1 Late-acting self-incompatible system, preferential allogamy and delayed selfing in the

2 ~~heterostylous~~heteromorphic invasive populations of *Ludwigia grandiflora* subsp. *hexapetala*

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4 Luis O. PORTILLO LEMUS[1], Marilyne HARANG[1], Michel BOZEC[1], Jacques HAURY[1], Solenn

5 STOECKEL[2], Dominique BARLOY[1]

6 Short title: ~~Mixed mating~~Breeding system in a European invasive *Ludwigia*

7 [1] ~~ESE, Ecology and~~ DECOD, (Ecosystem ~~Health,~~Dynamics and Sustainability), Institut Agro -

8 Agrocampus Ouest, IFREMER, INRAE, ~~35042,~~Rennes, France

9 [2] IGEPP, INRAE, Institut Agro, Univ Rennes, 35653, Le Rheu, France

10 Corresponding author: ~~dominique.barloy@agrocampus-ouest~~Dominique.Barloy@Agrocampus-

11 Ouest.fr

12 Orcid number:

13 Luis O. PORTILLO LEMUS: 0000-0003-2123-4714

14 Jacques HAURY: 0000-0002-8628-8265

15 Solenn STOECKEL: 0000-0001-6064-5941

16 Dominique BARLOY: 0000-0001-5810-4871

17

18

Abstract

20 ~~Mating~~Breeding system influences local population genetic structure, effective size,

21 offspring fitness and functional variation. Determining the respective importance of self- and

22 cross-fertilization in hermaphroditic flowering plants is thus important to understand their

ecology and evolution. The worldwide invasive species, *Ludwigia grandiflora* subsp. *hexapetala* (*Lgh*) presents two floral morphs: one self-compatible short-styled morph (S-morph) and one self-incompatible long-styled morph (L-morph). ~~Most invasive populations worldwide are only composed of self-incompatible L-morphs, which questions the importance of sexual reproduction during the invasion.~~ In this study, we identified the ~~mating~~breeding systems of western European experimental and natural populations of *Lgh* by comparing structural characteristics of pollen and style, by studying self- and cross-pollen tube elongations and the viability of the resulting seeds and seedlings in both floral morphs. Our results showed no differences in pollen shape and stigma surfaces among and between the two floral morphs. In the self-incompatible L-morph flowers, self-pollen tubes were stopped tardily, in the ovarian area, and were unable to fertilize the ovules. This first formal identification of a late-acting, prezygotic self-incompatible system in *Ludwigia* genus questions on the distribution of this ~~mating~~breeding system in the Myrtales order. In the self-compatible S-morph flowers~~, rarer in worldwide invasive populations~~, self-pollen always succeeded to self-fertilize the ovules that nearly all developed into viable seedlings. However, cross-pollen tubes always elongated faster than self-pollen tubes. S-morph individuals may thus advantage preferential allogamy over selfing when cross-pollen is available despite its self-compatibility. As expected in late-acting self-incompatible systems, L-morph flowers authorised 0.2‰ of selfed seeds during the uppermost flowering season, that increased to 1‰ at the end of the flowering season. Such delayed selfing ~~resulting~~resulted in a significant quantity of viable floating seeds. They may contribute to the local regeneration, seed bank and propagation of the L-morph, which may contribute to explain its invasion success worldwide. Management plans of *Lgh* would gain to consider the ~~mixed mating system~~breeding systems we identified.

Introduction

Around 50% of angiosperm species develop a variety of self-incompatible mechanisms (SI) that favour allogamy by avoiding self-fertilisation and promoting outcrossing (Igic et al. 2008; Fujii et al. 2016). Characterizing the type of SI by identifying the site of the pollen-pistil recognition within species is essential to categorize and assess the prevalence of SI systems across Angiosperms and beyond, and understand their phylogeny and evolution (Charlesworth et al. 2005; Igic et al. 2008; Santos-Gally et al. 2013; Gibbs 2014a; Fujii et al. 2016; Grossenbacher et al. 2017; Barrett 2019). Moreover, identifying the type of SI mechanism developed by specific species and populations helps understanding plant ecology and evolution, including how their mating systems may contribute to their adaptation in a context of global changes (Charlesworth et al. 2005; Takayama and Isogai 2005; Busch and Schoen 2008; Ferrer and Good 2012; Grossenbacher et al. 2017). Mating systems are indeed the main factor influencing the evolution of genetic diversity in populations and species with potential consequences for adaptation and peripatric speciation (Duminil et al. 2007; Ellegren and Galtier 2016).

We currently consider three major mechanisms of self-incompatibility in flowering plants, homomorphic gametophytic, homomorphic sporophytic and heteromorphic sporophytic, that differ in the site of the rejection of incompatible pollen tubes (Gibbs and Bryan 1986; Dickinson et al. 1992; Hinata et al. 1993; Barrett and Cruzan 1994b; Kao and McCubbin 1996; de Nettancourt 1997). Homomorphic SI systems imply that flowers with different SI types present the same flower shape and structure. By contrast, heteromorphic self-incompatibility (HetSI) associates different SI systems with different flower structure such as heterostyly (Darwin 1877; Barrett and Cruzan 1994a; Igic et al. 2004; Barrett 2019). Heterostyly defines the occurrence in a species of two or three floral morphs (di- and tristly) that exhibit reciprocal herkogamy (spatial distancing of the anthers and stigma in the 3D

74 ~~architecture of a flower, Barranco et al. 2019), sometimes associated with additional features~~

75 ~~such as differences in pollen sizes and shapes, and different lengths of stigmatic papillae~~

76 ~~(Barrett and Shore 2008). Commonly, species with style polymorphism have a sporophytic~~

77 ~~heteromorphic (*i.e.* di- or tri-allelic) incompatibility system that prevents self-fertilization and~~

78 ~~crosses between individuals of the same floral morph (Barrett 2019).~~

79 ~~In most studied species developing these three SI systems, pollen-tube growth is stopped~~

80 ~~from the stigma or in the style as a result of a rapid response to the pollen-pistil interaction.~~

81 ~~However, some species present a delayed reaction of self-pollen rejection, named ovarian or~~

82 ~~late-acting self-incompatibility (LSI) because occurring lately in the ovarian area (Seavey and~~

83 ~~Bawa 1986; Gibbs and Bianchi 1993; Sage et al. 1994; Gibbs 2014a).~~Around 50% of

84 angiosperm species develop a variety of self-incompatible mechanisms that favour allogamy

85 by avoiding self-fertilisation and promoting outcrossing (Igic et al. 2008; Fujii et al. 2016).

86 Self-incompatibility (SI) is the inability of functional male and female gametes to achieve self-

87 fertilization and fertilization in particular combinations of parents. Prezygotic SI involve

88 biochemical reactions resulting from the pollen-pistil interaction that blocks incompatible

89 pollen before sperm fertilizes an egg. Out of a handful of model species, the precise nature of

90 these reactions in most species is not clearly understood, with an intriguing paradox: recurrent

91 patterns of SI are ubiquitous across families but with a large diversity in the type of sites of

92 reaction (Charlesworth et al. 2005; Allen and Hiscock 2008; Shimizu and Tsuchimatsu 2015).

93 Characterizing the type of SI individuals develop in a species by identifying the site of the

94 incompatible pollen-pistil rejection is a first essential step to understand and predict how its

95 breeding system may shape the evolution of its populations in their ecological contexts

96 (Charlesworth et al. 2005; Takayama and Isogai 2005; Busch and Schoen 2008; Ferrer and

97 Good 2012; Grossenbacher et al. 2017). Moreover, characterizing the prevalence of SI systems

98 developed in species, genera and families across the Angiosperms and eucaryotes contribute

99 understanding which biological, ecological and evolutionary features may explain the
100 ubiquitous occurrence of mechanisms favouring allogamy across the tree of life (Charlesworth
101 et al. 2005; Igic et al. 2008; Santos-Gally et al. 2013; Gibbs 2014; Fujii et al. 2016;
102 Grossenbacher et al. 2017; Barrett 2019). Breeding systems are indeed the main factor
103 influencing the evolution of genetic diversity in populations and species with potential
104 consequences for adaptation and speciation (Duminil et al. 2007; Ellegren and Galtier 2016).

105   We currently consider three major mechanisms of SI in flowering plants, homomorphic
106 gametophytic, homomorphic sporophytic and heteromorphic sporophytic, that differ in the site
107 of the rejection of incompatible pollen tubes (Gibbs and Bryan 1986; Dickinson et al. 1992;
108 Hinata et al. 1993; Barrett and Cruzan 1994; Kao and McCubbin 1996; de Nettancourt 1997).
109 Homomorphic SI systems imply that flowers with different SI types present the same flower
110 shape and structure, or a continuous variation of floral morphologies not correlated with
111 compatibility. By contrast, heteromorphic self-incompatibility (HetSI) associates different
112 compatibility between individuals with different floral morphologies, sometimes associated
113 with additional features such as different patterns of spatial separation of anthers and stigmas
114 (Webb and Lloyd 1986; Opedal 2018), differences in pollen sizes and shapes, and different
115 lengths of stigmatic papillae (Darwin 1877; Barrett and Shore 2008; Igic et al. 2008; Barranco
116 et al. 2019; Barrett 2019; Matsui and Yasui 2020). Commonly, species with style polymorphism
117 have a sporophytic heteromorphic (*i.e.,* di- or tri-allelic) incompatibility system that prevents
118 self-fertilization and crosses between individuals of the same floral morph (Barrett 2019).

119   In most studied species developing these three SI systems, pollen-tube growth is stopped
120 from the stigma or in the style as a result of a rapid response to the pollen–pistil interaction.
121 However, some species present a delayed reaction of self-pollen rejection, named ovarian or
122 late-acting self-incompatibility (LSI) because occurring lately in the ovarian area (Seavey and
123 Bawa 1986; Gibbs and Bianchi 1993; Sage et al. 1994; Gibbs 2014). It often coincides with a

124 residual permeability of their SI systems resulting in a steady low level of selfing despite an

125 effective late-acting SI ~~(Seavey and Bawa 1986; Gibbs 2014a). It concerns both homomorphic~~

126 ~~and heterostylous~~(Seavey and Bawa 1986; Gibbs 2014). It concerns both homomorphic and

127 heteromorphic species ~~(Gibbs 2014a; Simon-Porcar et al. 2015)~~(Gibbs 2014; Simon-Porcar et

128 al. 2015). For example, the homomorphic LSI Theaceae species *Camelia oleifera* and *Camelia*

129 *sinensis,* allow 10% and ~2% of self-fertilization resulting in viable seeds, respectively (Chen

130 et al. 2012; Liao et al. 2014). In the ovarian LSI ~~heterostylous~~heteromorphic *Narcissus spp.*

131 from the Amarilidacea, from 4 to 30% of their seed-sets result from self-fertilization (Barrett et

132 al. 2004; Medrano et al. 2012; Simon-Porcar et al. 2015). Within ~~literally~~ pre-zygotic LSI,

133 Gibbs (2014) proposes to distinguish LSI mechanisms stopping pollen tubes before ovaries (as

134 found in *Melaleuca spp.* and *Thryptomene calycina,* Barlow and Forrester 1984; Beardsell et

135 al. 1993) from those stopping self-pollen tubes while penetrating the ovules (as found in *Acacia*

136 *retinodes,* Kenrick et al. 1986).

137   In the *Ludwigia* genus (Onagraceae), 8 of 82 species have been reported as "self-

138 incompatible", although the nature of their SI were not formally studied and established ~~(Raven~~

139 ~~1979). Water~~(Raven 1979). The water primrose, *Ludwigia grandiflora* subsp. *hexapetala*

140 (Hook. & Arn.) Nesom and Kartesz (2000), (hereafter *Lgh*), is one of the most invasive aquatic

141 plants in the world ~~(Thouvenot et al. 2013). Tackling how this species reproduces is crucial for~~

142 ~~understanding how to slow its expansion and alleviate its impacts in the invaded,~~ currently

143 spreading out of South America into North America, Europe and Eastern Asia (EPPO 2011,

144 Thouvenot et al. 2013; Portillo-Lemus et al. 2021). Tackling how this species reproduces is

145 crucial for understanding its expansion and alleviate its impacts in aquatic environments

146 (EPPO~~,~~ 2011). Yet, we still don't know how sexual reproduction ~~contributes to the invasiveness~~

147 ~~of the~~works in this invasive species ~~in the newly-colonized areas (Dandelot 2004; Ruaux 2008;~~

148 ~~Thouvenot et al. 2013). This species presents two types of heterostylous flowers in European~~

invasive(Dandelot 2004; Ruaux 2008; Thouvenot et al. 2013). *Lgh* presents two types of floral morphologies in European populations: a short-styled morph (S-morph) that is self- and intra-morph compatible (crosses produce viable seeds), and a long-styled morph (L-morph) that is self- and intra-morph incompatible (crosses do not produce seeds, Portillo-Lemus et al. 2021). In both floral morphs, inter-morph crosses always produce viable seeds. The two floral morphs of *Lgh* develop bowl-shaped non-tubular flowers with two whorls of stamens of different heights. In the L-morph flowers, stamens of the second whorl (inner whorl) are longer than these of the first whorl (outer whorl). These two characteristics contrast with typical heteromorphic SI species that most frequently present tubular flowers and only one whorl of stamens (Barrett and Shore 2008; Cohen 2010; Barrett 2019)(Barrett and Shore 2008; Cohen 2010; Barrett 2019). Interestingly, floral morphs are mostly found in allopatric monomorphic populations (*i.e.,* exclusively S-morph or exclusively L-morph populations) in Western Europe and other invasive worldwide populations (Hieda et al. 2020; Portillo-Lemus et al. 2021). Surprisingly, around 75% of the invasive populations worldwide areseem exclusively composed of self-incompatible L-morph individuals, tackling the paradigm that successful invasive species may be mostly composed of individuals able to reproduce using self-fertilization when mating (Baker 1955; Cheptou 2012; Razanajatovo et al. 2016). Recently, some isolated monomorphic L-morph self-incompatible populations were however found to produce seeds (Portillo-Lemus et al. 2021). These puzzling observations question the type of SI reaction involved in the self-incompatible L-morph and its level of permeability to self - fertilization. More broadly, they question the type of matingbreeding systems developed in these invasive populations and the respective importance of allogamy and selfing during invasion (Hieda et al. 2020; Portillo-Lemus et al. 2021).

