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ORIGINAL ARTICLE

Partial interfertility between independently originated populations of the neo-allopolyploid *Mimulus peregrinus*

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Abstract The reduction in genetic diversity in polyploid lineages, formed from whole-genome duplication of a few individuals, can affect their long-term evolutionary potential. Because most polyploids originate multiple times, secondary contact and gene exchange among independently formed polyploids can create novel genetic combinations and reduce the severity of genetic bottlenecks. However, independently originated polyploids may be reproductively isolated from each other due to genetic and chromosomal differences predating the polyploidisation event, or evolving subsequently in the dynamic genomes of young polyploid populations. Here we conducted experimental crosses to investigate the phenotype and interfertility between two independently originated populations of the allopolyploid *Mimulus peregrinus* (Phrymaceae). We found that individuals from the two populations are phenotypically distinct, but that inter- and intrapopulation crosses are not statistically different. Interpopulation crosses produce viable and fertile offspring, although our results suggest the existence of partial reproductive barriers in the form of reduced pollen viability. We found no difference in pollen viability between F1 and F2 generations. In contrast, we detected a reduction in floral and vegetative size, and in the proportion of plants that flowered, between F1 and F2 generations for both intra- and interpopulation crosses. Together, our results indicate that populations of independent origin can partially exchange genes, producing variable offspring, and that the phenotype of *M. peregrinus* may be unstable in the early generations. Natural selection on genetically based variation may be required for the evolution of more stable and fertile individuals of this nascent allopolyploid.

Keywords Hybridisation · *Mimulus* · Non-native species · Reproductive barriers · Speciation · Whole-genome duplication

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Introduction

Whole-genome duplication (WGD), or polyploidisation, is an important phenomenon that characterises the evolutionary history of many plants lineages (Levin 2002; Soltis et al. 2009; Jiao et al. 2011). Despite the widespread occurrence of WGD, polyploids need to surmount considerable challenges arising at their origin, including genetic and meiotic instability, minority cytotype disadvantage, and ecological competition with parental taxa (Levin 1975; Ramsey and Schemske 2002; Comai 2005; Arrigo and Barker 2012; Soltis et al. 2014b; Vallejo-Marín and Hiscock 2016). One of the challenges that polyploids need to overcome is the expected reduction in genetic diversity associated with the birth of a polyploid species. Because polyploidisation often results in strong reproductive barriers between the new polyploid and parental taxa due, in part, to differences in chromosome numbers and deregulated expression of imprinted genes (Coyne and Orr 2004; Mallet 2007; Köhler et al. 2010; Lafon-Placette and Kohler 2015), polyploids may be derived from only one or a few

individuals within a population. Thus, nascent polyploids may carry limited genetic diversity, which in turn might affect their long-term evolutionary potential (Soltis et al. 2014b). This expected reduction in genetic diversity has led to some authors to propose that polyploidisation should be an evolutionary dead end (e.g. Stebbins 1950), and recent work suggests that polyploid lineages have lower rates of speciation than diploid lineages (Mayrose et al. 2011; Arrigo and Barker 2012; but see Soltis et al. 2014a). Yet, the successful establishment of some polyploid lineages indicates that the severity of the challenges associated with the origin of polyploids, including the loss of genetic diversity due to small population sizes at their origin, can sometimes be circumvented.

A principal mechanism that may rescue polyploids from a fate of depauperate evolutionary potential is the fact that polyploid species often have multiple, independent origins (e.g. Wyatt et al. 1988; Soltis and Soltis 1991; Ashton and Abbott 1992; Doyle et al. 1999; Segraves et al. 1999; Beck et al. 2012; Modliszewski and Willis 2012; Sampson and Byrne 2012; Mavrodiev et al. 2015; Servick et al. 2015; Vallejo-Marín et al. 2015). Although each instance of polyploid formation may result in limited genetic diversity, mating between independently generated polyploids may introduce genetic variation into the nascent lineage and create new genetic combinations (Soltis and Soltis 1999). Interfertility between independently originated populations of polyploids is determined by multiple factors. On one hand, genetically distinct populations of polyploids may be reproductively compatible because they share the same parental species (autopolyploids) or combination of species (allopolyploids) and chromosome number. On the other hand, independently originated populations may be isolated from each other by postzygotic reproductive barriers due to genetic or chromosomal incompatibilities that either predate the polyploidisation event or evolve subsequently. For example, nuclear–nuclear and nuclear–cytoplasmic incompatibilities within or between species can result in postzygotic isolation barriers among genetically differentiated populations (Levin 2003; Coyne and Orr 2004; Martin and Willis 2010; Burton et al. 2013; Lindtke and Buerkle 2015). If genetically distinct parental populations of a given parental taxon give rise to separate polyploids, the derived polyploids may be reproductively isolated upon secondary contact. This type of pre-existing barriers may be particularly acute in allopolyploids, as the hybridisation event in each origin may involve different parental taxa as either the maternal or paternal parents, resulting in populations with different combinations of nuclear and cytoplasmic genomes which may interact epistatically (Levin 2003). In addition, postzygotic barriers between independently originated populations may evolve following the polyploidisation event. Polyploid and hybrid genomes can be highly dynamic often undergoing considerable change in gene content, gene expression, and chromosome structure and number in a few generations (Song et al. 1995; Wendel 2000; Ozkan et al. 2001; Tate et al. 2006; Chester et al. 2012; Buggs 2013; Hegarty et al. 2013; Lafon-Placette and Kohler 2015; Soltis et al. 2015; Yant and Bomblies 2015). These rapid genomic changes may result in postzygotic reproductive barriers that impede mating among independently originated populations after they come into secondary contact. Despite the wide recognition that multiple origins characterise many polyploids (but not all, see e.g. Ainouche et al. 2004), few studies have experimentally tested the extent to which populations of separate origin are interfertile (e.g. Modliszewski and Willis 2012).

