14	1.21	1.24	3.50	-0.294	33.70	0.096	1.44E-05	4.62E-03	0.06	0.09	40	0.726	21	0.137	0.535	0.917	0.275	0.176	0.104	0.726	0.185
Mt	SC	S	13	0.86	3.49	1.47	0.21	1.84	0.18	1.13	1.28	3.46	-0.194	21.02	0.510	9.87E-07	1.29E-03	0.49	0.19	28	0.537	52	0.61	0.521	0.553	1.000	0.667	0.812	0.537	0.826
PoS	SC	S	15	1.00	3.71	3.00	0.26	1.87	0.21	1.08	1.34	3.51	-0.235	15.86	0.093	9.51E-08	4.21E-04	0.29	0.14	10	0.202	41	0.26	0.000	0.405	0.270	0.466	0.476	0.202	0.404
Sa	SC	S	15	1.00	3.71	3.00	0.32	1.32	0.28	0.93	1.46	3.82	-0.148	2.98	0.152	5.34E-10	3.05E-05	0.18	0.21	3	0.018	43	0.293	0.000	0.035	0.373	0.892	0.289	0.018	0.518
Su	SC	S	15	1.00	3.71	3.00	0.20	1.99	0.17	1.41	1.29	4.95	-0.172	22.39	0.080	2.76E-06	1.94E-03	0.22	0.19	16	0.296	40	0.256	0.000	0.592	0.247	0.775	0.357	0.296	0.459
Vy	SC	S	10	0.64	3.15	0.95	0.19	2.15	0.15	1.24	1.24	3.64	-0.304	35.81	0.063	1.13E-05	4.06E-03	0.09	0.09	47	0.836	17	0.124	0.695	0.977	0.217	0.127	0.152	0.836	0.166

Table SI2: All genetic and genotypic indices computed on the 53 sampled populations. Each index was obtained from 15 individuals sampled in each population. (See spreadsheet supplementary material). *Mating* column indicates if the population was supposed to expressed a late-acting self-incompatible system (LSI) or be self-compatible (SC), the seven underlined populations being directly tested by controlled crosses using 15 plants randomly sampled in the population. *Morph* indicates the floral morph of the population. *G* is the number of multi-locus genotypes genotyped on the 15 sampled individuals per population; *R* is the genotypic diversity; *D** clonal heterogeneity; *Pareto's β* the clonal evenness; *HO*, the mean observed heterozygosity; *var(ho)*, the variance of the observed heterozygosity among loci; *HE*, the mean expected heterozygosity; *var(he)*, the variance of the expected heterozygosity among loci; *AE*, the mean effective number of alleles among loci; *var(Ae)*, the variance of the effective number of alleles among loci; *FIS*, the mean inbreeding coefficient; *var(FIS)*, the variance of the inbreeding coefficient among loci; *rd*, the population index of multilocus linkage disequilibrium; *PIDu* and *PIDsib*, unbiased probability of identity under panmixia and between sibs respectively; *Sg*, the estimate of selfing and *SE(Sg)* the standard error of estimate of selfing. Then, the relative rankings of populations according to different indices and the relative rankings of reproduction modes (see Material and Methods). Full table is available at https://doi.org/10.5281/zenodo.12760022, tabulation "*Genetic indices per pop*".

Population with missing genotype	nb_missing_genotypes	nb_individuals_concerned
Bléré_Ls	1	1
Chambéon_écopôle_du_Forez	2	2
Le_Port_Ls	1	1
Plougonver_Léguer	1	1
Quevillon	1	1
Rennes_apigné_prairie	1	1
Roost-Warendin	1	1
Saint_Aignan_Couflons_Ls	1	1
Vichy	1	1

Over single SNP genotypes	N	%age
single SNP genotype P(cad)>70%	28610	0.99965
single SNP genotype P(cad)≤70%	10	0.00035
total	28620	1

Over individuals	N	%age
nb_individuals_with_full_genotype	785	0.98742
nb_individuals_with_one_missing_genotype	10	0.01258
total	795	1

Table SI3: Summary of the allele dosage. First subtable lists populations with individual genotyped with at least one individual with missing genotype at one locus. Second subtable reports the frequency of single SNP genotypes with posterior probability of allele dosage strictly superior to 70%. Third subtable reports the frequency of individuals with their 36 SNP genotypes assigned with posterior probability superior to 70%.

Table SI4: Distribution of the different repeated Multi-Locus Genotypes (MLGs) found with 36 SNPs among populations considering their mating system (LSI or SC). Figure 1 presents a summarized plot of this table. (See spreadsheet supplementary material).