173   In this study, we thus aimed at better characterizing the ~~mating~~breeding system of the

174   two floral morphs in Western European populations of *Lgh* by tracking the fates of self- and

175   inter-morph pollen tubes in both floral morphs. First, we searched for structural evidences

176   through analysis of the morphologies of pollen grains and stigma surfaces of the two floral

177   morphs that may be characteristic of homomorphic and heteromorphic sporophytic self-

178   incompatibilities. Second, to better categorize the type of the SI developed by this species, we

179   aimed at identifying the site ~~of~~where the ~~self-~~incompatible pollen ~~rejection~~tubes were blocked

180   in the self-incompatible L-morph ~~individuals~~pistils. To achieve this goal, we followed the

181   germination and the progression of the self- and inter-morph-pollen tubes on histological

182   sections, prepared at different times after pollination. Third, to quantify the permeability of this

183   self-incompatible system, we counted the number of viable seeds and seedlings obtained from

184   self-fertilization in controlled conditions and in natural monomorphic L-morph populations.

185   Fourth, to test if the S-morph individuals truly reproduced using selfing, as expected in this self-

186   compatible morph, we followed the progression of the self- and inter-morph-pollen tubes, as

187   described for the morph-L, and studied the viability of obtained seeds and seedlings. Finally,

188   we ~~compared the results we obtained~~discuss on the contribution of the patterns of SI reactions

189   and breeding system found in *Lgh* with those already observed in other species, ~~especially~~

190   ~~from~~with a special focus on the Myrtales order ~~to discuss on the importance of our results for~~

191   ~~our phylogenetic comprehension of SI distribution in these families.~~ that includes the *Ludwigia*

192   genus.

193

Materials and Methods

Plant material and ~~sampling~~sampled populations

To better characterize the ~~mating~~breeding system of the two floral morphs that were previously reported in *Lgh*, (Portillo-Lemus et al. 2021), we ~~focused our study on~~ studied a total of 100 individuals ~~sampled from~~ growing along the Loire valley in France (Table S1 for GPS location). We sampled five monomorphic ~~fruitful~~ populations only composed of S-morph individuals (distance between them: min=40km, mean=146km, max=301km) and ~~from~~ five monomorphic ~~fruitless~~ populations only composed of L-morph individuals, ~~growing along the Loire valley in France (Table S1 for GPS location). We sampled~~ (distance between them: min=6km, mean=124km, max=274km). In each population, we randomly sampled 10 stems per population. *Lgh* partly reproduce using clonal budding and rhizomes (Thouvenot et al 2013). We also know using allele sharing distances between pairs of individuals from 30 individuals genotyped using 38 polymorphic SNPs that, at least in one of these monomorphic populations (Mazerolle), populations present different genotypes within a same floral morph though with a restricted number of ancestors (Genitoni et al. 2020). Sampled stems of all S-morph and L-morph populations were transplanted into a common greenhouse (location: ~~Agrocampus Ouest~~L'institut Agro, Rennes, France. 48°06'47.7"N 1°42'30.2"W~~). All~~) where individuals ~~sampled~~ from one population were growth together in an 80L container. to avoid environmental influences on pollen shapes, pollen tube elongations, ovule and seed developments. The 10 containers were watered every 15 days with a commercial nutrient solution (6% nitrogen, 6% phosphorus and 6% potassium) during growth and flowering periods to avoid nutritional deficiencies.

217 **Measure of herkogamy**

218 To link breeding systems with floral morphs, we measured the spatial separation on a vertical
219 axis of anthers and stigmas within flowers (herkogamy) from the 10 *Lgh* populations we
220 sampled, as this floral trait would have a negative relationship with the rate of autofertility in
221 plant species (Webb and Lloyd 1986). Herkogamy is usually quantified as the difference
222 between pistil and stamen lengths (respectively, $x_{\female}$ and $x_{\male}$) with their natural twists and
223 curvatures (Opale 2018). Flowers with stigmas positioned above or protruding beyond the
224 anthers would have higher probability to be contacted first by a visiting pollinator which
225 corresponds to approach herkogamy. Flowers in which anthers are above the pistil and with
226 higher probability to be contacted first by pollinators which corresponds to reverse herkogamy.
227 *Lgh* flowers present two whorls of stamens. We thus did 150 measures, i.e., the length of the
228 pistil ($x_{\female}$) and the length of the inner ($x_{\male i}$) and outer ($x_{\male o}$) stamens per floral morph, and
229 calculate difference between length of pistil and length of anther.

230

231 **Histological preparations**

232 To observe pollen and stigmatic papillae morphology, to follow pollen tube growth and to
233 achieve histological sections of ovaries for studying embryo development, we fixed flowers in
234 FAA 1: 2: 20 (formalin: acetic acid: ethanol V/V). Pollen germination, pollen tube growth and
235 ovule penetration were observed using the aniline blue fluorescence method (Martin 1959). The
236 fixed samples were washed several times with distilled water and each flower was placed in
237 1mL of staining solution (0.1% w/v aniline blue, 1% v/v tween-20[(R)], 0.2 M K_2PO^4, 0.1 M
238 NaOH) for 1 hour at 95°C. After squashing the pistils between the slide and coverslip,
239 observations were made under ultraviolet light (335-364 nm) with a microscope Leica DM4000
240 B[©], and a camera 190HD. To observe embryo development, fixed ovaries were dehydrated,

241   embedded in paraffin, sectioned at 15 μm with a microtome, and mounted on glass slides (Sakai,

242   1973). Sections were placed in 0.05% toluidine blue O in distilled water for 2–30 min, rinsed

243   once in water for 1 min, and air-dried. Paraffin was removed using two xylene baths and the

244   cover slip was mounted with resin. Histological preparations were realized by Nublat

245   Laboratory (https://www.laboratoire-nublat.com/118botanique.html).

246

Pollen and stigmatic papillae morphologies

248   To assess if ~~heterostylous flowers~~the two floral morphs found in western Europe populations of

249   *Lgh* showed difference in pollen shapes and sizes in association with floral morphs, we studied

250   the shape of 500 pollen grains per floral morph. For each morph, we sampled 25 flowers in the

251   5 corresponding populations. For each of these flowers, we measured 20 pollen grains from a

252   mix of the stamen verticels, resulting into a total of 1000 pollen grain measures for both morphs.

253   We measured their diameter under an optical microscope. To assess if ~~heterostylous~~S-morph

254   and L-morph flowers of *Lgh* showed difference in stigmatic papillae structure as

255   ~~expected~~sometimes found in ~~classical~~some heteromorphic SI species, we also noted the long or

256   short shape of stigmatic papillae in 30 histological stylar sections for each of the two floral

257   morphs (Dulberger 1992).

258

Pollen tube growth, ovule fertilisation and embryo-sac development

260   To identify ~~the site of the self-pollen rejection~~ in the self-incompatible L-morph pistil where

261   self-pollen tubes were blocked and to characterize the type of ~~mating~~breeding preferentially

262   achieved in the two floral morphs, we performed hand-controlled pollinations to track and

263   compare the self- and inter-morph pollen tube growth in both floral morphs. As experimental

264 pollinations in *Lgh* have previously shown that self- and intra-morph pollinations in L-morph

265 flowers ~~lead to a~~gave similar ~~incompatible reaction~~rates of rejections (Portillo Lemus et al

266 2021), here we focused our experiments ~~here~~ on a comparison between self-pollinations (pollen

267 grains from the same flowers) and reciprocal inter-morph pollinations. To perform controlled

268 self-pollinations, we enclosed early flower buds in cellophane bags to protect them from

269 external pollen. To effect self-pollination, we dissected mature anthers using tweezers to place

270 pollen directly on its own receptive stigma and re-enclosed the flowers in cellophane bags. For

271 reciprocal inter-morph pollination, to simulate free random crosses, we selected five pollen-

272 donor flowers from ~~short-style or long-style flowers~~the other morph to ~~create~~generate a pollen

273 mix. Flowers to receive inter-morph pollen were emasculated before anthesis and then

274 pollinated with this mix of pollen. After pollination, the pollinated flowers were once again

275 enclosed in cellophane bags in order to protect them from any contaminant pollen.

276 To study self- and inter-morph pollen tube growth in both floral morphs, we made 150 self-

277 pollinations per morph and 300 reciprocal ~~disassortative~~ inter-morph pollinations between S-

278 morph and L-morph. At 2, 3, 7, 16, 24 hours after the hand-controlled pollination, we randomly

279 sampled and fixed 30 flowers per morph and pollination type. For each flower, pollen tube

280 growth and ovule fertilisation were observed using aniline blue fluorescence method with

281 squashed pistils. We measured the growth of the pollen tubes, *i.e.,* the distance between the

282 stigma and the tip of pollen tube (d, μm) at 2, 3, 7, 16, 24 hours (*t*) after the hand-controlled

283 pollinations. From these measures, we calculated the speed of pollen tube (speed) between two

284 measures in micrometres per hour (μm/h) as $speed = \frac{d_{t_2} - d_{t_1}}{t_2 - t_1}$ and the acceleration of pollen

285 tube growth in micrometres per hour square (μm/h²) as $acceleration = \frac{speed_{t_2} - spee\ t_1}{t_2 - t_1}$.

To evaluate the viability of fertilisation and study the embryo-sac development, we analysed a total of 2520 ovary sections corresponding to 14 sections of five flowers per floral morph and cross type, over nine-time steps (1, 2, 3, 4, 5, 6, 7, 15, 21 days after pollination (*dap*).

Fruit and seed set production

To assess the incidence of self-fertilisation in the self-incompatible L-morph individuals, 1600 self-pollinations were carried out from July to August (summer), and from September to October (fall), respectively. Self-pollinated L-morph flowers were randomly selected within groups of individuals growing in the greenhouse. As a control, we made 30 self-pollinations with self-compatible S-morph flowers, 30 L x S and 30 S x L-morph pollinations during summer and repeated this sequence during the fall.

We also assessed the rate of self-fertilisation in self-incompatible L-morph from *in situ* populations at the beginning of October. We collected the fruits of five S-morph and five L-morph *in situnatura* populations to evaluate the fruit and seed sets. To quantify the fruit-set *in situnatura*, we counted fruits produced in five quadrats of 1 m^2, 10 m away on a linear transect. As S-morph populations showed a massive fruit production, we estimated the seed-set production per m^2 as the mean number of seeds produced by 25 fruits randomly picked in the quadrats (5 fruits per quadrat). As the L-morph *in-situ natura* populations produced few fruits, we collected and counted all the seeds produced in all the fruits found in quadrats. We sowed all the *in-situnatura* produced seeds in the greenhouse to calculate the germination rate as the number of seedlings over the number of sowed seeds.

Statistical analysis

To examine possible differences in pollen tube elongations at each time step (time=[2, 3, 7, 16, 24 hours]), and the changes in speed and acceleration of elongations along time in the styles of the two floral morphs considering the four possible types of crosses (cross_types=[S-morph self-pollination; S-morph x L-morph; L-morph self-pollination; L-morph x S-morph]), we used ANOVA (type = III) tests. For each time step, we computed three ANOVA models: pollen_tube_length~cross_types, speed~cross_types*time and acceleration~cross_types*time. For each test, we verified the homoscedasticity of distributions between groups and the normality of residuals using Shapiro–Wilk's tests. When ANOVAs were significant, we applied Tukey's honestly significant difference (HSD) posthoc comparisons of the means to identify which groups and parameters caused those significant differences. We compared measures of herkogamy between S-morph and L-morph flowers using a Mann-Whitney U test and discussed considering their compatibility and realised breeding systems. All analyses were performed using the stats package in R 4.0.4 software (R Development Core Team, 2014). All results were summarised in the supplementary information table S2.

Results

Measures of herkogamy

The measures of herkogamy (pistil length – stamen length in a same flower, Figure S1) showed that, in the L-morph flowers, the heights of the anthers of the 150 outer and 150 inner measured whorls were, respectively always (U=22500, p<0.001) and significantly (U=21050, p<0.001) below the heights of the pistil. In the S-morph flowers, the heights of the anthers of the 150 outer measured whorls were significantly below the heights of the pistil (U=19930, p<0.001),

while the heights of the anthers of the 150 inner measured whorls were all above the heights of the pistil (U=22500, p<0.001).