The recent discovery of the allopolyploid *Mimulus peregrinus* (Phrymaceae) (Vallejo-Marín 2012) provides an opportunity to experimentally test whether independently originated populations are interfertile. *Mimulus peregrinus* evolved via hybridisation and genome duplication between two closely related taxa (Vallejo-Marín 2012): the, mostly, diploid *Mimulus guttatus* ($2n = 28$) and the ancient tetraploid *Mimulus luteus* ($2n = 60$–64) (Vickery 1995). These two species are allopatrically distributed in their native range (North and South America, respectively), but came into secondary contact after they were introduced into the British Isles in the nineteenth century. In the British Isles, *M. guttatus* and *M. luteus* form the triploid hybrid *Mimulus* × *robertsii* ($2n = 44$–46) (Stace 2010), which is highly sexually sterile, but can propagate vigorously through clonality (Vallejo-Marín and Lye 2013). *Mimulus* × *robertsii* is currently well established and widely distributed in Great Britain (Stace et al. 2015) and can be found in approximately 40% of all *Mimulus* populations (Vallejo-Marín and Lye 2013). In the British Isles, *M.* × *robertsii* has given rise, twice, to a new species: the sexually fertile allohexaploid *M. peregrinus* ($2n = 92$) (Vallejo-Marín et al. 2015). Because the earliest records of naturalised populations of *M.* × *robertsii* date back to the 1870s (Preston et al. 2002), it is likely that *M. peregrinus* is a recently formed allopolyploid less than 150 years old. *Mimulus peregrinus* (= *Erythranthe peregrina*) has been found in southern Scotland (South Lanarkshire) and the Orkney Isles off the north coast of Scotland, two regions separated by approximately 400 km. Within each of these regions, *M. peregrinus* occurs in multiple localities separated by a few kilometres. Genomic analyses of *M. peregrinus* from South Lanarkshire and Orkney indicate that this allopolyploid species has originated in each of these two regions, independently, and is closely related to sympatric (Leadhills) or nearby (Orkney) populations of *M.* × *robertsii* (Vallejo-Marín et al. 2015).

Recent analyses of the cytoplasmic genomes of *M. peregrinus* have shown that all sampled individuals carry

chloroplast and mitochondria inherited from *M. guttatus*, suggesting that the hybridisation event involved in the origin of this allopolyploid had *M. guttatus* as the maternal parent and *M. luteus* as the paternal one (Vallejo-Marín et al. 2016). Consistent with this observation, natural populations of *M.* × *robertsii* also tend to have *M. guttatus* mitochondria and chloroplasts, and experimental crosses between *M. guttatus* and *M. luteus* show that hybrids are much easier to produce when *M. guttatus* is the mother than in the opposite direction (Vallejo-Marín et al. 2016). The observed pattern of asymmetric hybridisation in *M. peregrinus* is common in interploidy crosses (Vallejo-Marín and Hiscock 2016), although in many cases, hybrids are more likely to be viable when the maternal parent is of higher ploidy than the paternal parent (Köhler et al. 2010), opposite to what has been observed in *M.* × *robertsii* (Vallejo-Marín et al. 2016). The mechanism for the formation of *M. peregrinus* is unknown, but it may have evolved either via somatic doubling or via mating of unreduced gametes of *M.* × *robertsii*. Although highly sterile, *M.* × *robertsii* occasionally produces unreduced gametes (M. Vallejo-Marin and A. Laing unpublished data), which potentially could produce a polyploid individual. In summary, *M. peregrinus* is a newly evolved allopolyploid for which the origin and genetic composition of different populations are relatively well characterised. Moreover, *M. peregrinus* can be easily grown in experimental conditions and has a short generation time (as fast as 8 weeks from seed to seed), which makes it suitable for experimental tests carried over multiple generations. For the present study, we conducted experimental pollinations and grew individuals from both intra- and interpopulation crosses in common gardens to determine whether independently originated populations of a newly evolved allopolyploid *M. peregrinus* are interfertile. We also analysed germination, fertility, phenology, and floral and leaf morphology of parental populations and two generations of intra- and interpopulation crosses to characterise the phenotype of *M. peregrinus.*

Materials and methods

We studied two populations of *M. peregrinus*. The first population (LED; latitude: 55.423°, longitude: −3.735°) is located in southern Scotland and is the place where *M. peregrinus* was first discovered (Vallejo-Marín 2012). The second population (STR) is in the Orkney Isles near the town of Stromness (58.969°, −3.283°). We have previously shown that these two populations are genetically differentiated and represent separate, independent origins of *M. peregrinus*, from local triploid *M.* × *robertsii* (Vallejo-Marín et al. 2015). For each of the two populations, we selected five maternal seed-families, i.e. groups of seeds derived from the same maternal parent. The maternal families were either directly collected from in the field (STR) or derived from 1–2 generations of selfing of field-collected individuals (LED) (Table 1).

Seeds were planted in 9-cm-diameter plastic pots filled with Modular Seed compost (Sinclair, Lincoln Lincolnshire, UK) on 1 July 2014 and placed in a controlled environment cabinet (Snijder Microclima). The growth cabinets were kept at 24 °C/16 h light and 16 °C/8 h dark cycles with 70% relative humidity and 80% illumination. After approximately 9 days, individual seedlings were transplanted to 9 cm pots filled with General Purpose compost (Sinclair) and placed in flooded plastic trays in the glasshouse. Supplemental lighting was provided by compact fluorescent lamps during 16 h each day. These individuals formed the parental (P) generation.