	G	H_O	H_E	A_E	R	β	F_{IS}	$VarF_{IS}$	r_d	PID_{SIB}	S_g	Σ_{clon}	Σ_{self}
G	-	0.438	**0.013**	**0.009**	***	***	***	***	0.057	**0.008**	**0.008**	***	***
H_O	0.076	-	***	***	0.397	0.462	0.122	**0.025**	0.653	***	**0.032**	0.065	0.142
H_E	**0.244**	**0.742**	-	***	**0.01**	**0.014**	0.212	***	**0.026**	***	0.844	***	0.372
A_E	**0.255**	**0.722**	**0.82**	-	**0.008**	**0.013**	0.353	***	0.094	***	0.347	***	0.845
R	**0.991**	0.083	**0.251**	**0.258**	-	***	***	***	0.052	**0.007**	**0.005**	***	***
β	**0.91**	0.071	**0.238**	**0.24**	**0.919**	-	***	***	0.126	**0.009**	**0.004**	***	***
F_{IS}	**0.602**	-0.144	0.116	0.086	**0.603**	**0.557**	-	***	***	0.163	***	***	***
$VarF_{IS}$	**-0.579**	**-0.209**	**-0.435**	**-0.346**	**-0.589**	**-0.551**	**-0.626**	-	***	***	***	***	***
r_d	0.187	0.042	**0.207**	0.156	0.19	0.148	**0.432**	**-0.365**	-	**0.021**	***	**0.006**	***
PID_{SIB}	**0.259**	**0.729**	**0.981**	**0.833**	**0.265**	**0.251**	0.13	**-0.443**	**0.214**	-	0.936	***	0.286
S_g	**0.264**	**-0.202**	-0.019	-0.089	**0.277**	**0.28**	**0.542**	**-0.379**	**0.353**	-0.008	-	***	***
Σ_{clon}	**-0.79**	**-0.171**	**-0.376**	**-0.347**	**-0.799**	**-0.809**	**-0.604**	**0.756**	**-0.254**	**-0.387**	**-0.34**	-	***
Σ_{self}	**0.388**	**-0.136**	0.083	0.018	**0.399**	**0.361**	**0.723**	**-0.524**	**0.631**	0.099	**0.69**	**-0.451**	-

Table SI5: Correlations between genetic indices using non-parametric Kendall partial rank-order correlation. Below the diagonal, the values of the coefficient of correlation τ are consigned. Above, the related p-values are provided. Values inferior to the threshold 0.05, and the related coefficient τ were bold. The parameters Σ_{CLON} and Σ_{SELF} correspond to the sum of normalized indices sensitive to clonality and selfing, respectively.

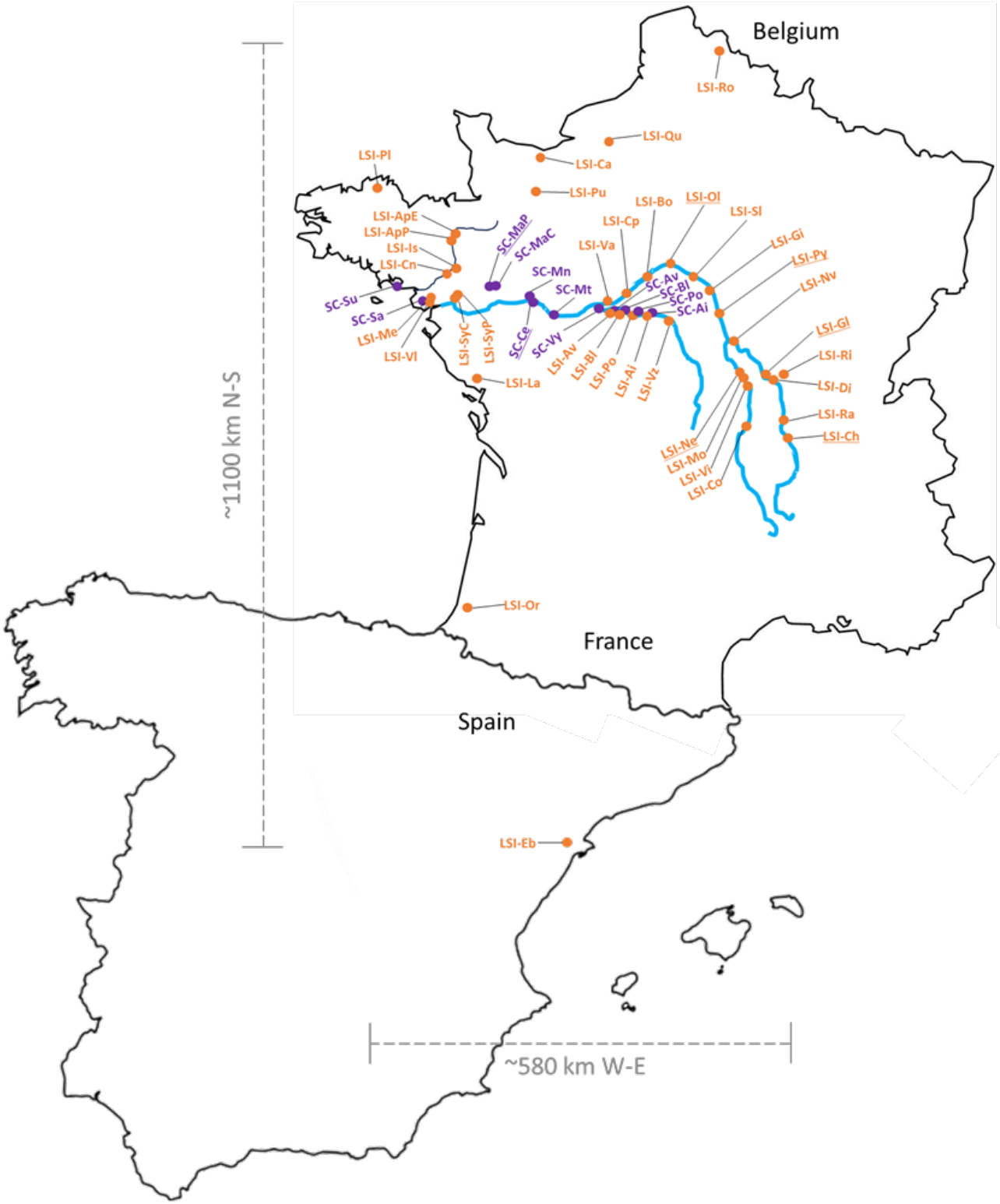

Figure SI1: Map of the locations of the 53 sampled populations across France and Spain. In orange, populations with only L-morph individuals, supposed to mate under the control of a late-acting self-incompatible (LSI); In purple, populations with only S-morph individuals, supposed to be self-compatible (SC). In light blue, the Loire river system. In grey, the North-to-South (N-S) and West-to-East (W-E) scale distances of the sampled populations. Underlined, the seven populations in which 15 individuals were tested for the self-incompatibility (Portillo-Lemus et al. 2021) and one individual was karyotyped to count the chromosome numbers.