Pollen and stigmatic papillae morphologies

Pollen grains were all released in triporate monads and connected to the anthers by viscin threads (Fig.1; a3). In both floral morphs and in all the studied populations, pollen grains were all smooth and had the same sizes ranging from 60 to 95 μm whatever their floral morphs (ANOVA, p-value=0.228, Fig. 1 b3, c3). The styles of both floral morphs had capitate stigmas, which were all wet during anthesis and had stigmatic papillae which were submerged by mucilage (Fig. 1; a1, b1). In both floral morphs, all the 60 longitudinal sections of the stigma receptive surface (30 per morph) showed the same morphology of elongated unicellular papillae (Fig. 1; a2, b2). The only difference we found between styles of floral morphs concerned their lengths: styles of S-morph flowers were significantly wider (2.27mm ± 0.19) and shorter (8.04 mm ± 0.19) than those of L-morph flowers (width: 1.53 mm±0.09; length: M=8.97±0.25; p-value=<10^-15<0.001, Fig.1; c1, c2), resulting in a difference of around 1mm between long- and short-style lengths.

Pollen tube growth and fertilisation

All pollen grains germinated, elongated down the style (Fig. 2a-c), and reached the ovules 24 hours after pollen deposition on stigmas (Fig. 2d-e), in self and inter-morph crosses and whateverindependent of the pollen origin. In the self-pollinated L-morph flowers, only six ovules of 300 studied (2 %) were penetrated by a pollen tube with pollen tube tips located in the area of the embryo sac (Fig. 2f). Figure 2f shows an ovule surrounded by several pollen

tubes, but it is unclear whether any of them have penetrated the micropyle. The other 294 unpenetrated ovules showed tips of pollen tubes with a small bulge, stopped between the ovarian tissues and the beginning of the first cell layers of the ovule integuments and we found no traces of pollen tubes in the area of the embryo sac (Fig. 2d, e). All the 300 L-morph ovules that were cross-pollinated with S-morph pollen were penetrated by pollen tubes 24 hours after pollination. All S-morph ovules were penetrated by one pollen tube 24 hours after self and inter-morph pollinations (Fig. 2g).

Kinetic elongation of pollen tube

In the styles of both floral morphs, until reaching the ovules, inter-morph pollen tubes outdistanced self-pollen tubes (HDS-test, p-value=~~<10^-15~~<0.001; Mean and SD values reported in Table S2; Fig. 3). Significant advances of inter-morph pollen tubes were observed from two hours after pollinations. (Fig. ~~S1a~~S2a). Speed of elongations of pollen tubes in both morphs quickly increased in the first two hours after germination while penetrating the stigma tissues to reach an elongation of 0.3-0.4 mm/hours all along the style, and then doubled their speed of elongation when entering the ovary area (Fig. ~~S1b~~S2b, Table S2).

Fertilisation and embryo development

In both floral morphs, each locule of the pentacarpellate ovary has 12 ± 2 ovules (Fig. ~~S2~~S3). The anatropous and bitegmic ovules were disposed along a single longitudinal axis on placentae (Figs. 4, 5 and ~~S2~~S3). The embryo sac (female gametophyte) was an *Oenothera*-type and composed of only 4 cells: 2 synergids and an egg-cell on the micropyle side, and a single polar nucleus in the central cell, matching previous observations made on other *Ludwigia* species and

377 other Onagraceae genera (Fig. 4, 5; Rigakishi 1918; Eyde 1977; Tilquin and de Brouwer 1982;

378 Tobe and Raven 1986).

379 In L-morph flowers, 24 hours after pollination, 98% of self-pollen tubes stopped their

380 elongations, lately, in between the ovarian area and the beginning of the first integuments (Fig.

381 4.a, b). In these figures, the pollen tubes seem to be located before the nucellus-epistase out of

382 the embryo sac and did not succeed to pass the synergids. However, it is always difficult to

383 obtain sections showing all together, the synergids, egg cell and central cell which compromise

384 the possibility to identify precisely where the pollen tube stopped. The embryo sacs found with

385 self-pollen tubes stopped didn't develop and degenerated three days after pollination (Fig. 4.c).

386 In the greenhouse, self-pollinated L-morph flowers abscised three days after pollination which

387 is consistent with the temporality of our histological observations (Fig. 4a). In comparison, the

388 abscission of the unpollinated flowers of L-morphs occurred four days after emasculation, when

389 protected with a cellophane bag. Under aniline blue fluorescence, we observed that 2% of self-

390 pollen tubes (6/300) in L-morph flowers succeeded to reach the embryo sac and that only one

391 self-fecundation event was observed over the 300 self-pollinated L-morph studied ovules from

392 the ovary sections (Fig. 4b).

393 In S-morph, regardless of pollen origin, and in L-morph when inter-morph cross-pollinated, the

394 first cell divisions of the embryos began two to three days after pollination (Fig. 5). From five

395 days after pollination, the nucellus gradually began to disintegrate while the embryo continued

396 to develop and grow. At 15 days, the nucellus disappeared completely, giving way to

397 exalbuminous embryo development, which concluded with a viable seed 45 days after

398 pollination.

399

Fruit production in self-incompatible L-morph

In the greenhouse, during summer, only four very "small fruits" containing one, four, five and nine seeds respectively developed over 1600 studied L-morph flowers that were hand-pollinated with self-pollen only (Fig. 6). Considering that 60 ovules on average were initially available per flower, the rate of self-fertilisation was 0.02% (i.e., 19 seeds from 1600 x 60=96000 potential ovules available). In the greenhouse, during the fall, at the end of the flowering season, 25 very "small fruits" containing between one and ten seeds for a total of 98 seeds (Fig. 6), gave a rate of self-fertilisation of 0.1% (98 on 96000 potential ovules), thus 5-fold more than obtained in July, during the high fruiting period. All 30 S-morph flowers, which were self-pollinated as a control, developed into 30 fruits containing a total of 1800 seeds, with a constant number of 60 seeds per fruit both in July and September (Fig. 6).

To validate the results obtained in experimental conditions, we then assessed the production of "small fruits" in five L-morph and five S-morph *in situnatura* populations (Table S1). We found between 15 and 35 "small fruits" per m^2 in self-incompatible monomorphic L-morph populations. All the "small fruits" collected *in situnatura* contained one to 15 seeds per fruit, as we obtained in experimental conditions in greenhouse. We counted a production of 92 to 217 seeds per m^2. In the five self-compatible S-morph populations, we counted 384 to 864 fruits per m^2. On five of these fruits per population, we counted 50 to 70 seeds per fruit, giving an estimation of 19200 to 51840 seeds per m^2 (Table S1). All seeds from "small fruits" obtained in greenhouse and *in situ natura* germinated, and developed into viable seedlings (Table S1).

Discussion

~~Our~~The results we obtained from a common garden and from natural populations showed that both floral morphs found in European invasive populations of *Lgh* reproduced

using a mixed ~~mating~~breeding system, ~~whatever~~ with mechanisms that may result in the ~~floral morph, both in a common garden and in natural populations.~~production of allogamous and delayed autogamous viable seeds. Pollen grains and stigma surfaces of both floral morphs showed similar sizes and shapes. In the self-incompatible L-morph flowers with approach herkogamy, self-pollen tubes were only stopped when reaching the ovaries, before penetrating the ovules. These observations argue for a prezygotic, ovarian, ~~late acting self-incompatibility~~ LSI system in L-morph flowers of *Lgh*, ~~literally~~ corresponding to Gibbs (2014) classification ~~(2014).~~. As commonly observed in this kind of SI system ~~(Seavey and Bawa 1986; Gibbs 2014b)~~(Seavey and Bawa 1986; Gibbs 2014), a small proportion of self-pollen (0.02%), increasing by a factor of 5 at the end of the flowering seasons, succeeded to fertilize the ovules and to develop into seeds. All ~~the resulting~~these seeds ~~obtained~~ then developed into viable seedlings. In the self-compatible S-morph~~, all~~ with reverse herkogamy, self-~~pollen~~pollen grains succeeded in fertilizing the embryo-sacs ~~and~~that successfully developed into viable seeds and then into viable seedlings. Yet, in the two *Lgh* floral morphs, inter-morph pollen tubes always elongated faster than self-pollen tubes, which may give advantage to intermorph crosses when ~~inter-morph~~intermorph pollen is available.

The prezygotic LSI system in *Lgh*: a rare but already observed SI system

In European invasive populations, *Lgh* ~~is a heterostylous species with two floral morphologies, a long-styled prezygotic late-acting self-incompatible L-morph and a short-styled self-compatible S-morph. Most heterostylous species with SI systems also often present differences in size and shape of pollen and stigmas papillae (Dulberger et al. 1975; Dulberger 1992; Barrett and Shore 2008) which was not the case of Lgh. Less frequently, as we observed in Lgh populations, heterostylous SI species show similar pollen shape and papillae structure between floral morphs, as previously observed in Turnera joelii and Turnera scabra (Turneraceae,~~

Safavian and Shore 2010) and in the heterostylous LSI species *Narcissus tazetta* L. (Dulberger, 1964),present two floral morphologies, a long-styled prezygotic late-acting self-incompatible L-morph (approach herkogamy) and a short-styled self-compatible S-morph (reverse herkogamy). LSI systems are *Narcissus triandrus* L. (Sage et al. 1999), and *Narcissus papyraceus* (Barrett and Shore 2008; Simon-Porcar et al. 2015). In these last three Amaryllidaceae species, self-pollen tubes also stopped lately and prezygotically, as we observed in *Lgh*. Such LSI system is not specific to heterostylous species as it was also observed in homomorphic species like in *Ipomopsis aggregate* from the Polemoniaceae (Sage et al. 2006).

Many HetSI species present differences in size and shape of pollen and stigmas papillae (Dulberger et al. 1975; Dulberger 1992; Barrett and Shore 2008). Less frequently, as we observed here in European populations of *Lgh*, HetSI species show similar pollen shape and papillae structure between floral morphs, as previously observed in *Turnera joelii* and *Turnera scabra* (Turneraceae, Safavian and Shore 2010).

Three style-polymorphic LSI Amaryllidaceae species, *Narcissus tazetta* L., *Narcissus triandrus* L. and *Narcissus papyraceus* also present similar pollen shape and papillae structure between floral morphs. However, their LSI systems are not related to the heteromorphy of their flowers, as crosses between individuals of the same floral type are fertile, and only strict self-pollination results into ovarian prezygotic pollen rejection (Dulberger 1964; Sage et al. 1999; Barrett and Shore 2008; Simon-Porçar et al. 2015). In the invasive *Lgh* populations we studied here, the genetic ancestrality between individuals may be limited (Genitoni et al. 2020), and we didn't assess the genetic identity of the individuals we sampled along the 530km west-east transect of the Loire watershed. We were thus not able to identify if the LSI we identified in western European *Lgh* populations would be truly heteromorphic, or as in *Narcissus sp.*, nonheteromorphic.

In the order of Myrtales, 76 of 674 species of the Onagraceae family were reported to be self-incompatible ~~(Raven 1979). Two of these species, *Oenothera organensis* and *Oenothera rhombipetala*, were formally demonstrated to be self-incompatible by studying their pollen tube progression after self and cross pollinations and~~(Raven 1979). Two of these species, *Oenothera organensis* and *Oenothera rhombipetala*, were formally demonstrated to be self-incompatible by studying their pollen tube progression after self and cross pollinations and were reported to involve a homomorphic GSI system (Emerson 1939; Bali and Hecht 1965). In *Epilobium obcordatum,* another self-incompatible species of the Onagraceae family, most of the self-pollen tubes stopped before entering the embryo sac (Seavey and Carter 1996) and were suspected to mate using an ovarian, LSI system (Seavey and Bawa 1986). However, contrary to our observations in *Lgh*, the rare self-fertilised ovules of *E. obcordatum* present substantial rates of post-zygotic failures (Seavey and Carter 1996).

Overall, LSI have been previously reported in two other families of the Myrtales order: in the Myrtaceae family, in *Acca sellowiana* ~~(Finatto et al. 2011), in *Melaleuca alternifolia* (Baskorowati et al. 2010) and in *Eucalyptus globulus* (Pound et al. 2002), and in six Vochysia species of the Vochysiaceae family (Oliveira and Gibbs 1994). Together with our results, these observations question a possible wider occurrence of LSI in the Myrtales order. Our results may add a third family of the Myrtales order as developing an LSI system.~~(Finatto et al. 2011), in *Melaleuca alternifolia* (Baskorowati et al. 2010) and in *Eucalyptus globulus* (Pound et al. 2002), and in six *Vochysia* species of the Vochysiaceae family (Oliveira and Gibbs 1994). If confirmed, our results may add a third family of the Myrtales order as developing an LSI system. Together with our results, these observations question a possible wider occurrence of LSI in the Myrtales order.

Permeability of LSI L-morph flowers: a delayed selfed mating system?

499 Our results showed that, despite the LSI developed in the L-morph flowers, self-fertilisation

500 still occurred at a low, stable rate in both experimental and *in ~~situ~~natura* populations. This type

501 of ~~mating~~breeding system enabling for some self-fertilisation especially at the end of flowering

502 season, named delayed selfing, has been reported in multiple angiosperm species as a recurrent

503 ~~mating~~breeding strategy (Lloyd 1992; Sakai 1995; Goodwillie and Weber 2018). In such

504 species, self-pollination is blocked or delayed until the opportunity for outcrossing has passed.