To generate the F1 generation, we conducted controlled pollinations both within and between populations in the P

Table 1 Parental accessions (P) of *Mimulus peregrinus* used in the experiment

Maternal seed family ID	Population	Source of individuals in P generation
11-LED-seed-1-54	Leadhills	S2
11-LED-seed-2-14	Leadhills	S2
11-LED-seed-2-19	Leadhills	S2
11-LED-seed-4-5	Leadhills	S2
11-LED-seed-5-17	Leadhills	S2
11-LED-seed-19	Leadhills	S1
13-STR-seed-1	Stromness	Field collected
13-STR-seed-2	Stromness	Field collected
13-STR-seed-3	Stromness	Field collected
13-STR-seed-4	Stromness	Field collected
13-STR-seed-5	Stromness	Field collected

The source of each maternal family is indicated as field collected or derived from one (S1) or two (S2) generations of selfing

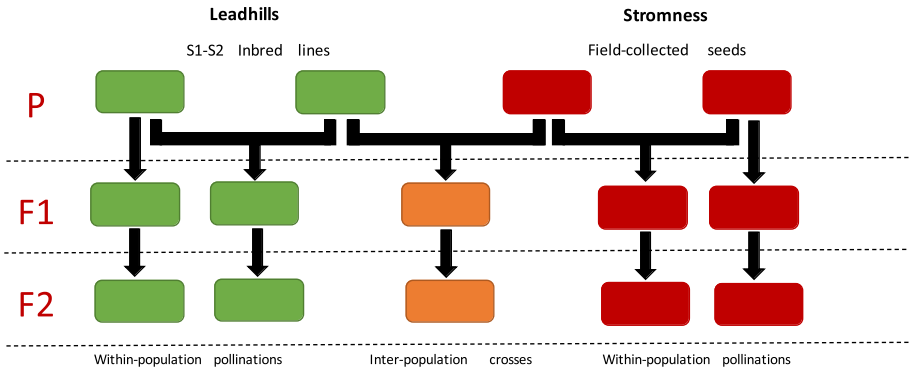

Fig. 1 Simplified diagram of the crossing design used in the experiment. *Straight arrows* denote self-pollination. *Joining arrows* are cross-pollinations. The Leadhills individuals used in the parental (P) generation are first- and second-generation lines from field-collected seed (bulk collected) in 2011. The Stromness individuals in the P generation were grown from field-collected seed sampled in 2013 from individually collected maternal parents

plants in August 2014 (Fig. 1). Pollen was collected from individual plants, placed on glass coverslips, and applied directly to the stigmas of flowers using a wooden toothpick. As in other *Mimulus* species (e.g. Fetscher and Kohn 1999), *M. peregrinus* has bi-lobed thigmotropic stigmas, which close after touching them. Stigmas remain closed following successful pollination. All pollinations were made using plants kept in pollinator-free growth cabinets. To generate the F2 generation, we self-pollinated F1 individuals in February 2015 using the same protocol. Seeds were harvested from mature fruits 17–20 days after pollination.

Phenotypic measurements

Parental populations were measured in the glasshouse in August 2014 with growing conditions as described above. For the filial generations, we simultaneously grew seeds from both F1 and F2 generations. Seeds were sown on 30 March 2015 in a controlled environment cabinet at 24 °C/18 h light and 16 °C/6 h dark cycles with 70% relative humidity and 80% illumination. F1 and F2 seedlings were transplanted on 17 April 2015 to 9 cm pots and placed in flooded trays in the greenhouse as in the P generation and measured in May 2015. The following phenotypic variables were measured on each plant: (1) corolla width; (2) corolla height; (3) corolla tube's length; (4) calyx length; (5) pedicel length; (6) length and (7) width of the leaf at the second node; (8) flowering node (the number of nodes in the main stem, counted from the first node above the soil surface, to the node where the first flower appears); (9) anther–stigma distance; and (10) date to first flower. Floral measurements were taken in the first two flowers produced by each individual. Morphological measurements were taken with a digital calliper (Absolute Digimatic, 500-196-20; Mitutoyo, Japan) to the nearest 0.01 mm, except leaf size, which was measured with a ruler to the nearest 0.5 cm.

The viability of F1 seeds resulting from controlled pollinations was assessed in seed families from 10 inter-population (five in each direction) and eight intrapopulation crosses (four crosses per population). Here we use seed germination as an estimate of seed viability. For each family, we planted an average of 74 seeds (range = 30–157) in a controlled environment cabinet at 24 °C/18 h light and 16 °C/6 h dark cycles with 70% relative humidity and 80% illumination. We counted the number of emerged seedlings 14 days after planting and calculated the probability of germination (number of seeds that germinated over the total number of seeds planted) per family.

To estimate pollen fertility in F1 and F2 individuals, we collected five mature anthers from a single flower in 1.5-mL microcentrifuge tubes with 1 mL of 70% ethanol. Pollen was stained with 100 µL lactophenol-aniline blue (Kearns and Inouye 1993) and scored in a compound microscope (Olympus, Hamburg, Germany) at 400× magnification. Pollen that was evenly stained blue was considered viable, and pollen that was not stained or with a few irregular blue patches was considered inviable. Non-stained pollen was usually smaller and/or appeared malformed (not spherical). Hereafter we use this measure of pollen stainability as an estimate of pollen fertility. In each sample, we counted at least 200 pollen grains (mean = 230 grains). Biomass was estimated in F1 and F2 plants after plants had begun senescing in July–August 2015. All above-ground biomass was harvested from each individual and placed in a drying oven at 40 °C for 72 h, and weighted in an OHAUS-Analytical Plus digital balance (GmbH, Nanikon, Switzerland).