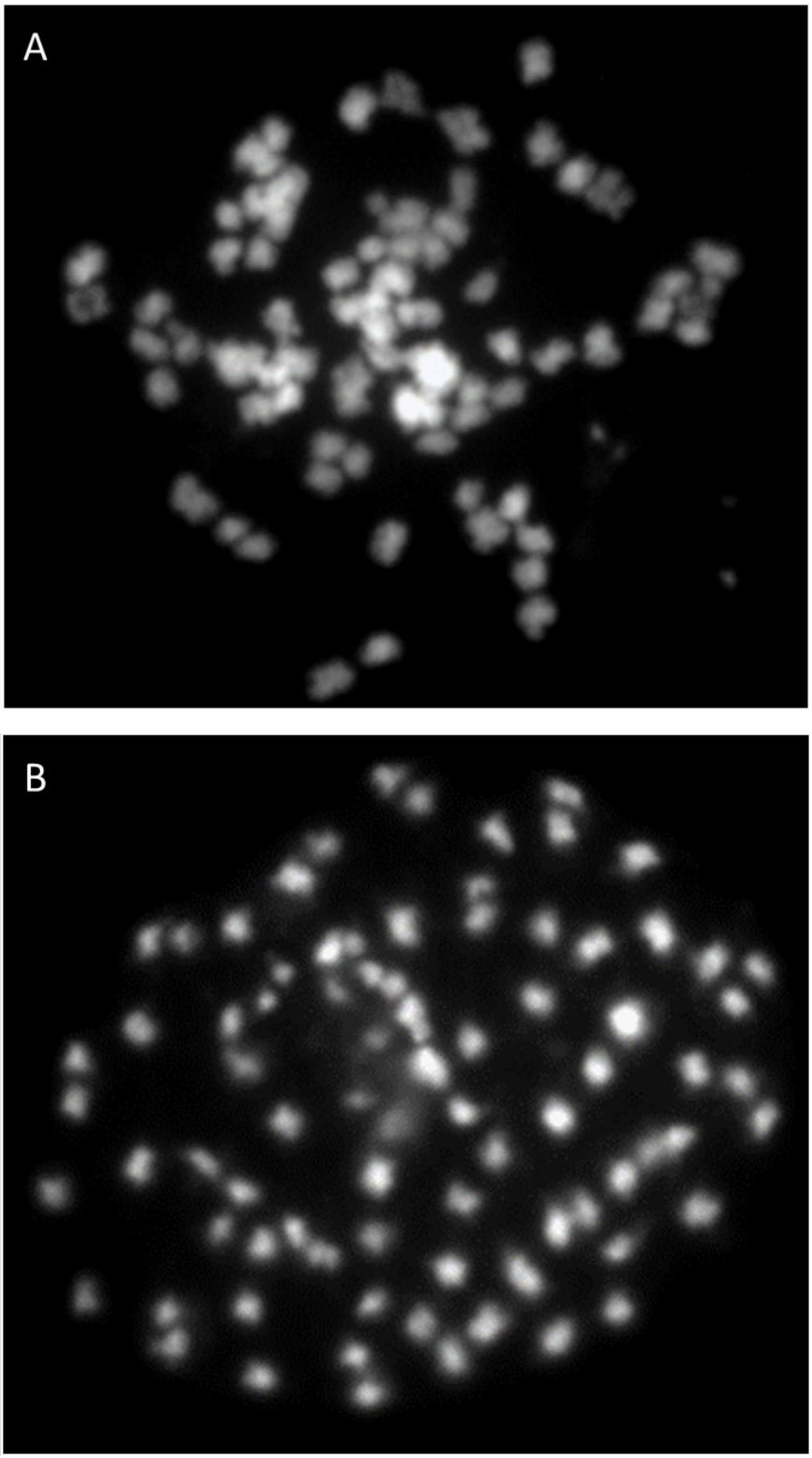

Figure SI2: Photos of somatic metaphase chromosomes indicating that L- and S-morph individuals that succeed to mate and to give full fruit set and 100% viable first- and second-generation descendants (Portillo-Lemus et al. 2021) also present the same number of chromosomes (2n=10X=80), belonging to the same taxon *Ludwigia grandiflora* subsp. *hexapetala*. We still didn't find *Ludwigia grandiflora* subsp. *grandiflora* karyotype in France (2n=6X=48, see Dandelot 2005, Barloy et al. 2024). **A**: somatic metaphase chromosomes from L-morph (2n=10X=80); **B**: somatic metaphase chromosomes from S-morph (2n=10X=80).