505 Delayed selfing is thought to have evolved in preferentially allogamous species because it

506 would provide some reproductive assurance when populations suffer from the lack of

507 compatible pollen (Goodwillie and Weber 2018; Ruane et al. 2020; Xu 2021).

508

509 **A preferential allogamy ~~mating~~breeding system even in the self-compatible S-morph?**

510 Before our study, we expected that individuals of the self-compatible S-morph preferentially

511 mates using selfing (Kerbs et al. 2020). Surprisingly, the elongation dynamics of pollen tubes

512 highlighted another process susceptible to favour allogamy in *Lgh*, even in the self-compatible

513 S-morph. ~~Inter~~In both floral morphs, inter-morph pollen tubes elongated significantly faster

514 than self-pollen tubes while progressing along the style. This phenomenon has already been

515 described in several species with a LSI mating system, like in *Camellia oleifera* and in

516 *Crotalaria juncea* (Liao et al. 2014; Rangappa Thimmaiah et al. 2018). But faster elongation

517 of inter-morph pollen tubes does not occur in all LSI species. For example, self- and cross-

518 pollen tubes present similar elongation speed in *Aconitum kusnezofii* or *Cyrtanthus breviflorus*

519 (Vaughton et al. 2010; Hao et al. 2012). Further studies should determine whether the advantage

520 of inter-morph pollen tube elongation also applies when flowers are pollinated with a mixture

521 of self and inter-morph pollens in controlled pollinations, as reported in *Campsis radicans*

522 (Bertin et al. 1989) or using paternity analysis on seeds produced after free pollination in *in*

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situnatura populations that mix the two floral morphs, as reported in *Luculia pinceana* (Zhou et al. 2015).

Consequences of LSI for invasive populations of *Lgh*

Our results showed that invasive populations only composed of the self-incompatible L-morph still produced few small fruits containing few seeds resulting from self-fertilization, in similar proportions to what we obtained in experimental conditions. If these self-fertilized seeds remained limited in number at the scale of an individual, we estimated from our measures in quadrats that populations in Loire watershed still produced from 92 to 217 seeds per m^2 which can all potentially germinate and resulted into viable seedlings. Delayed selfing in the L-morph *Lgh* populations resulted in a consequential quantity of viable floating seeds that may contribute to their local regeneration, seed bank and dissemination. In the invasion context, individuals capable of self-fertilization or uniparental reproduction can regenerate a new population in the absence of compatible partners (Baker 1955; Barrett et al. 2008). Accordingly, a recent survey on 1,752 angiosperm species showed that selfing ability fosters directly and indirectly alien plant establishment (Razanajatovo et al. 2016). In contrast, 75% of the worldwide *Lgh* invasive populations are monomorphic L-morph self-incompatible populations (Portillo-Lemus et al. 2021) which may constitute an anomaly to 'Baker's Law' (Baker 1955). But, we highlighted hereOur results showed that the L-morph individuals certainly mated using a LSI system but also used delayed selfing in European invasive populations, better fitting Baker hypotheses. This mixed matingbreeding system with preferential allogamy and delayed selfing, never described before this study, must be considered for the future management plan of *Lgh* invasive populations.

Beyond a system with a compatible S-morph and a self-incompatible L-morph, that may have suggested simple ~~mating~~breeding systems, our study revealed a more complex picture ~~with a mixed mating system combining~~, where the two floral morphs combined different mechanisms that may result in preferential allogamy and delayed selfing ~~in a different way in the two floral morphs.~~. Our results encouraged to explore in more details fertilization in plants to better characterise their ~~mating system~~breeding systems and their consequences for populations. Concerning *Lgh*, we still lack of a clear picture of the respective importance of its reproductive modes in native and invasive populations worldwide in different ecological contexts, including in these monomorphic populations found along the recent European invasion front. Genetic marker-based analyses of experimental crosses and parentage analyses in natural populations should help validating the relevance of our first results and clarifying the reproductive modes of both floral morphs and natural populations as recently achieved in two Oleaceae species (Besnard et al. 2020, De Cauwer et al. 2021).

part of the research infrastructure Analysis and Experimentations on Ecosystems-France, for

help with the maintenance of plants.

Data availability

Here lien Zenodo

Author Contribution

LP and DB designed this project. MH, MB, LP and DB performed all experiments. JH

participated in capsule husking. LP, SS and DB analysed data and wrote the manuscript. All

authors approved the manuscript.

References

Allen AM, Hiscock SJ (2008) Evolution and phylogeny of self-incompatibility systems in

angiosperms. In: Self-incompatibility in flowering plants. Springer, pp 73–101

Baker HG (1955) Self-compatibility and establishment after'long-distance'dispersal.
Evolution 9:347–349

Bali PN, Hecht A (1965) The genetics of self-incompatibility in *Oenothera rhombipetala*.
Genetica 36:159–171. https://doi.org/10.1007/BF01557151

Barlow BA, Forrester J (1984) Variation in Indumentum Morphologyindumentum
morphology in the *Melaleuca leucadendra* Complexcomplex (Myrtaceae). Brunonia
7:277–288. https://doi.org/10.1071/bru9840277

591   Barranco D, Arroyo J, Santos-Gally R (2019). Avoiding sexual interference: herkogamy and

592   dichogamy in style dimorphic flowers of *Narcissus broussonetii* (Amaryllidaceae),

593   AoB PLANTS, 11 (4): plz038,. https://doi.org/10.1093/aobpla/plz038

594   Barrett SC, Colautti RI, Eckert CG (2008) Plant reproductive systems and evolution during

595   biological invasion. Molecular ecology 17:373–383

596   Barrett SC, Cole WW, Herrera CM (2004) Mating patterns and genetic diversity in the wild

597   daffodil *Narcissus longispathus* (Amaryllidaceae). Heredity 92:459–465

598   Barrett SCH (2019) 'A most complex marriage arrangement': recent advances on heterostyly

599   and unresolved questions. New Phytologist 224:1051–1067.

600   https://doi.org/10.1111/nph.16026

601   Barrett SCH, Cruzan ~~M (1994a) Variation in incompatibility in heterostylous plants. In:~~

602   ~~Genetic control of self-incompatibility and reproductive development in flowering~~

603   ~~plants, Springer. Dordrecht, pp 189–219~~

604   ~~Barrett SCH, Cruzan~~ MB (~~1994b~~1994) Incompatibility in heterostylous plants. In: Williams

605   EG, Clarke AE, Knox RB (eds) Genetic control of self-incompatibility and

606   reproductive development in flowering plants. Springer Netherlands, Dordrecht, pp

607   189–219

608   Barrett SCH, Shore JS (2008) New ~~Insights~~insights on ~~Heterostyly: Comparative Biology,~~

609   ~~Ecology~~heterostyly: comparative biology, ecology and ~~Genetics~~genetics. In: Self-

610   ~~Incompatibility~~incompatibility in ~~Flowering Plants~~flowering plants. Springer Berlin

611   Heidelberg, Berlin, Heidelberg, pp 3–32

Mis en forme : Police :Italique

Mis en forme : Police :Italique

612 Baskorowati L, Moncur MW, Cunningham SA, et al (2010) Reproductive biology of

613 *Melaleuca alternifolia* (Myrtaceae) 2. Incompatibility and pollen transfer in relation to

614 the breeding system. Aust J Bot 58:384–391. https://doi.org/10.1071/BT10036

615 Beardsell DV, Knox RB, Williams EG (1993) Breeding ~~System~~system and ~~Reproductive~~

616 ~~Success~~reproductive success of *Thryptomene calycina* (Myrtaceae). Aust J Bot

617 41:333–353. https://doi.org/10.1071/bt9930333

618 Bertin RI, Barnes C, Guttman SI (1989) Self-~~Sterility~~sterility and ~~Cryptic Self-Fertility~~cryptic

619 self-fertility in *Campsis radicans* (Bignoniaceae). Botanical Gazette 150:397–403.

620 https://doi.org/10.1086/337785

621 Besnard, G, Cheptou, P-O, Debbaoui, M, et al. Paternity tests support a diallelic self-

622 incompatibility system in a wild olive (*Olea europaea* subsp. *laperrinei*, Oleaceae).

623 Ecology and Evolution 10:1876– 1888. https://doi.org/10.1002/ece3.5993

624 Busch JW, Schoen DJ (2008) The evolution of self-incompatibility when mates are limiting.

625 Trends in Plant Science 13:128–136. https://doi.org/10.1016/j.tplants.2008.01.002

626 Charlesworth D, Vekemans X, Castric V, Glémin S (2005) Plant self-incompatibility systems:

627 a molecular evolutionary perspective: ~~Research~~research review. New Phytologist

628 168:61–69. https://doi.org/10.1111/j.1469-8137.2005.01443.x

629 Chen X, Hao S, Wang L, et al (2012) Late-acting self-incompatibility in tea plant (*Camellia*

630 *sinensis*). Biologia 67:347–351. https://doi.org/10.2478/s11756-012-0018-9

631 Cheptou P-O (2012) Clarifying Baker's law. Annals of botany 109:633–641

Mis en forme : Police :Italique

Mis en forme : Police :Italique

Mis en forme : Police :Italique

Mis en forme : Français (France)

Mis en forme : Police :Italique

Mis en forme : Anglais (États-Unis)

632 Cohen JI (2010) "A case to which no parallel exists": The influence of Darwin's Different

633   Forms of Flowers. American Journal of Botany 97:701–716.

634   https://doi.org/10.3732/ajb.0900395

635 Dandelot S (2004) Les *Ludwigia* spp. invasives du Sud de la France : historique,

636   biosystématique, biologie et écologie. Université d'Aix-Marseille

637 Darwin C (1877) The different forms of flowers on plants of the same species. D. Appleton

638 ~~de~~De Cauwer I, Vernet P, Billiard S, Godé C, Bourceaux A, Ponitzki C, Saumitou-Laprade P

639   (2021) Widespread coexistence of self-compatible and self-incompatible phenotypes

640   in a diallelic self-incompatibility system in *Ligustrum vulgare* (Oleaceae). Heredity

641   127:384–392. https://doi.org/10.1038/s41437-021-00463-4

642 De Nettancourt D (1997) Incompatibility in angiosperms. ~~Sex~~Sexual Plant

643   ~~Reprod~~Reproduction 10:185–199. https://doi.org/10.1007/s004970050087

644 Dickinson HG, Crabbe MJC, Gaude T (1992) Sporophytic ~~Self-Incompatibility Systems: S~~

645   ~~Gene Products.~~self-incompatibility systems: S-gene products. International Review of

646   Cytology 140:525–561. https://doi.org/10.1016/S0074-7696(08)61108-0

647 Dulberger R (1964) Flower dimorphism and self-incompatibility in *Narcissus tazetta* L.

648   Evolution 18:361–363. https://doi.org/10.2307/2406347

649 Dulberger R (1992) Floral ~~Polymorphisms and Their Functional Significance~~polymorphisms

650   and their functional significance in the ~~Heterostylous Syndrome.~~heterostylous

651   syndrome. In: Barrett SCH (ed) Evolution and ~~Function~~function of

652   ~~Heterostyly~~heterostyly. Springer, Berlin, Heidelberg, pp 41–84

653 Dulberger R, Heslop-Harrison J, Lewis D (1975) Intermorph structural differences between
654   stigmatic papillae and pollen grains in relation to incompatibility in Plumbaginaceae.
655   Proceedings of the Royal Society of London Series B Biological Sciences 188:257–
656   274. https://doi.org/10.1098/rspb.1975.0018

657 Duminil J, Fineschi S, Hampe A, et al (2007) Can population genetic structure be predicted
658   from life-history traits? The American Naturalist 169:662–672

659 ~~Dutartre MA, CEMAGREF F, Secretariat E, Brunel MS (2011) PEST RISK ANALYSIS~~
660   ~~FOR: Ludwigia grandiflora. EPPO~~

661 Ellegren H, Galtier N (2016) Determinants of genetic diversity. ~~Nat Rev Genet~~Nature
662   Reviews Genetics 17:422–433. https://doi.org/10.1038/nrg.2016.58

663 Emerson S (1939) A Preliminary Survey of the Oenothera Organensis Population. Genetics
664   24:524–537

665 EPPO, 2011. Dutartre MA, CEMAGREF F, Secretariat E, Brunel MS (2011) Pest risk
666   analysis for: *Ludwigia grandiflora*. EPPO

667 Eyde RH (1977) Reproductive structures and evolution in *Ludwigia* (Onagraceae). I.
668   Androecium, placentation, merism. Annals of the Missouri Botanical Garden 644–655

669 Ferrer MM, Good SV (2012) Self-sterility in flowering plants: preventing self-fertilization
670   increases family diversification rates. Annals of Botany 110:535–553.
671   https://doi.org/10.1093/aob/mcs124

672 Finatto T, Dos Santos KL, Steiner N, et al (2011) Late-acting self-incompatibility in *Acca*
673   *sellowiana* (Myrtaceae) 1. Australian journal of botany 59:53–60

Mis en forme : Police :Italique

Mis en forme : Police :Italique

674 Fujii S, Kubo K, Takayama S (2016) Non-self- and self-recognition models in plant self-
675   incompatibility. Nature Plants 2:16130. https://doi.org/10.1038/nplants.2016.130