Statistical analyses

Parental generation

We conducted a principal component analysis (PCA) of nine morphological variables (traits 1–9 above) using the function *princomp* in the statistical package *R* (R Development Core Team 2016). We analysed statistical differences in the first two principal components between populations (LED and STR) using a generalised linear mixed effects model in the package *lme4* (Bates et al. 2015). Plant identity and maternal family were used as random effects, and population of origin as a fixed effect. Days to flower were compared between populations using maternal family as a random effect. Statistical significance of the fixed effect was assessed with *lmerTest* (Kuznetsova et al. 2016) using a Satterthwaite approximation for degrees of freedom.

Seed viability of F1 generation

The viability of F1 seeds was analysed using a binomial model with logit link. The significance of cross type (LED × LED, STR × STR or STR × LED) was assessed using a likelihood ratio test with two degrees of freedom.

Phenotype and fertility of the F1 and F2 generations

We analysed phenotype and fertility for the F1 and F2 generations jointly. We conducted a PCA on the same nine morphological variables as in the P analysis. The first principal component, as well as pollen viability and biomass, was analysed using generalised linear Markov Chain Monte Carlo models using the *R* package *MCMCglmm* (Hadfield 2010). We chose to use generalised mixed effects models in *MCMCglmm* because it allowed us to incorporate complex pedigree information in the specification of the error structure. Thus, this analysis explicitly accounts for the relationships between individuals in F1 and F2 generations. In all analyses, cross type (i.e., LED × LED, STR × LED, STR × STR) was included as a fixed effect. Cross type and generation (F1, F2) were included as fixed effects, and individual as a random effect. For the analysis of the first principal component, days to flower, and biomass, we used a Gaussian distribution and default priors, and for pollen viability we used a multinomial distribution ("multinomial2") and the following priors for the random effect (individual): $V = 1$, $v = 0.002$. All models were run for 1000,000 iterations with a 50,000 burn-in period, and thinning every 100 iterations. The statistical significance of the fixed effects was assessed using MCMC "*P* values". Pairwise comparisons between specific "cross type" levels (e.g. STR × LED vs. LED × LED) were assessed by using one of those levels as the reference (intercept) in the model, calculating the difference in posterior parameter estimates between each two levels (i.e., STR × LED–LED × LED) for the 1,000,000 iterations of the model, and calculating whether the 95% credible interval of this difference overlapped zero.

Results

Phenotypic differentiation between populations of *Mimulus peregrinus*

The first (PC1) and second principal components (PC2) explained 27 and 24% of the variation in the nine measured floral and vegetative traits of the parental, P, generation (Table 2a). The first principal component was positively associated with larger values of all nine traits, and thus serves as an overall indicator of size and of flowering at later nodes. The second principal component was correlated with larger leaves and flowering at later nodes, and smaller calyces and corollas. A bivariate plot of PC1 and PC2 suggests a clear overall differentiation between the population of *M. peregrinus* from southern Scotland (LED) and the Orkney population (STR) (Fig. 2). Individuals

Table 2 Loadings of the first two principal components (PC1 and PC2) of a principal component analysis of floral and vegetative characters of (**a**) two parental (P) populations of *Mimulus peregrinus* and (**b**) F1 and F2 generations of both within- and between-population crosses of these populations, grown in a common garden

	PC1	PC2
(a)		
Flowering node	0.394	0.388
Pedicel length	0.230	−0.316
Corolla width	0.519	−0.161
Corolla height	0.424	−0.298
Corolla tube	0.044	−0.592
Anther–stigma distance	0.406	0.135
Calyx length	0.168	−0.312
Second node leaf length	0.367	0.354
Second node leaf width	0.114	0.206
(b)		
Flowering node	0.253	−0.422
Pedicel length	0.397	0.009
Corolla width	0.493	0.022
Corolla height	0.486	0.079
Corolla tube	0.419	0.010
Anther–stigma distance	0.178	0.082
Calyx length	0.286	0.003
Second node leaf length	0.095	0.627
Second node leaf width	−0.039	0.645

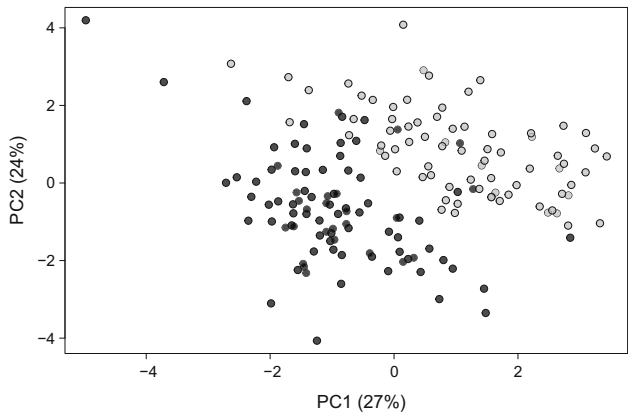

Fig. 2 Scatterplot of the first two components of a PCA of floral and vegetative traits in the parental generation (P) of two independently originated populations of *Mimulus peregrinus*. *Grey symbols*: population from Stromness, Orkney (STR); *black symbols*: population from Leadhills, South Lanarkshire (LED), Scotland. The proportion of variance explained by each component is given in parenthesis. Sample size: 168 flowers, 84 individuals (46 from LED, 38 from STR)

from STR had significantly larger values than LED individuals for both PC1 (STR regression coefficient = 2.02 ± 0.28, mean ± S.E.; $P < 0.001$) and PC2 (STR regression coefficient = 1.56 ± 0.32; $P < 0.01$). Among plants that flowered in our experiment, STR individuals took an average of 3 days longer to produce their first flower (41.76 ± 0.31 vs. 38.00 ± 0.44, for STR and LED, respectively; $P < 0.01$). Together, our results suggest that *M. peregrinus* from Orkney is generally larger and produces flowers later and in higher nodes than the southern population, although there is variation among individual traits (Fig. 3).