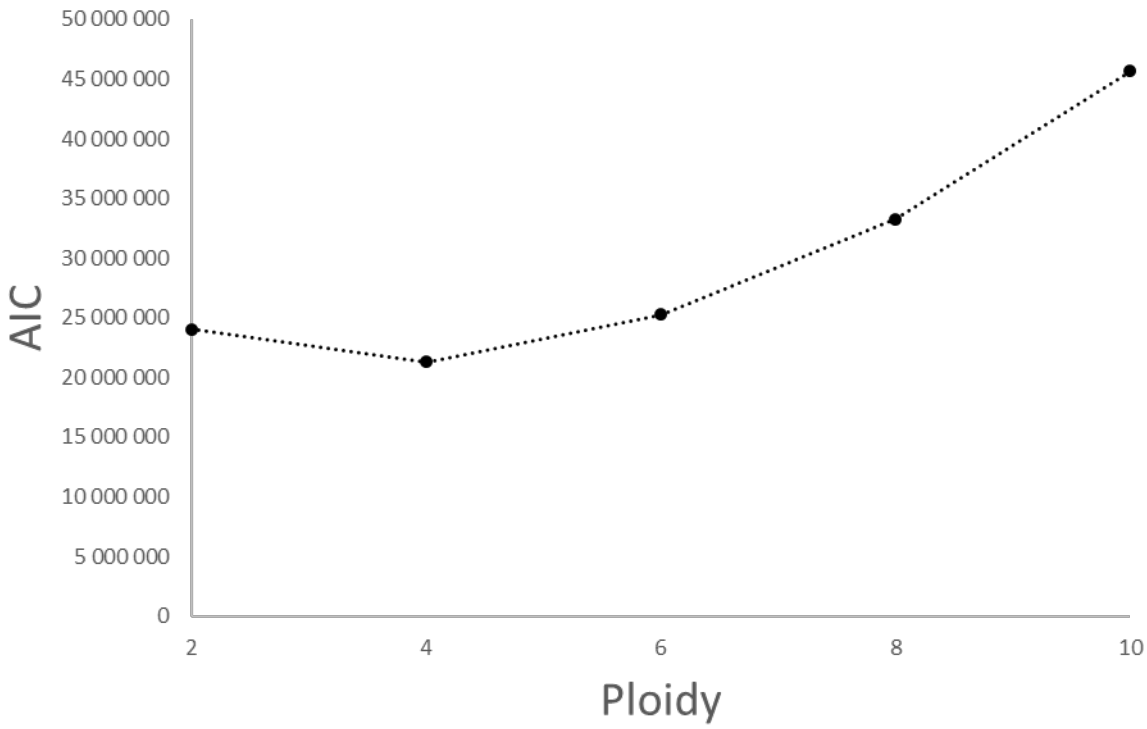

Figure SI3: Akaike's information criterion (AIC) as function of the ploidy level on the likelihoods of the sequenced allele countings over all individuals. Tetraploidy, a ploidy 4x, presents the lowest AIC, and thus the best supported model to explain distribution of sequenced allele countings over all individuals, which confirm our initial SNP development to be sure of their ploidy level.