676 Genitoni J, Vassaux D, Delaunay A, Citerne S, Portillo Lemus L, Etienne M-P, Renault D,
677   Stoeckel S, Barloy D and Maury S (2020), Hypomethylation of the aquatic invasive
678   plant, *Ludwigia grandiflora subsp. hexapetala* mimics the adaptive transition into the
679   terrestrial morphotype. Physiologia Plantarum, 170: 280-298.
680   https://doi.org/10.1111/ppl.13162

681 Gibbs PE (~~2014a~~2014) Late-acting self-incompatibility – the pariah breeding system in
682   flowering plants. New Phytologist 203:717–734. https://doi.org/10.1111/nph.12874

683 ~~Gibbs PE (2014b) Late-acting self-incompatibility – the pariah breeding system in flowering~~
684   ~~plants. New Phytologist 203:717–734. https://doi.org/10.1111/nph.12874~~

685 Gibbs PE, Bianchi M (1993) Post-pollination ~~Events~~events in ~~Species~~species of *Chorisia*
686   (Bombacaceae) and *Tabebuia* (Bignoniaceae) with ~~Late~~late-acting ~~Self~~self-
687   incompatibility. Botanica Acta 106:64–71. https://doi.org/10.1111/j.1438-
688   8677.1993.tb00339.x

689 Gibbs PE, Bryan GW (1986) Reproductive ~~Failure~~failure in ~~Populations~~populations of the
690   ~~Dog-Whelk~~dog-whelk, N*ucella Lapillus*, ~~Caused~~caused by ~~Imposex Induced~~imposex
691   induced by ~~Tributyltin~~tributyltin from ~~Antifouling Paints~~antifouling paints. Journal of
692   the Marine Biological Association of the United Kingdom 66:767–777.
693   https://doi.org/10.1017/S0025315400048414

694 Goodwillie C, Weber JJ (2018) The best of both worlds? A review of delayed selfing in
695   flowering plants. American Journal of Botany 105:641–655

Grossenbacher DL, Brandvain Y, Auld JR, et al (2017) Self-compatibility is over-represented on islands. New Phytol 215:469–478. https://doi.org/10.1111/nph.14534

Hao Y-Q, Zhao X-F, She D-Y, et al (2012) The ~~Role of Late-Acting Self-Incompatibility and Early-Acting Inbreeding Depression in Governing Female Fertility~~role of late-acting self-incompatibility and early-acting inbreeding depression in governing female fertility in Monkshood, *Aconitum kusnezoffii*. PLOS ONE 7:e47034. https://doi.org/10.1371/journal.pone.0047034

Hieda S, Kaneko Y, Nakagawa M, Noma N (2020) *Ludwigia grandiflora* (Michx.) Greuter & Burdet *subsp. hexapetala* (Hook. & Arn.) GL Nesom & Kartesz, an invasive aquatic plant in Lake Biwa, the largest lake in Japan. Acta Phytotaxonomica et Geobotanica 71:65–71

Hinata K, Watanabe M, Toriyama K, Isogai A (1993) A ~~Review~~review of ~~Recent Studies~~recent studies on ~~Homomorphic Self-Incompatibility~~homomorphic self-incompatibility. International Review of Cytology 143:257–296. https://doi.org/10.1016/S0074-7696(08)61877-X

Igic B, ~~Bohs L, Kohn JR (2004) Historical inferences from the self-incompatibility locus. New Phytologist 161:97–105. https://doi.org/10.1046/j.1469-8137.2003.00952.x~~

~~Igic B,~~ Lande R, Kohn JR (2008) Loss of ~~Self-Incompatibility~~ self-incompatibility and ~~Its Evolutionary Consequences~~its evolutionary consequences. International Journal of Plant Sciences 169:93–104. https://doi.org/10.1086/523362

Kao TH, McCubbin AG (1996) How flowering plants discriminate between self and non-self pollen to prevent inbreeding. PNAS 93:12059–12065

Kenrick J, Kaul V, Williams EG (1986) Self-incompatibility in *Acacia Retinodes*: site of pollen-tube arrest is the nucellus. Planta 169(2):245–50. http://www.jstor.org/stable/23378114.

Kerbs B, Crawford DJ, White G, et al (2020) How rapidly do self-compatible populations evolve selfing? Mating system estimation within recently evolved self-compatible populations of Azorean Tolpis succulenta (Asteraceae). Ecology and Evolution 10:13990–13999. https://doi.org/10.1002/ece3.6992

Liao T, Yuan D-Y, Zou F, et al (2014) Self-sterility in *Camellia oleifera* may be due to the prezygotic late-acting self-incompatibility. PLoS One 9:e99639

Lloyd DG (1992) Self- and Cross-Fertilization in Plants. II. The Selection of Self-Fertilization. International Journal of Plant Sciences 153:370–380. https://doi.org/10.1086/297041

Martin FW (1959) Staining and observing pollen tubes in the style by means of fluorescence. Stain Technology 34(3):125-128. https://doi.org/10.3109/10520295909114663

Matsui K, Yasui Y (2020). Buckwheat heteromorphic self-incompatibility: genetics, genomics and application to breeding. Breeding science 70(1):32–38. https://doi.org/10.1270/jsbbs.19083

Medrano M, Requerey R, Karron JD, Herrera CM (2012) Herkogamy and mate diversity in the wild daffodil Narcissus longispathus: beyond the selfing–outcrossing paradigm in the evolution of mixed mating. Plant Biology 14:801–810

Nesom GL, Kartesz JT (2000) Observations on the Ludwigia uruguayensis Complex (Onagraceae) in the United States. Castanea 65:123–125

Oliveira P, Gibbs P (1994) Pollination biology and breeding systems of six Vochysia species (Vochysiaceae) in Central Brazil. Journal of Tropical Ecology 509–522

Opedal OH (2018) Herkogamy, a Principal Functional Trait of Plant Reproductive Biology. International Journal of Plant Sciences 179(9):677-687. https://doi.org/10.1086/700314

Portillo-Lemus LO, Bozec M, Harang M, et al (2021) Self-incompatibility limits sexual reproduction rather than environmental conditions in an invasive water primrose. Plant-Environment Interactions 2:74–86. https://doi.org/10.1002/pei3.10042

Pound LM, Wallwork MAB, Potts BM, Sedgley M (2002) Self-incompatibility in Eucalyptus globulus ssp. globulus (Myrtaceae). Australian Journal of Botany 50:365–372

Rangappa Thimmaiah M, Choudhary SB, Sharma HK, et al (2018) Late-acting self-incompatibility: a barrier to self-fertilization in sunnhemp (Crotalaria juncea L.). Euphytica 214:19. https://doi.org/10.1007/s10681-017-2096-9

Raven PH (1979) A survey of reproductive biology in Onagraceae. New Zealand Journal of Botany 17:575–593. https://doi.org/10.1080/0028825X.1979.10432572

Razanajatovo M, Maurel N, Dawson W, et al (2016) Plants capable of selfing are more likely to become naturalized. Nature communications 7:1–9

Rigakishi MI (1918) Studies on the Embryo Sac and Fertilization in Oenothera. Annals of Botany 279–318

Ruane LG, Mangum SM, Horner KM, Moeller DA (2020) The opportunity for outcrossing varies across the geographic range of the primarily selfing Clarkia xantiana ssp.

761   parviflora. American Journal of Botany 107:1198–1207.

762   https://doi.org/10.1002/ajb2.1510

763   Ruaux B (2008) Les plantes envahissantes des corridors fluviaux: traits biologiques, impacts

764   de *Ludwigia peploides* et *L. grandiflora* en Loire moyenne et implications pour la

765   gestion. Université François Rabelais

766   Safavian D, Shore JS (2010) Structure of styles and pollen tubes of distylous Turnera joelii

767   and T. scabra (Turneraceae): are there different mechanisms of incompatibility

768   between the morphs? Sexual plant reproduction 23:225–237

769   Sage TL, Bertin RI, Williams EG (1994) Ovarian and other late-acting self-incompatibility

770   systems. In: Williams EG, Clarke AE, Knox RB (eds) Genetic control of self-

771   incompatibility and reproductive development in flowering plants. Springer

772   Netherlands, Dordrecht, pp 116–140

773   Sage TL, Price MV, Waser NM (2006) Self-sterility in Ipomopsis aggregata (Polemoniaceae)

774   is due to prezygotic ovule degeneration. American Journal of Botany 93:254–262.

775   https://doi.org/10.3732/ajb.93.2.254

776   Sage TL, Strumas F, Cole WW, Barrett SCH (1999) Differential ovule development

777   following self- and cross-pollination: the basis of self-sterility in Narcissus triandrus

778   (Amaryllidaceae). American Journal of Botany 86:855–870.

779   https://doi.org/10.2307/2656706

780   Sakai WS. Simple method for differential staining of paraffin embedded plant material using

781   toluidine blue o. Stain Technol. 1973 Sep;48(5):247-9. doi:

782   10.3109/10520297309116632.

783 Sakai S (1995) Evolutionarily Stable Selfing Rates of Hermaphroditic Plants in Competing

784   and Delayed Selfing Modes with Allocation to Attractive Structures. Evolution

785   49:557–564. https://doi.org/10.1111/j.1558-5646.1995.tb02287.x

786 Santos-Gally R, Gonzalez-Voyer A, Arroyo J (2013) Deconstructing Heterostyly: The

787   Evolutionary Role of Incompatibility System, Pollinators, and Floral Architecture.

788   Evolution 67:2072–2082. https://doi.org/10.1111/evo.12087

789 Seavey SR, Bawa KS (1986) Late-acting self-incompatibility in angiosperms. Bot Rev

790   52:195–219. https://doi.org/10.1007/BF02861001

791 Seavey SR, Carter SK (1996) Ovule fates in Epilobium obcordatum (Onagraceae). American

792   Journal of Botany 83:316–325. https://doi.org/10.1002/j.1537-2197.1996.tb12712.x

793 Shimizu KK, Tsuchimatsu T (2015) Evolution of selfing: recurrent patterns in molecular

794   adaptation. Annual Review of Ecology, Evolution, and Systematics 46:593–622

795 Simon-Porcar VI, de Castro A, Herrero M, Arroyo J (2015) Ovarian self-incompatibility in

796   Narcissus papyraceus (Amaryllidaceae) is the result of a pre-zygotic response.

797   Botanical Journal of the Linnean Society 177:629–643

798 Takayama S, Isogai A (2005) ~~SELF-INCOMPATIBILITY IN PLANTS.~~Self-incompatibility

799   in plants. Annu Rev Plant Biol 56:467–489.

800   https://doi.org/10.1146/annurev.arplant.56.032604.144249

801 Thouvenot L, Haury J, Thiebaut G (2013) A success story: water primroses, aquatic plant

802   pests. Aquatic Conservation: Marine and Freshwater Ecosystems 23:790–803

803 Tilquin JP, de Brouwer K (1982) Aspects cytologiques de la reproduction sexuée dans le

804   genre Fuchsia. Bulletin de la Société Royale de Botanique de Belgique/Bulletin van de

805   Koninklijke Belgische Botanische Vereniging 33–42

806 Tobe H, Raven PH (1986) A comparative study of the embryology of Ludwigia

807   (Onagraceae): characteristics, variation, and relationships. Annals of the Missouri

808   Botanical Garden 768–787

809 Vaughton G, Ramsey M, Johnson SD (2010) Pollination and late-acting self-incompatibility

810   in Cyrtanthus breviflorus (Amaryllidaceae): implications for seed production. Annals

811   of Botany 106:547–555. https://doi.org/10.1093/aob/mcq149

812 Webb CJ, Lloyd DJ (1986) The avoidance of interference between the presentation of pollen

813   and stigmas in angiosperms II. Herkogamy. New Zealand Journal of Botany

814   24(1):163-178, https://doi.org/10.1080/0028825X.1986.10409726

815 Xu K (2021) The coevolution of flower longevity and self-fertilization in hermaphroditic

816   plants. Evolution n/a: https://doi.org/10.1111/evo.14303

817 Zhou W, Barrett SCH, Wang H, Li D-Z (2015) Reciprocal herkogamy promotes

818   disassortative mating in a distylous species with intramorph compatibility. New
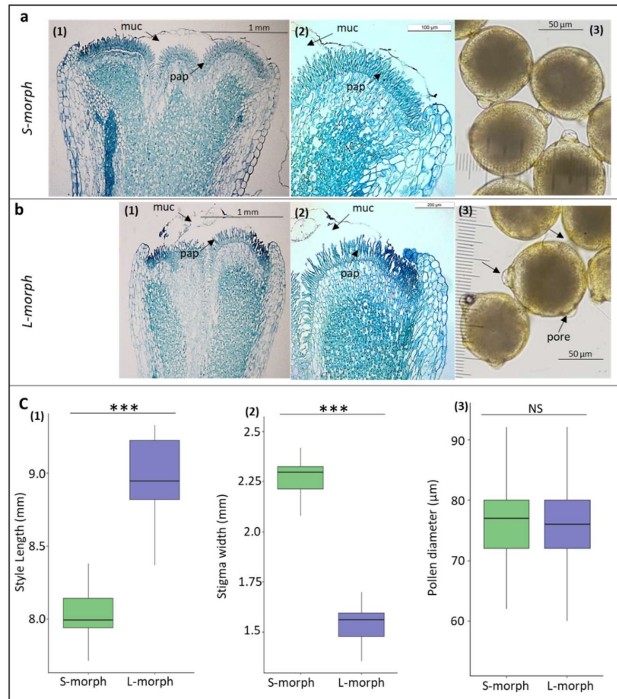
819   Phytologist 206:1503–1512. https://doi.org/10.1111/nph.13326