Germination of within- and between-population crosses

Both within- and between-population crosses produced viable seeds and had similar germination rates. Germination for crosses between populations (LED × STR) seeds was 75% ± 7, and for within population crosses was 71% ± 10 (LED × LED) and 74% ± 9 (STR × STR). These differences were not statistically significant as assessed with a likelihood ratio test ($P = 0.561$).

Floral and vegetative phenotype of F1 and F2 generations

Scatterplots of individual floral and vegetative traits in the F1 and F2 generation are shown in Online Resources 1 and 2. The PCA of the morphological data across the F1 and F2 generations summarised 59% of the variation in the first and second principal components. The first principal component (PC1) was correlated with larger values of all floral traits and with flowering at later nodes, and only weakly with vegetative traits (longer and narrower leaves), explaining 37% of the variation (Table 2b). As in the parental generation, STR individuals had larger PC1 values indicating larger floral traits, and flowering at later nodes than LED individual (0.88 ± 0.18 vs. −0.76 ± 0.17, for STR and LED, respectively). The interpopulation crosses had PC1 values intermediate between the parental populations (0.29 ± 0.20). However, the MCMC analysis of PC1 using the pedigree information did not find statistically significant differences between the different cross types ($P > 0.13$; Fig. 4a). The second principal component explained 22% of the variation; it was negatively and strongly associated with leaf size, and only weakly and negatively associated with floral traits. The second principal component (PC2) was positively associated with flowering at later nodes. PC2 was smaller for LED than for STR (−0.42 ± 0.15 vs. 0.05 ± 0.16, respectively), suggesting that LED produces shorter and narrower leaves than STR. The cross LED × STR had the largest average values for PC2 (0.40 ± 0.14) suggesting that the interpopulation cross had larger leaves and flowered at later nodes than the intrapopulation crosses. Nevertheless, the MCMC analysis did not show statistically significant differences between the crosses ($P > 0.05$). Finally, we found a significant effect of generation on PC1 but not on PC2, with F2 individuals having smaller PC1 values than F1 plants ($P < 0.001$; Fig. 4a).

Average days to flower in the F1 and F2 generations were nearly identical between LED × LED and STR × LED crosses (42.45 ± 0.26 days vs. 42.39 ± 0.24 days, respectively), while STR × STR individuals took slightly longer to produce the first flower (43.89 ± 0.21 days). These differences were not statistically significant (95% credible intervals of effect size differences overlap zero).

Pollen viability and biomass of F1 and F2 generations

We found no statistically significant differences in pollen viability between LED × LED and STR × STR (Fig. 4c). Average pollen viability in STR x STR individuals across both F1 and F2 generations was 0.76 ± 0.04 vs. 0.63 ± 0.04 for LED × LED individuals. The pollen viability of interpopulation hybrids STR × LED was 0.50, which was significantly lower than STR × STR, but not distinguishable from LED × LED plants (Fig. 4c). We found no significant differences in pollen viability between F1 and F2 (Fig. 4c).

Average dry biomass across F1 and F2 generations was 3.19 g ± 0.15 (STR × STR), 2.51 g ± 0.10