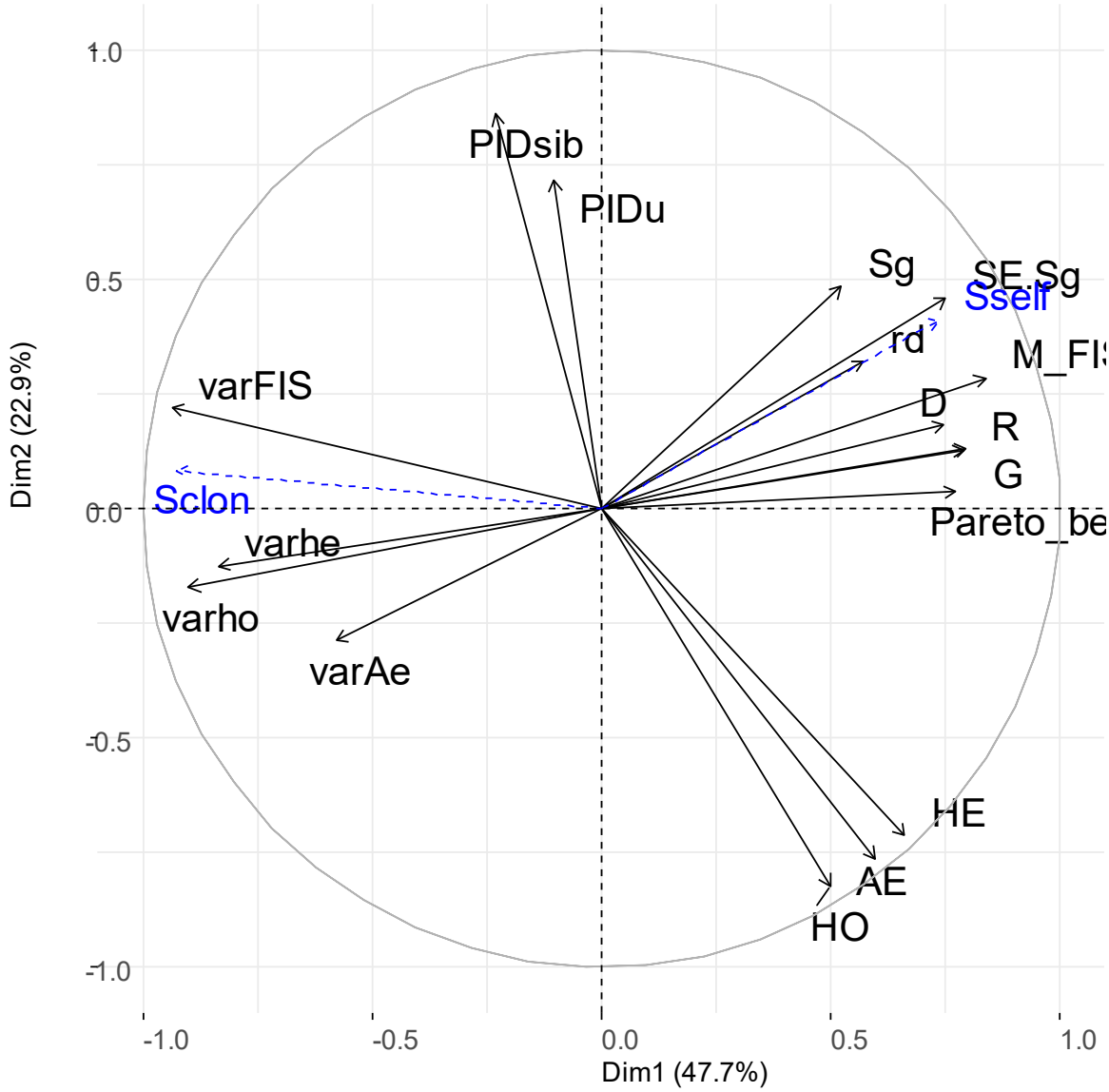

Figure SI4.A: Correlation circles of the principal component analysis on the 17 centered and scaled genetic indices measured in 53 *Lgh* populations in western Europe. The first horizontal dimension accounting for 47.7% of the variance regroups indices related to clonality (number of genotypes *G*, genotypic diversity *R*, clonal heterogeneity *D*, clonal evenness *Beta_Pareto*, variance of Fis *varFis*, variance of expected heterozygosity *varHe*, variance of observed heterozygosity *varHo* and variance of effective number of alleles varAe between loci). The second vertical dimension accounting for 22.9% of the variance with a main contribution of estimate of selfing Sg. Other indices, *i.e.*, mean observed heterozygosity *Ho*, mean expected heterozygosity *He*, effective number of alleles *Ae*, inbreeding coefficient *Mfis*, linkage disequilibrium *rd*, standard error of estimate of selfing *SE.Sg*, unbiased probability of identity under panmixia *PIDu* and between sibs *PIDsib*. In blue and dashed lines, the predictions of Σclon (*Sclon*) and Σself (*Sself*) provided as supplementary variables.

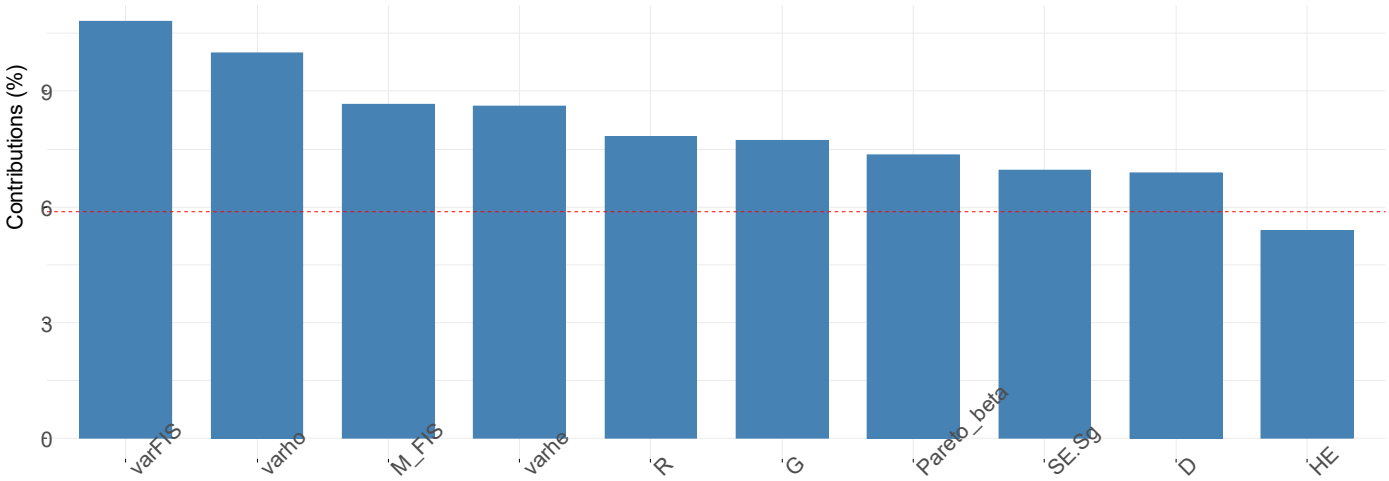

Figure SI4.B: Bar plot of the genetic index contributions to the first principal component. The expected average contribution *1/(number of genetic indices)* is plotted as the red dashed line. VarFis and indices of genotypic diversity (Pareto , R, G & D) are the main contributors to the first component.