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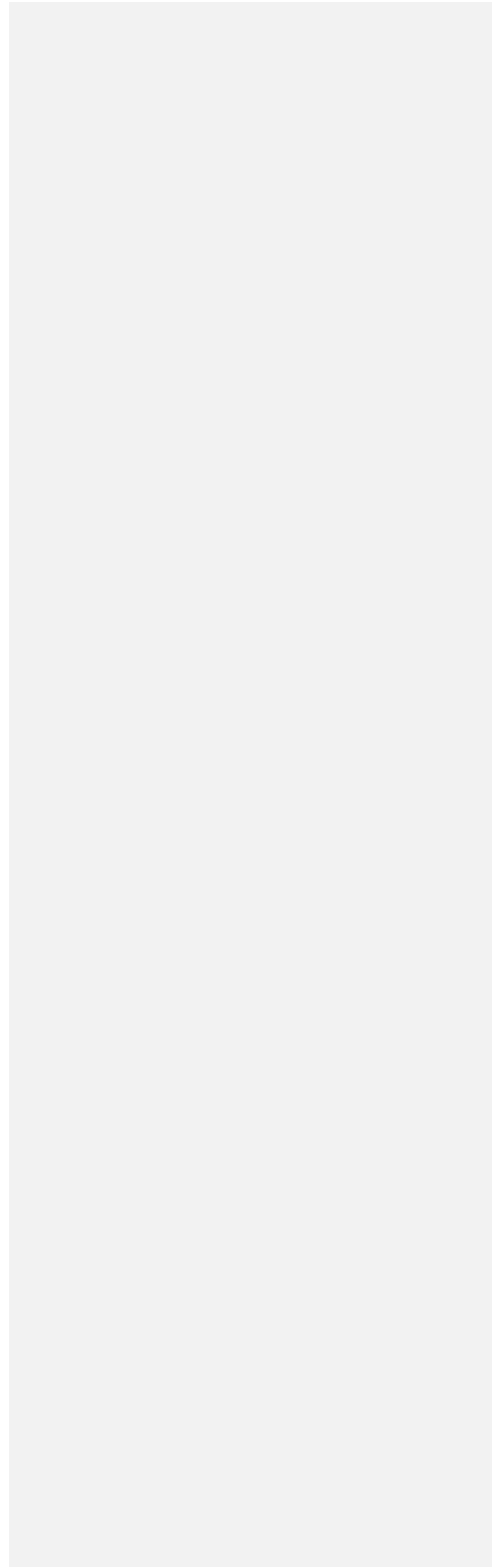
Figures and Support information

823

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Figure 1: Stigma and pollen morphologies in both floral morphs of *Ludwigia grandiflora* subsp. *hexapetala*.

- Stigma section of S-morph (a 1, 2) and L-morph (b 1, 2) under the 10x (a1, b1) and 20x (a2, b1) objectives of optic microscope stained with toluidine blue O. Stigma sections of the stigma have a thickness of 15 μm. In both floral morphs, the stigma was filled with mucilage (mu) (a1-2, b1-2,). Both floral morphs showed the same shape of stigmatic papillae (pap).

- Pollen morphologies of S-morph (a3) and L-morph (b3) under 20X objective of optical microscope. Both morphotypes shared the same shape and size of pollen (a3, b3) and the same diameter of pollen (c3).

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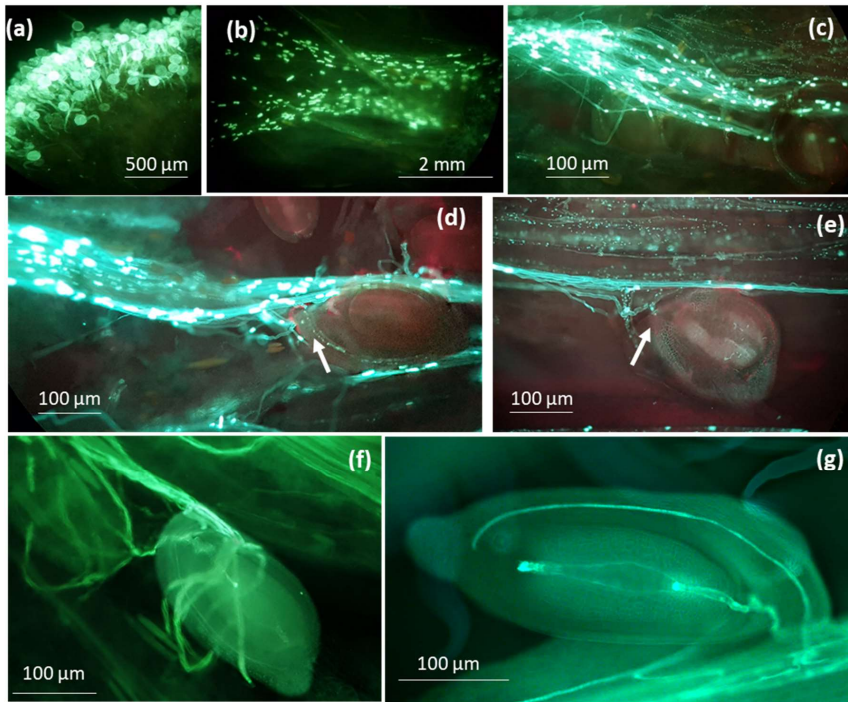
Figure 2: Fluorescence aniline blue staining of self-pollen tube in L-morph pistils (a-f).

a) Pollen grains germination on a stigma surface (zoom 10X); b) Pollen tubes elongating in a style (zoom 2.5X); c, d, e) Pollen tubes in the ovarian area (zoom 20X); f) Pollen tubes in the area of embryo sac (noticed for 6 pollen tubes over 300 studied). g) Pollen tubes in the area of embryo sac in S-morph ovary after self-pollination.
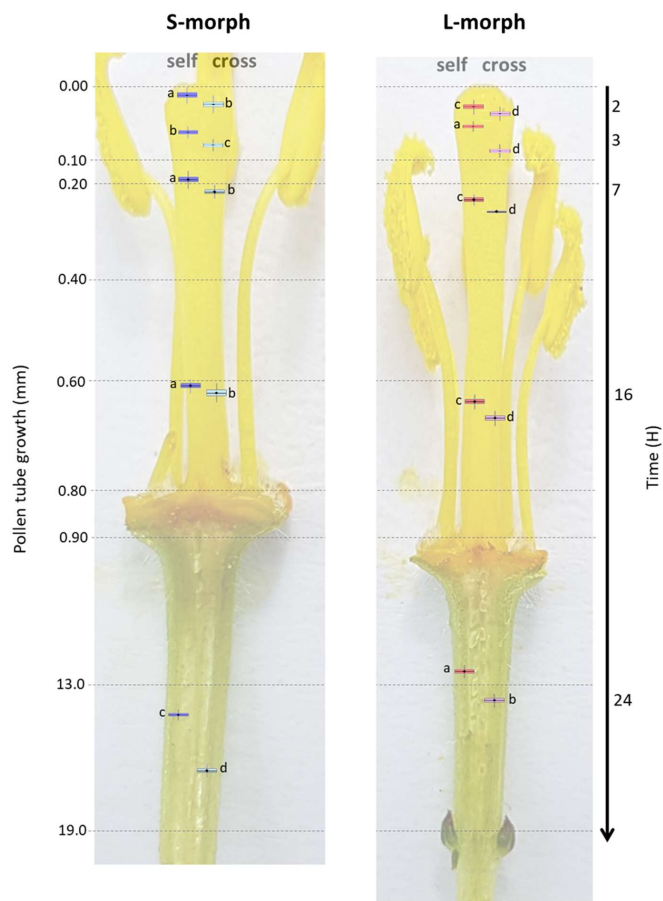
Figure 3: Kinetic of pollen tube elongations in both floral morphs in *Ludwigia grandiflora*

subsp. *hexapetala*.

a) Kinetic of pollen tube elongations in S-morph styles when self- and cross-pollinated (left, blue and sky-blue boxes respectively); b) Kinetic of pollen tube elongations in L-morph style when self- and cross-pollinated (right, red and pink boxes respectively). Significance letters were obtained using a Tukey's honestly significant difference (HSD) post-hoc comparisons of the means. In both floral morphs, cross-pollen tubes elongated faster than self-pollen.

849
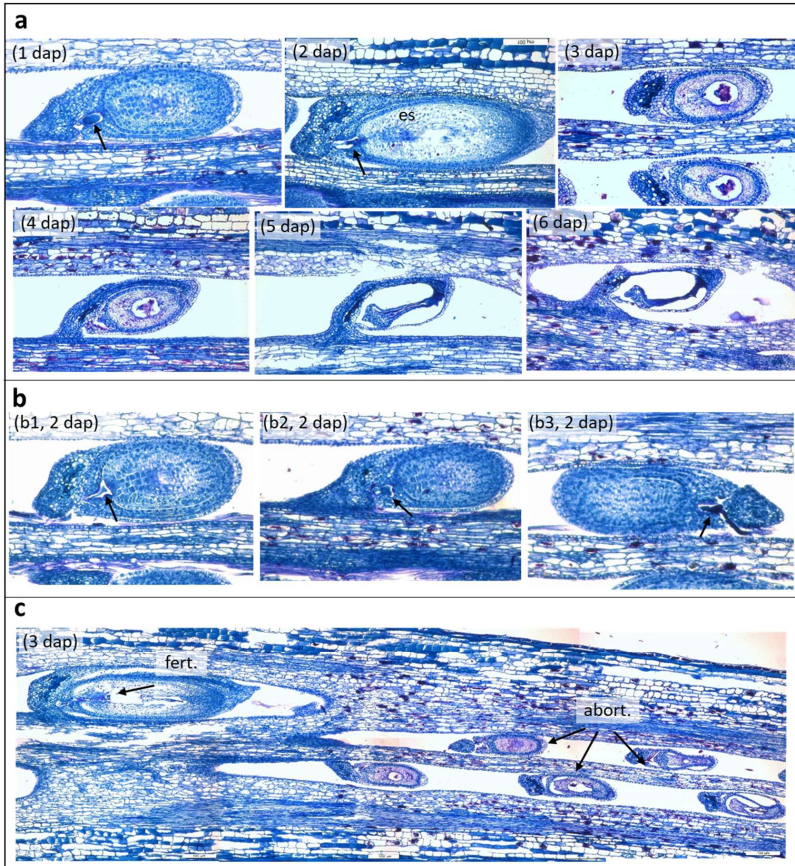
Figure 4: Temporal evolution of embryo sacs, ovules and ovaries in self-incompatible L-morph flowers after self-pollination.

a) Abortive ovules sections after 1, 2, 3, 5 and 6 days after self-pollination (dap); b) Self-pollen tubes that stopped their elongations two days after self-pollination (dap) in three different ovules (1 to 3). The arrows indicate the tips of the self-pollen tube stopped between the ovarian tissues and the beginning of the first cell layers of the ovule integuments (see figure 5a for a comprehensive representation of the ovule parts); c) Example of a rare self-fertilisation event (fert.) obtained three days after self-pollination (dap) informing the "small fruit" formation.
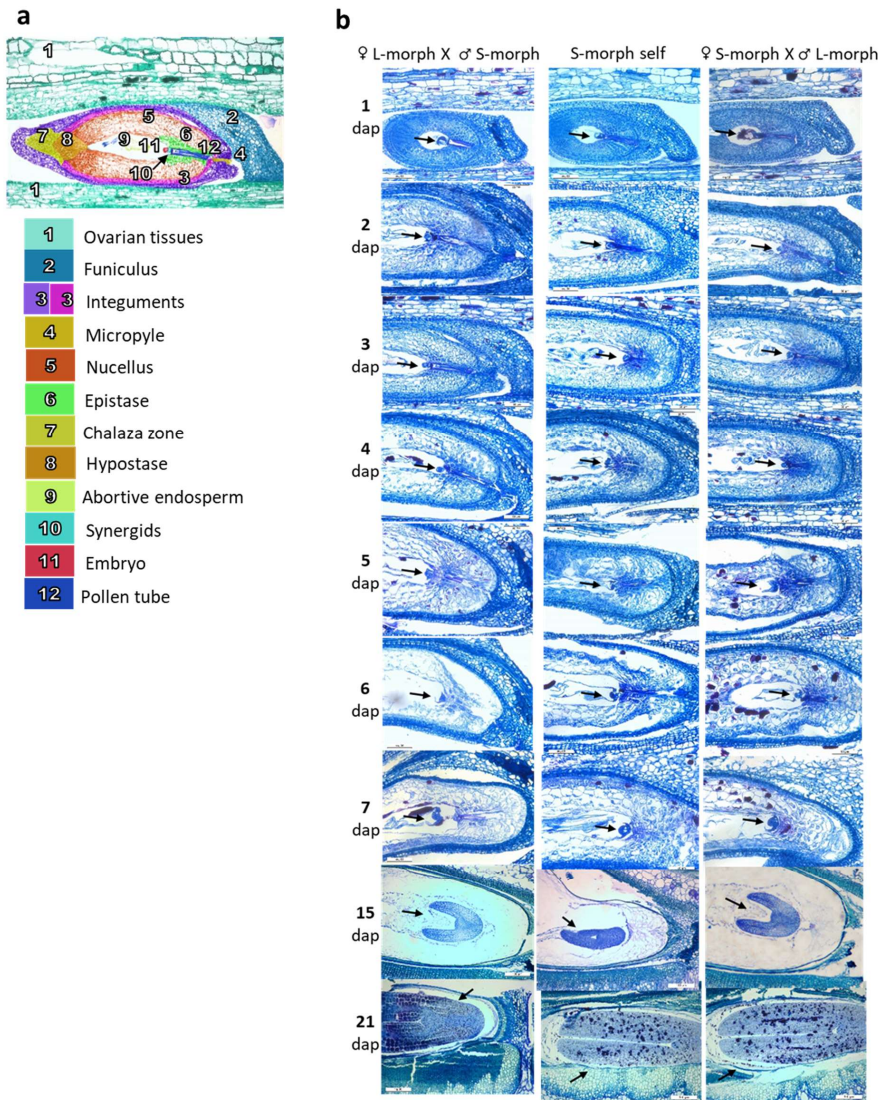
858   Only ~0.33% ovules were fertilised (1/300 ovules observations) (fertilisation = fert; abortive

859   ovules= abort). All sections stained with toluidine blue O (zoom 10X). See figure 5 a

860   comprehensive representation of the ovule parts.