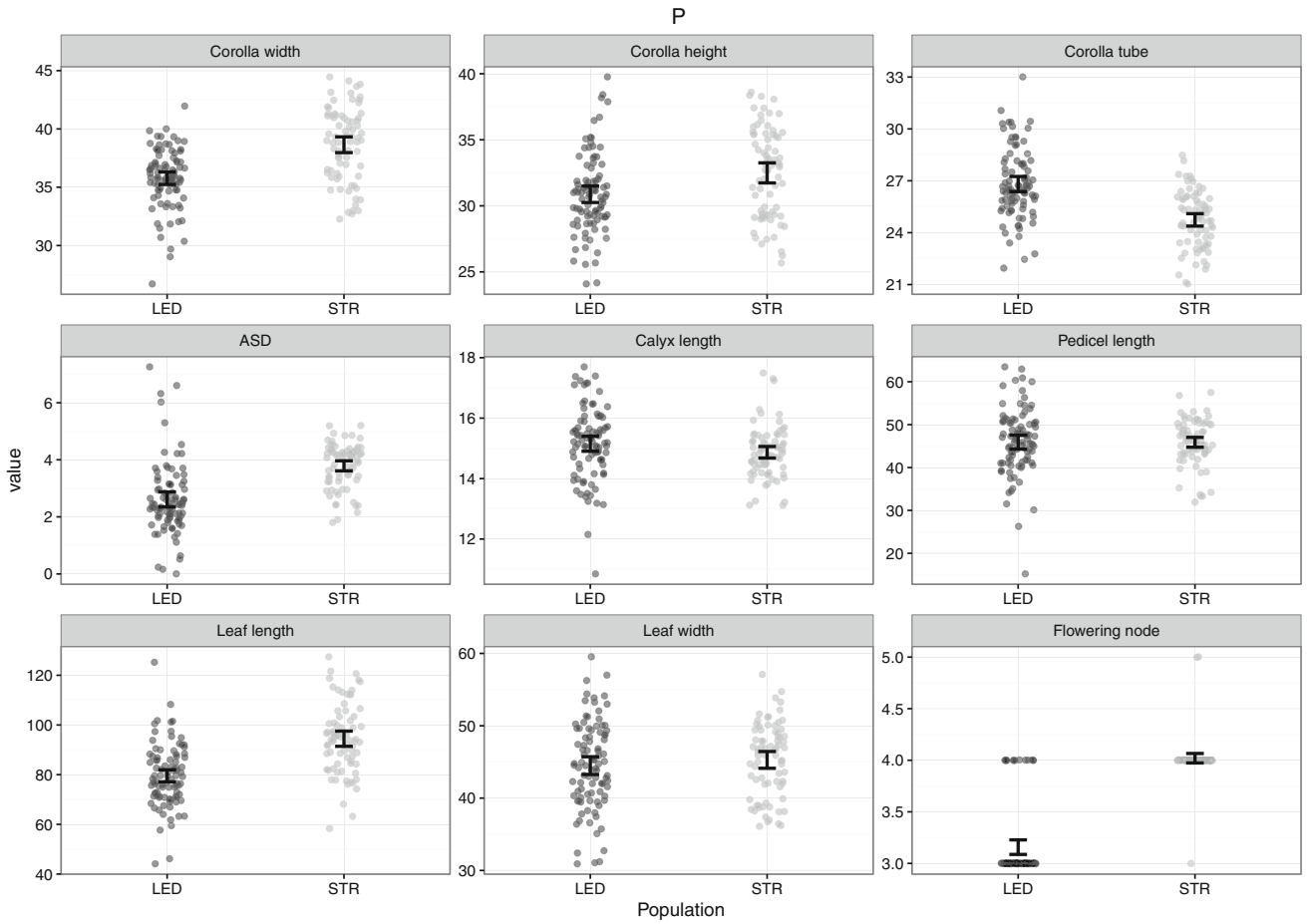

Fig. 3 Floral and vegetative characteristics of individuals of *Mimulus peregrinus* from two independently originated populations: Leadhills, South Lanarkshire (LED); and Stromness, Orkney (STR). The error bars represent the means and 95% confidence intervals calculated using a nonparametric bootstrap (1000 replicates). A small amount of jitter has been added to the *x*-axis to improve the visualisation of data points. All measurements in mm, except flowering node, which is the number of nodes counted from the base of the plant to the first flower. *ASD* anther–stigma distance, *P* parental generation. Sample size as in Fig. 1

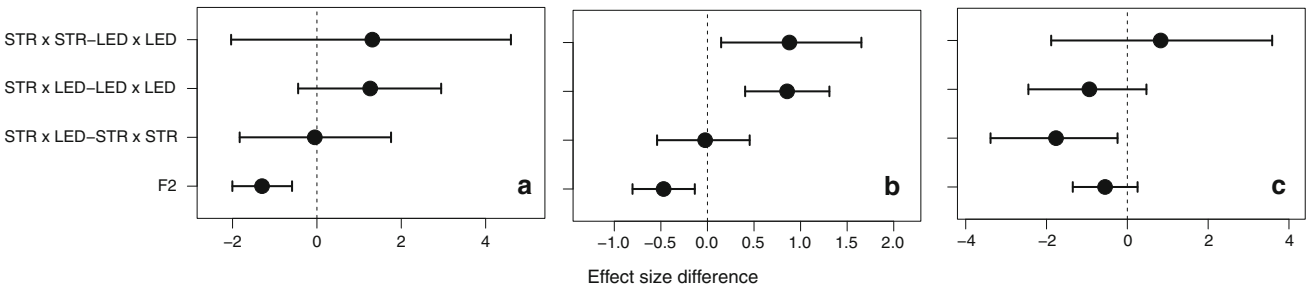

Fig. 4 Effect size differences in pairwise comparisons of phenotype and fertility between different types of intra- and interpopulation crosses of independently originated populations of *Mimulus peregrinus* (LED and STR) obtained using MCMC generalised linear mixed models. Pairwise comparisons between specific "cross type" levels (e.g. STR × LED vs. LED × LED) were assessed by calculating the difference in posterior parameter estimates between each two levels (i.e., STR × LED–LED × LED) for 1,000,000 iterations of the model, and calculating the 95% credible interval. Intervals that do not overlap zero are interpreted as statistically significant. The credible interval for F2 indicates differences in trait value between the F1 and F2 generation across all cross types. **a** Results for the model of the first principal component (PC1). Sample sizes: F1 generation: 81 (49), 57 (37), and 30 (24) [number of flowers (number of individuals) for LED × LED, STR × LED, and STR × STR, respectively]; F2 generation: 13 (7), 35 (22), and 20 (13). **b** Model results for above-ground dry biomass. **c** Model results for pollen viability

(LED × LED), and 3.24 g ± 0.12 (STR × LED). In pairwise comparisons, dry biomass of STR × STR was significantly higher than LED × LED, but no different from interpopulation crosses. In contrast, STR × LED produced significantly more dry biomass than LED × LED (Fig. 4b). We also found a significant reduction in dry biomass in the F2 (3.03 g ± 0.9 vs. 2.79 g ± 0.15, for F1 and F2, respectively; Fig. 4b), but no interaction between cross type and generation (results not shown).