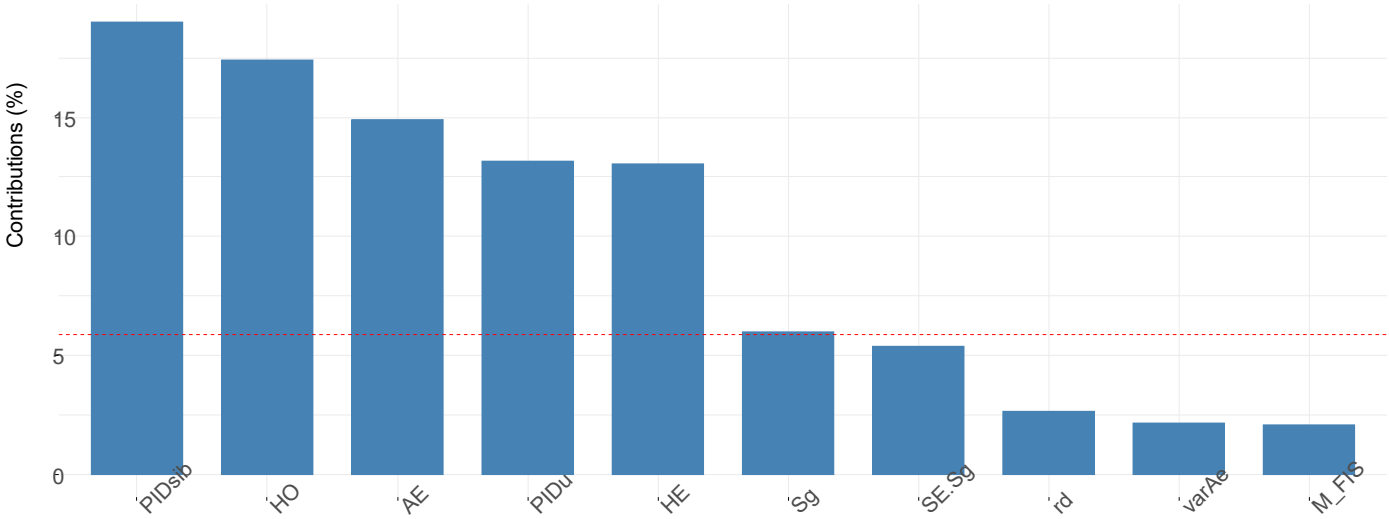

Figure SI4.C: Bar plot of the genetic index contributions to the second principal component. The expected average contribution *1/(number of genetic indices)* is plotted as the red dashed line. Estimates of selfing (Sg), mean observed heterozygosity (H_O), mean effective number of alleles (A_E) and probabilities of identities (pid_u and pid_{sib}) are the main contributors to the second component.

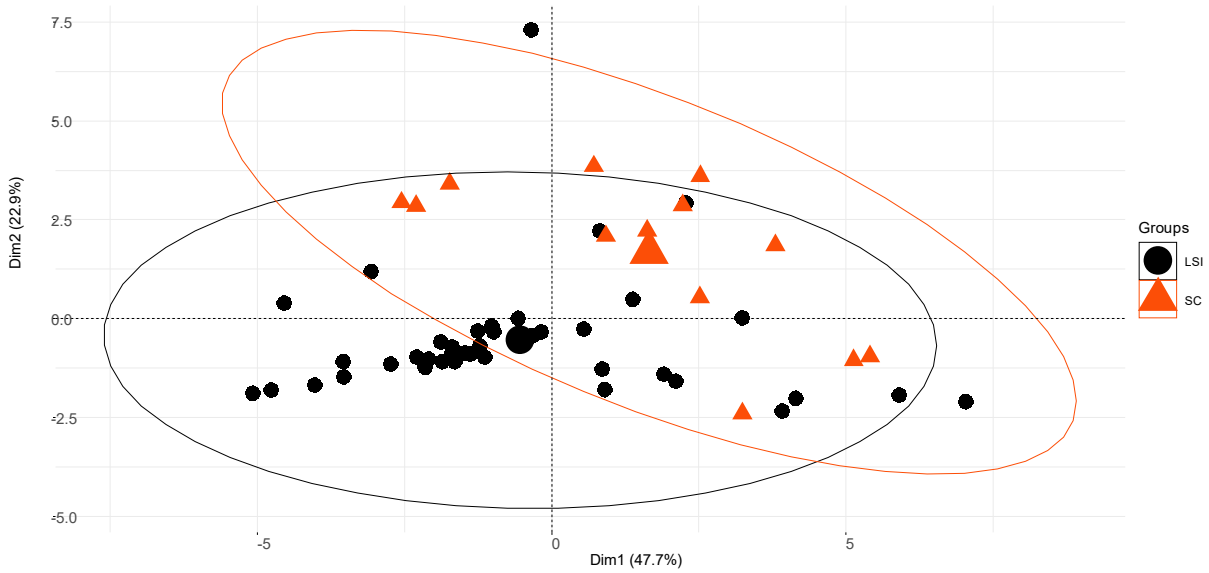

Figure SI4.D: Plot of the projection of each population onto the two first principal components. SC populations are plotted as red triangles and LSI populations as black diamonds. Concentration ellipses around LSI and SC populations are plotted in black and red, respectively. Barycenters of the two groups are represented as bigger black diamond and red triangle, respectively.