861

Figure 5: Fertilisation and embryonic development after cross and self-pollinating the self-incompatible L-morph and the self-compatible S-morph flowers.

Mis en forme : Police :Gras

866　a - A comprehensive, colorized scheme of the different tissue parts of a L-morph ovule 3 days

867　after disassortative pollination.

868　b- Evolution of the ovules 1, 2, 3, 4, 5, 6, 7, 15, and 21 days after pollen deposition on the

869　stigmas (dap): (1) L-morph ovules after cross-pollination (♀ L-m X ♂ S-m); (2) S-morph ovules

870　after self-pollination; (3) S-morph ovules after cross-pollination (♀ S-m X ♂ L-m).

871　The arrows indicate the development of embryo. All sections were stained with toluidine blue

872　O, 20X zoom under optical microscope.

873
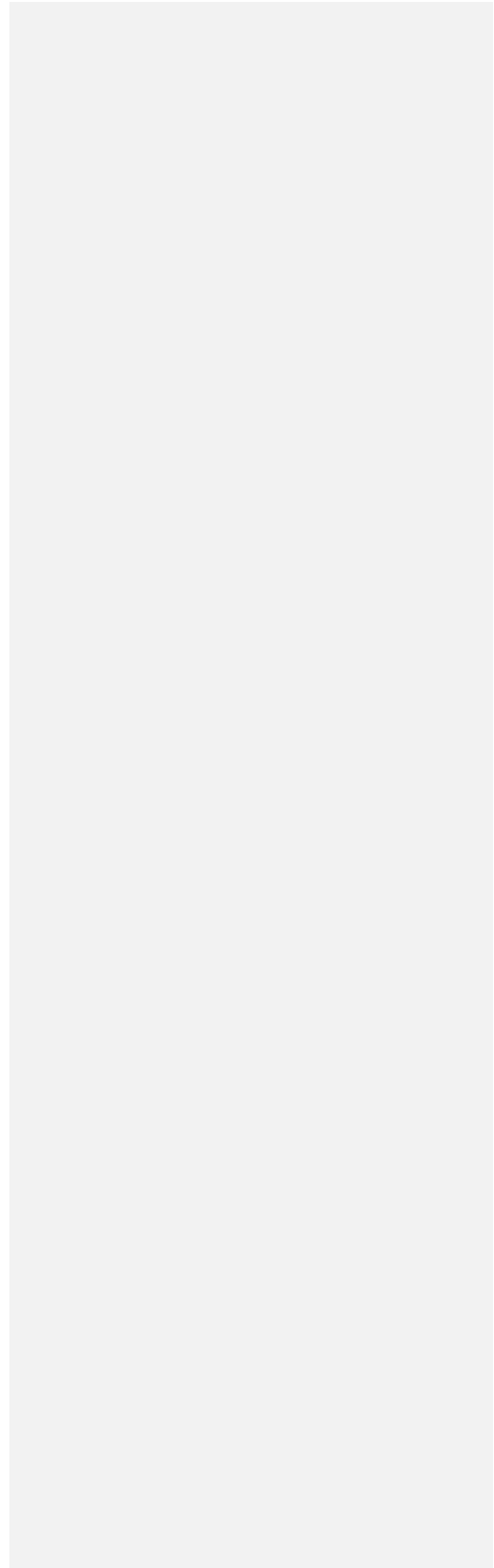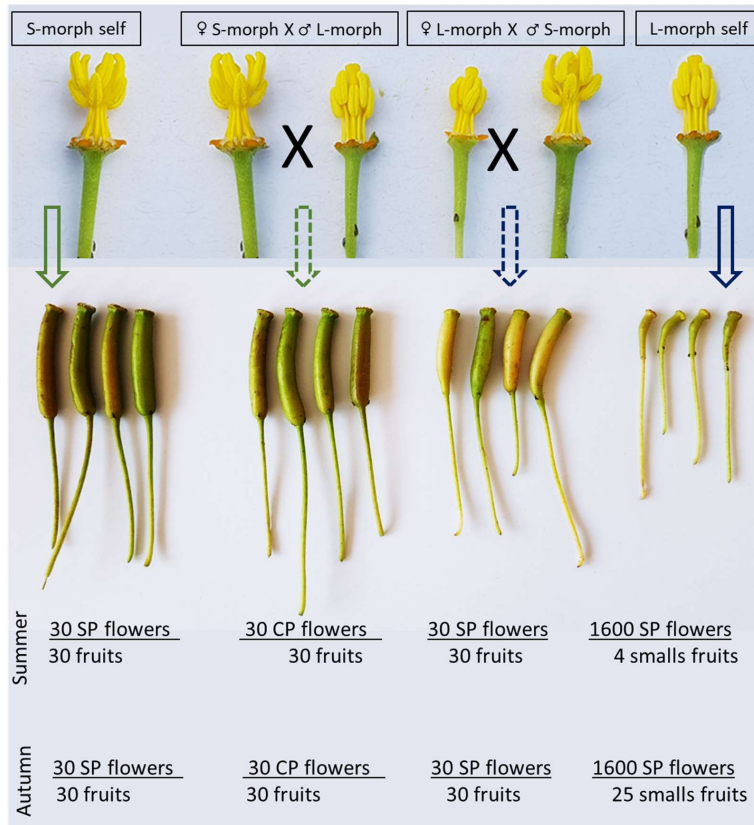
Figure 6: Summary of the fruit-set obtained from S-morph and L-morph flowers after hand-controlled pollination (SP = Self-pollination; CP = Cross-pollination) in summer and autumn.

- All S-morph flowers produced fully-seeded fruits, in summer and autumn, both after self- and cross-pollinations.

- All L-morph flowers produced fully-seeded fruits, in summer and autumn, when pollinated with S-morph pollen (L-morph x S-morph).

- 0.25% and 1.56% of L-morph flowers produced "small fruits" in summer and autumn when self-pollinated. These "small fruits" contained one to nine seeds that all successfully germinated and grew into viable seedlings.

Table S1: Fruit-set, seed-set and germination rate in five S-morph and five L-morph monomorphic *in ~~situ~~natura* populations in western Europe.
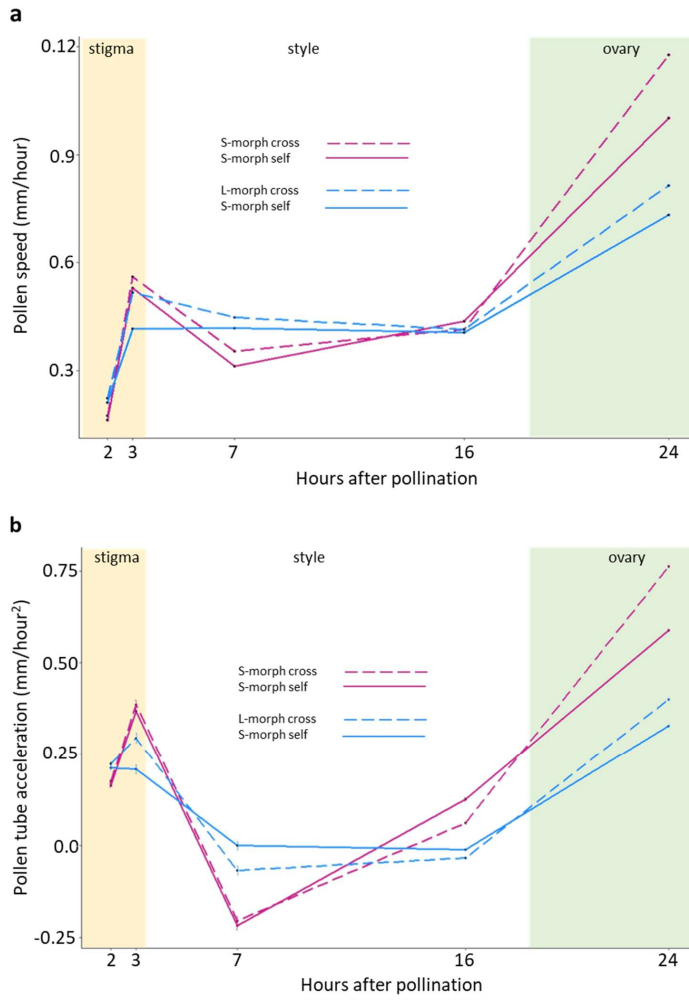
Fruit-set, seed-set and mean number of seeds produced in the studied *in ~~situ~~natura* monomorphic populations of *Lgh*. Seed-set productions per m^2 in monomorphic S-morph populations were estimated by counting the number of seeds produced by fruits in 5 quadrats of 1m^2 and multiplied by the mean number of counted fruits per quadrat. Seed-set productions per quadrat in L-morph populations are reported as the exhaustive number of seeds within all the fruits found in the quadrat.

| Population	GPS Location		Fruitfulness & Floral morph	average number of seeds per fruit	Fruitset per m^2	Seedset per m^2	germination percentage
Chambéon	45°41'03.4"N	4°12'12.1"E	Fruitless L-morph	4.18	22	92	95.67%
Châtel-de-Neuvre	46°24'05.4"N	3°19'10.1"E	Fruitless L-morph	6.40	15	96	93.75%
Gilly-sur-Loire	46°31'40.6"N	3°48'25.7"E	Fruitless L-morph	6.79	28	190	93.68%
Pouilly-sur-Loire	47°16'48.4"N	2°57'25.2"E	Fruitless L-morph	5.68	19	108	89.81%
Orléans	47°53'41.3"N	1°55'48.6"E	Fruitless L-morph	6.20	35	217	94.47%
Pont-de-Cé	47°25'40.7"N	0°31'28.9"W	Fruitful S-morph	55	792	43560	90.30%
Mazerolles	47°23'17.3"N	1°28'07.4"W	Fruitful S-morph	60	864	51840	96.71%
Saint-Aignan-sur-Cher	47°16'19.8"N	1°22'32.1"E	Fruitful S-morph	55	528	29040	89.46%
Lac-de-Maine	47°27'34.7"N	0°35'30.8"W	Fruitful S-morph	50	624	31200	98.12%
Sabot-d'Or	47°19'14.1"N	2°15'24.4"W	Fruitful S-morphe	50	384	19200	91.55%

a

b

Figure S1

Population	GPS Location		Floral morph	Mean number of seeds per fruit	Fruit-set per m²	Seed-set per m²	Germination percentage
Chambéon	45°41'03.4"N	4°12'12.1"E	L	4.18	22	92	95.67%
Châtel-de-Neuvre	46°24'05.4"N	3°19'10.1"E	L	6.40	15	96	93.75%

Gilly-sur-Loire	46°31'40.6"N	3°48'25.7"E	L	6.79	28	190	93.68%
Pouilly-sur-Loire	47°16'48.4"N	2°57'25.2"E	L	5.68	19	108	89.81%
Orléans	47°53'41.3"N	1°55'48.6"E	L	6.20	35	217	94.47%
Pont-de-Cé	47°25'40.7"N	0°31'28.9"W	S	55	792	43560	90.30%
Mazerolles	47°23'17.3"N	1°28'07.4"W	S	60	864	51840	96.71%
Saint-Aignan-sur-Cher	47°16'19.8"N	1°22'32.1"E	S	55	528	29040	89.46%
Lac-de-Maine	47°27'34.7"N	0°35'30.8"W	S	50	624	31200	98.12%
Sabot-d'Or	47°19'14.1"N	2°15'24.4"W	S	50	384	19200	91.55%

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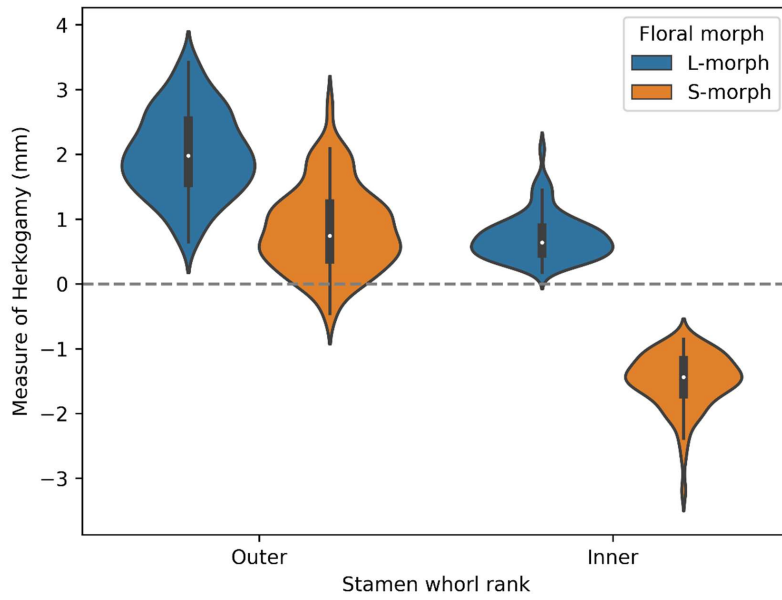
898

L-morph flower showing approach herkogamy   S-morph flower showing reverse herkogamy

899   **Figure S1:** Measures and photos of herkogamy in self-incompatible L-morph and self-

900   compatible S-morph flowers found in Western European populations of *Lgh*.