Discussion

Interfertility of *Mimulus peregrinus* of independent origin

Here we have shown that independently originated populations of the neo-allopolyploid *Mimulus peregrinus* are interfertile, i.e., produce viable, fertile offspring. Although there is ample genetic evidence of multiple origins across a variety of allopolyploid taxa (e.g. Wyatt et al. 1988; Ashton and Abbott 1992; Franzke and Mummenhoff 1999; Segraves et al. 1999; Soltis et al. 2004; Beck et al. 2012; Mavrodiev et al. 2015; Servick et al. 2015; Vallejo-Marín et al. 2015), fewer studies have experimentally compared the interfertility of independently originated populations. For instance, Modliszewski and Willis (2012) investigated the interfertility of populations of independent origin of the allotetraploid *Mimulus sookensis* ($2n = 4x = 56$), which is the polyploid derivative of hybrids between *M. guttatus* and *M. nasutus* ($2n = 28$) (Benedict et al. 2012). Modliszewski and Willis crossed four populations of *M. sookensis* and compared the pollen viability of F1 and outbred F2 individuals grown in a common garden. The average pollen viability of F1 individuals was similar to mean parental values (95.2 vs. 96.8%, for F1 and the average of both parents, respectively) and was slightly lower in F2s (93.3%, range: 89–94%) (Modliszewski and Willis 2012). The reduction in pollen fertility in the F2s was statistically significant in three of the six crosses analysed in *M. sookensis*. In the case of *M. peregrinus,* pollen viability of interpopulation crosses was reduced by approximately 30% (50 vs. 69% pollen viability for interpopulation crosses vs. average pollen viability of both parental populations), while pollen viability was not significantly different between F1 and F2 generations.

Although interpopulation crosses of *M. peregrinus* are fertile, the observed reduction in pollen viability indicates the existence of partial postzygotic isolation barriers to gene exchange between independently originated polyploid populations. Such barriers could arise from a variety of mechanisms and either predate the formation of the allopolyploids or evolve subsequently. For example, postzygotic reproductive barriers occur among some genetically differentiated populations of the same species, due to nuclear–nuclear or nuclear–cytoplasm incompatibilities that are expressed in the interpopulation crosses (Rieseberg and Willis 2007; Burton et al. 2013). In *Mimulus guttatus,* one of the parental species of *M. peregrinus*, reduced pollen viability is sometimes expressed in interpopulation crosses (Fishman and Willis 2006; Sweigart et al. 2007). Reduced pollen fertility in intraspecific crosses of *M. guttatus* is thought to arise through Dobzhansky–Muller incompatibilities between populations either from nuclear–nuclear or nuclear–cytoplasmic interactions (Martin and Willis 2010). *Mimulus peregrinus* could inherit these preexisting genetic incompatibilities from its parental taxa causing lower pollen fertility when independently originated populations are crossed. Currently there is no data to evaluate the level of reproductive isolation between populations of *M. guttatus* in the UK. However, genomic analyses indicate that UK populations of *M. guttatus* are closely related to each other, and that most UK populations may be derived from a similar geographic region (Puzey and Vallejo-Marín 2014; Pantoja et al. 2017). Future work addressing potential hybridisation barriers within introduced *M. guttatus* would help understand the extent to which gene flow among independently originated populations of allopolyploids is limited by preexisting genetic incompatibilities.

An additional mechanism that could explain the observed reduction in pollen viability in interpopulation crosses of *M. peregrinus* is the evolution of genomic differences between populations following the allopolyploidization events. For example, polyploid genomes often undergo extensive modification through the loss of genes (Buggs et al. 2009; Koh et al. 2010), chromosome fragments, and entire chromosomes (Lim et al. 2008; Chester et al. 2013). Moreover, even in the absence of gene loss, chromosomal rearrangements evolving in hybrid and polyploid genomes can result in postzygotic isolation in crosses between populations fixed for different chromosome rearrangements (Soltis and Soltis 1999; Rieseberg 2001). Genome fractionation (the loss of duplicated genes), chromosome loss, and genomic rearrangements can occur rapidly, in a few generations, as has been shown in studies of synthetic allopolyploids in *Tragopogon* (Lim et al. 2008; Buggs et al. 2009), *Brassica* (Song et al. 1995), *Triticum,* and *Aegilops* (Liu et al. 1998), although other synthetic allopolyploids such as those recreating *M. sookensis* show little evidence of gene loss in the first generations (Modliszewski and Willis 2014). In *M. peregrinus*, there is preliminary evidence suggesting that this young allopolyploid has undergone some level of genome reorganisation (Vallejo-Marín et al. 2015). Using a sequence-capture approach to sequence a small subset of the genome at high

depth, Vallejo-Marín et al. (2015) compared the genotypes of *M. peregrinus* from the Leadhills (LED) and Stromness (STR) populations to their corresponding local triploid ancestors (*M.* × *robertsii*). They found that for 40 out of 6434 SNPs that were heterozygous in *M.* × *robertsii,* one of the parental alleles was missing in the corresponding *M. peregrinus*. Interestingly, most of the loci with a missing allele (24/40) were restricted to an individual from the STR population and were mapped to a single chromosome (linkage group 14). To the extent that the missing alleles represent the local loss of part of one of the parental subgenomes, these results would indicate that LED and STR have genetically diverged following the polyploidisation event. This type of genetic divergence through the loss of genetic material following polyploidisation could provide a mechanism to explain the partial postzygotic barriers observed among independently originated populations of *M. peregrinus*.

Changes in phenotype across two consecutive allopolyploid generations

The analysis of the phenotype and fertility of both F1 and F2 generations of inter- and intrapopulation crosses grown simultaneously in a common garden offers some insight into the phenotypic stability of *M. peregrinus* across generations. The observed reduction in size from the F1 to the F2 generation in both intra- and interpopulation crosses may indicate the early symptoms of hybrid breakdown (Burton et al. 2013) in *M. peregrinus.* The traits involved included floral and leaf characters—as measured by the principal component analysis—and above-ground biomass. The reduction in these traits affected equally inter- and intrapopulation crosses (generation × cross type interaction was not statistically significant; $P > 0.28$ for both PC1 and biomass). A reduced performance of F2 individuals was also suggested by the lower fraction of individuals that flowered in our experiment in the F2 versus the F1 generation (46% ± 0.04 vs. 30% ± 0.06, in the F1 and F2 generations, respectively), although the average day to first flower of those individuals that flowered was nearly identical in the F1 and F2. However, despite the reduction in size and proportion of flowering individuals in the F2, we found no statistically significant evidence of a reduction in pollen viability, suggesting that changes in plant size are not necessarily associated with reduced fertility in *M. peregrinus*.