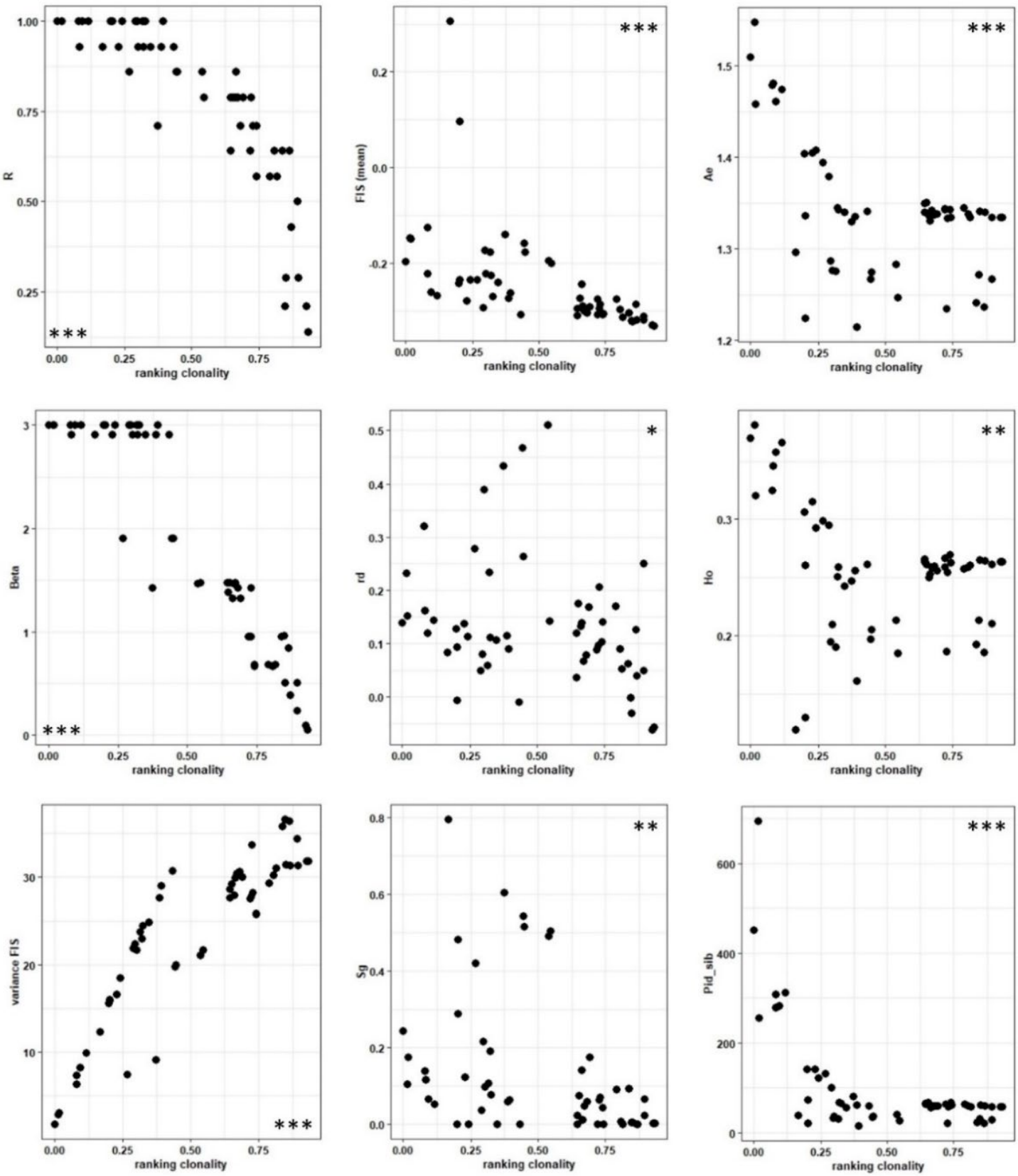

Figure SI5: Relationship and correlation between Σ_{CLON} and genetic indices (R, Pareto β, MFis, VarFis, Ho, Ae, Sg, pidsib and $\bar{r}_d$). Per graph, all the 53 populations were plotted as black dots. *: p<0.05, **: p<0.01, ***: p<0.001.