901 Measures are reported for the outer and inner whorls of anthers. 150 measured flowers per

902 violin plot, 5 populations sampled across the Loire watershed per morph.

a

b

903

Mis en forme : Police :Gras

906 Red = S-morph, blue = L-morph; solid line = self-pollination (self) and dotted line=cross-

907 pollination (cross). a) Speed of pollen tube elongations in both S-morph and L-morph styles

908 after self and cross pollinations. b) Acceleration of pollen tube elongations in both S-morph and

909 L-morph styles after self and cross pollinations.

910

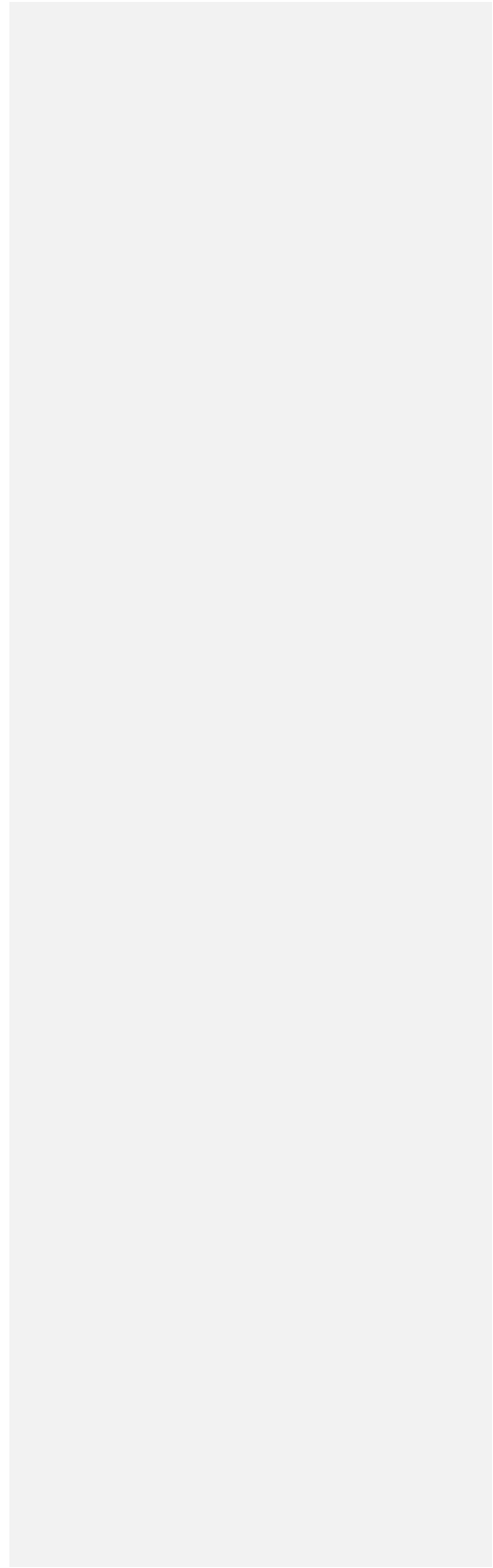**Table S2:** Analyses of the variations of pollen tube elongation along time (a), speed (b) and acceleration of pollen tube elongation (b) in both floral morphs in *Ludwigia grandiflora* subsp. *hexapetala* after self- and cross-pollinations.

(a)

```
Analysis of Variance Table
Response: Growth of pollen tubes => anova(lm(Length ~ Pollination_condition * Hours_after_pollinations))
```

Anova Table (Type III tests)

	Df	Sum_Sq	Mean_Sq	F_value	Pr(>F)
Pollination_conditions	3	1.94571	0.64857	6094	< 2.2e-16 ***
Residuals	796	0.08472	0.00011		
Pollination_conditions	3	1.89545	0.63182	4700.7	< 2.2e-16 ***
Residuals	796	0.10699	0.00013		
Pollination_conditions	3	46.067	15.3558	80721	< 2.2e-16 ***
Residuals	796	0.151	0.0002		
Pollination_conditions	3	26.1592	8.7197	65513	< 2.2e-16 ***
Residuals	796	0.1059	0.0001		
Pollination_conditions	3	1315.28	438.43	3306413	< 2.2e-16 ***
Residuals	796	0.11	0		

Significance codes: .001 "***"; .01 "**"; .05 "*"; non significant "NS."

```
Analysis of Variance Table
Tukey test; alpha = 0.05
Response: length of pollen tubes
```

Pollination_conditions	HAP	mean	SD	groups of signif
S-morph_self	2	0.3251328	0.0102478	a
S-morph x L-morph	2	0.349352	0.0099562	b
L-morph_self	2	0.4207731	0.0103041	c
L-morph x S-morph	2	0.4449497	0.0106426	d
S-morph_self	3	0.8550793	0.0103665	b
S-morph x L-morph	3	0.909661	0.0124761	c
L-morph_self	3	0.8374607	0.0101908	a
L-morph x S-morph	3	0.9615144	0.0129607	d
S-morph_self	7	2.1001969	0.0163765	a
S-morph x L-morph	7	2.3251743	0.0136882	b
L-morph_self	7	2.5056041	0.0113881	c
L-morph x S-morph	7	2.7542894	0.0131105	d
S-morph_self	16	6.0289593	0.0117017	a
S-morph x L-morph	16	6.0539117	0.0136438	b
L-morph_self	16	6.1577077	0.0098296	c
L-morph x S-morph	16	6.4826446	0.0104896	d
S-morph_self	24	14.048939	0.0099906	c
S-morph x L-morph	24	15.473202	0.0099158	d
L-morph_self	24	12.01793	0.0130931	a
L-morph x S-morph	24	12.993781	0.0125769	b

Signif. codes: a -> b -> c -> d = p-value <2.2e-16

917   (b)

Analysis of Variance Table
Response: speed of pollen tubes => anova(lm(speed ~ Pollination_condition))

Anova Table (Type III tests)

	Df	Sum_Sq	Mean_Sq	F_value	Pr(>F)
Pollination_conditions	3	0.48643	0.162142	6094	< 2.2e-16 ***
Residuals	796	0.02118	0.000027		
Pollination_conditions	3	2.26998	0.75666	5629.5	< 2.2e-16 ***
Residuals	796	0.10699	0.00013		
Pollination_conditions	3	2.30357	0.76786	64582	< 2.2e-16 ***
Residuals	796	0.00946	0.00001		
Pollination_conditions	3	0.104195	0.034732	21137	< 2.2e-16 ***
Residuals	796	0.001308	0.000002		
Pollination_conditions	3	23.7891	7.9297	3827337	< 2.2e-16 ***
Residuals	796	0.0016	0		

Analysis of Variance Table
Tukey test; alpha = 0.05
Response: Speed of pollen tubes

Pollination_conditions	HAP	mean	SD	groups of signif
morph-S_self	2	0.1625664	0.0051239	d
morph-S x morph-L	2	0.174676	0.0049781	c
morph-L_self	2	0.2103866	0.005152	b
morph-L x morph-S	2	0.2224748	0.0053213	a
morph-S_self	3	0.5298404	0.0103665	b
morph-S x morph-L	3	0.559422	0.0124761	a
morph-L_self	3	0.4176024	0.0101908	d
morph-L x morph-S	3	0.5166561	0.0129607	c
morph-S_self	7	0.3109203	0.0040941	d
morph-S x morph-L	7	0.3534147	0.0034221	c
morph-L_self	7	0.417389	0.002847	b
morph-L x morph-S	7	0.4483104	0.0032776	a
morph-S_self	16	0.4366003	0.0013002	a
morph-S x morph-L	16	0.4143728	0.001516	b
morph-L_self	16	0.4057916	0.0010922	c
morph-L x morph-S	16	0.4143976	0.0011655	b
morph-S_self	24	1.0024951	0.0012488	b
morph-S x morph-L	24	1.177403	0.0012395	a
morph-L_self	24	0.7325065	0.0016366	d
morph-L x morph-S	24	0.8138629	0.0015721	c

Significance codes: .001 "***"; .01 "**"; .05 "*"; non significant "NS."

Signif. codes: a -> b -> c -> d = P value <2.2e-16

918

919   (c)

Analysis of Variance Table
Response: accelleration of pollen tubes => anova(lm(acceleration~ Pollination_condition))

Anova Table (Type III tests)

	Df	Sum_Sq	Mean_Sq	F_value	Pr(>F)
Pollination_conditions	3	1.94571	0.64857	6094	< 2.2e-16 ***
Residuals	796	0.08472	0.00011		
Pollination_conditions	3	1.89545	0.63182	4700.7	< 2.2e-16 ***
Residuals	796	0.10699	0.00013		
Pollination_conditions	3	46.067	15.3558	80721	< 2.2e-16 ***
Residuals	796	0.151	0.0002		
Pollination_conditions	3	26.1592	8.7197	65513	< 2.2e-16 ***
Residuals	796	0.1059	0.0001		
Pollination_conditions	3	1315.28	438.43	3306413	< 2.2e-16 ***
Residuals	796	0.11	0		

Analysis of Variance Table
Tukey test; alpha = 0.05
Response: acceleration of pollen tubes

Pollination_conditions	HAP	mean	SD	groups of signif
morph-S_self	2	0.1625664	0.0051239	d
morph-S x morph-L	2	0.174676	0.0049781	c
morph-L_self	2	0.2103866	0.005152	b
morph-L x morph-S	2	0.2224748	0.0053213	a
morph-S_self	3	0.3672739	0.0112325	b
morph-S x morph-L	3	0.384746	0.0129094	a
morph-L_self	3	0.2072158	0.0116108	d
morph-L x morph-S	3	0.2941812	0.0137249	c
morph-S_self	7	-0.21892	0.0107856	d
morph-S x morph-L	7	-0.206007	0.0131338	c
morph-L_self	7	-0.0002130	0.0106224	a
morph-L x morph-S	7	-0.068346	0.0132985	b
morph-S_self	16	0.12568	0.0044317	a
morph-S x morph-L	16	0.0609581	0.0038266	b
morph-L_self	16	-0.011597	0.0029275	c
morph-L x morph-S	16	-0.033913	0.0034387	d
morph-S_self	24	0.5880975	0.0016601	b
morph-S x morph-L	24	0.7630302	0.0019039	a
morph-L_self	24	0.3267149	0.0018812	d
morph-L x morph-S	24	0.3994653	0.0019593	c

Significance codes: .001 "***"; .01 "**"; .05 "*"; non significant "NS."

Signif. codes: a -> b -> c -> d = P value <2.2e-16

920
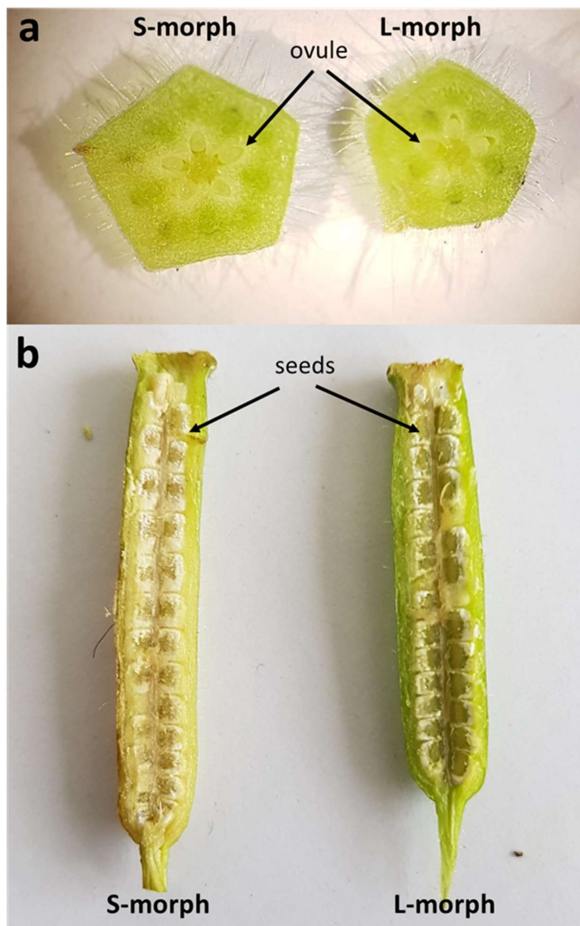
921

922

923 Figure S2S3: a) transversal and b) longitudinal sections of petaloculate ovary from 5-merous

924 floral morphs of *Ludwigia grandiflora* subsp. *hexapetala*.