Reduced viability or fertility in some F2 genotypes is well documented in homoploid hybrids (Grant 1966; Rieseberg et al. 1996; Burke et al. 1998; Burke and Arnold 2001), but less is known about how the fitness of allopolyploid taxa changes across early generations (Grant 1971; Comai et al. 2000; Madlung et al. 2005; Henry et al. 2014). Artificially created allopolyploids often suffer from low fertility and viability and display phenotypic instability (Madlung et al. 2005). In theory, allopolyploids may display reduced vigour and pollen viability in early generations as they accumulate chromosomal abnormalities, e.g. aneuploidy, due to the complexity of pairing and segregation during mitosis and meiosis (Grant 1971; Comai 2005; Madlung et al. 2005; Buggs et al. 2009; Madlung 2013). In addition, variation in patterns of gene loss (Buggs et al. 2012) and/or levels of gene expression could also rapidly produce phenotypic variation and lower fitness in early generation allopolyploids, even in the absence of large chromosomal abnormalities (Comai et al. 2000; Hegarty et al. 2006; Hegarty and Hiscock 2008; Buggs et al. 2011). For instance, synthetic allopolyploids of *Arabidopsis thaliana* × *Arabidopsis arenosa* ("*Arabidopsis suecica*") display phenotypic variation in the F2 in flower and leaf morphology, phenology, and pollen fertility, and at least some of this variation is thought to be associated with gene silencing (Comai et al. 2000) and may involve changes in transposon activity (Madlung et al. 2005). Variation in fertility in the F2 generation of allopolyploids can also arise due to segregation of Mendelian loci in interpopulation crosses of different origins. Again, in the allopolyploid *A. suecica*, F2s between natural and synthetic lines display variation in pollen viability. This variation is associated with the stability of meiosis in F2s and correlates with segregation at a single quantitative trait locus (BOY NAMED SUE) (Henry et al. 2014). Natural allelic variation for genes controlling meiotic stability are well studied in autopolyploids (Yant et al. 2013; Wright et al. 2015) and in a few allopolyploids (Hollister 2015). In addition, because F2s in our experiment were generated by self-fertilisation, it is also possible that phenotypic changes are partly due to increased homozygosity within subgenomes compared to the F1 generation. Therefore, the phenotype and genotype of recently formed allopolyploids may vary across generations due to the action of one or more of several mechanisms including rapid changes in chromosome number and structure, modification of gene expression, inbreeding, and segregation of genes involved in meiotic stability.

Variation in the phenotype of advanced generation allopolyploids in fitness-related traits, including pollen viability, has important implications for the evolutionary fate of nascent polyploid lineages. For instance, although average fertility in the F2 is ~50%, some of the F2 individuals from both inter and intrapopulation crosses in *M. peregrinus* have high fertility (>70%) (Fig. 5). It has long been recognised that even when hybrids have on average lower fitness than their parental taxa (Coyne and Orr 2004), some hybrids can have equal or higher fitness than parental classes (Arnold and Hodges 1995; Burke and Arnold 2001; Arnold and Martin 2010). In these cases, natural selection

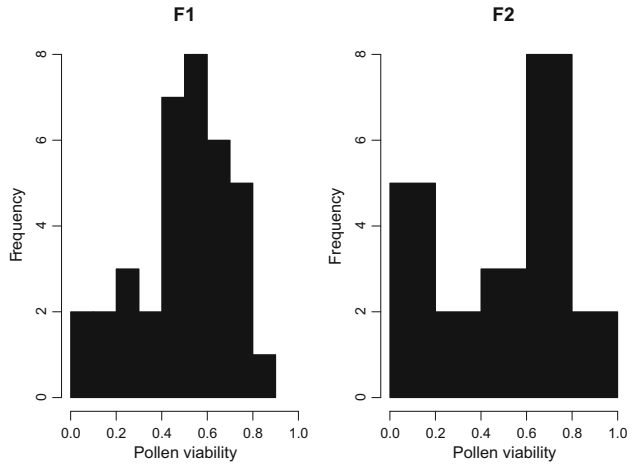

Fig. 5 Frequency distribution of the proportion of viable pollen in interpopulation crosses between two independently originated populations of *Mimulus peregrinus*, measured in the F1 and F2 generations (110 flowers and 42 flowers, respectively)

on the viability and fertility of segregating allopolyploids may favour the persistence of nascent lineages, allowing them to escape a "bottleneck of instability" and to become established in the long term (Ramsey and Schemske 2002; Comai 2005). The extent to which *M. peregrinus* can evolve higher fertility and viability through natural or artificial selection remains to be determined. Further work comparing fitness-related traits of *M. peregrinus* and its parents, including survivorship, pollen and seed viability, and clonal propagation, ideally under field conditions, is needed to establish the evolutionary fate of this nascent species. Furthermore, the increased availability of genomic resources in *Mimulus* offers the opportunity to search for links between phenotype and genotype in *M. peregrinus.* Such studies are needed to identify how potential changes in genome structure and expression may translate into phenotypic differences that can be acted upon by natural selection during the evolution of early generation allopolyploids.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Information on Electronic Supplementary Material

Online Resource 1. Floral and vegetative characteristics of F1 individuals produced through crosses within- (LED and STR), and between populations of *Mimulus peregrinus* (STR × LED).
Online Resource 2. Floral and vegetative characteristics of F2 individuals produced through self-pollination of F1 individuals from crosses within- (LED and STR), and between populations (STR × LED) of *Mimulus peregrinus*.

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