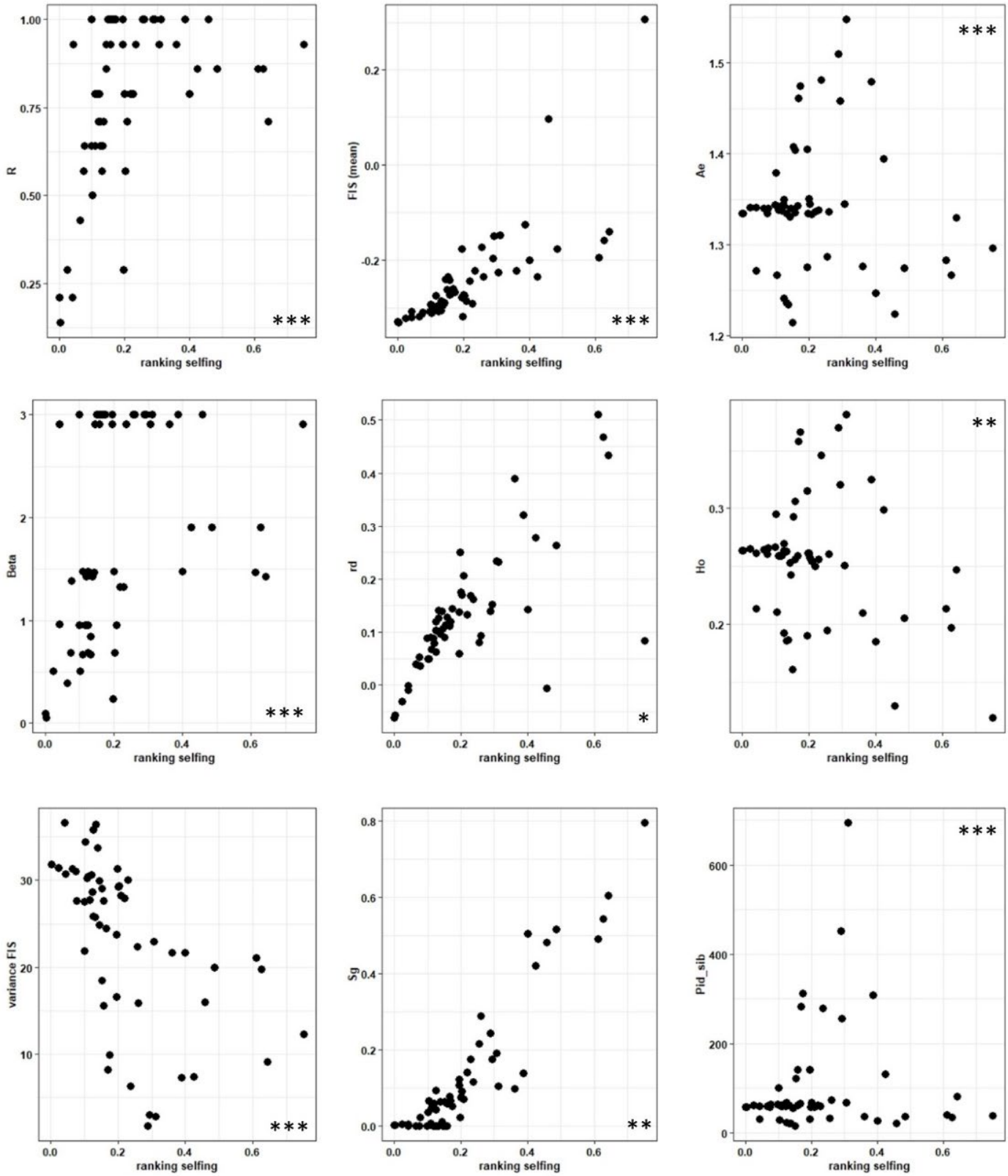

Figure SI6: Relationship and correlation between Σ_{SELF} and genetic indices (R, Pareto β, MFis, VarFis, Ho, Ae, Sg, pidsib and $\bar{r}_d$). Per graph, all the 53 populations were plotted as black dots. *: p<0.05, **: p<0.01, ***: p<0.001.

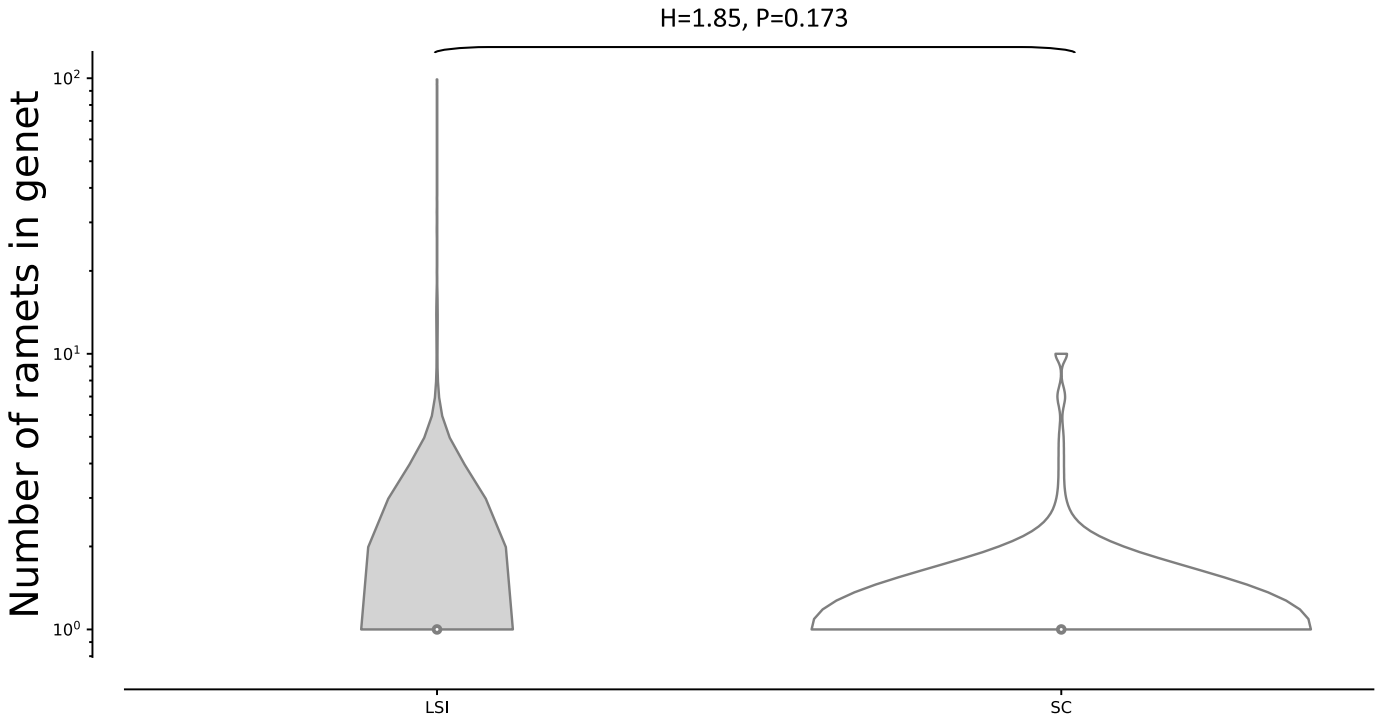

Figure SI7: Distributions of the logarithms of the number of ramets per genet in LSI (grey) and SC (white) populations. We report the Kruskal-Wallis probability that the two distributions are identical.