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Supplemental Information

Effect of Whole-Genome Duplication on the Evolutionary Rescue of Sterile Hybrid Monkeyflowers

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2 sterile hybrid monkeyflowers

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20 **Running title:** Evolutionary rescue by whole-genome duplication

21 **Short Summary:** Hybridisation and whole-genome duplication can be creative evolutionary forces
22 and fuel adaptation and speciation. Here we use natural and synthetically-produced hybrid and
23 polyploid monkeyflowers (*Mimulus spp.*) to study how polyploidy contributes to the fertility,
24 reproductive isolation, phenotype and performance of hybrids in the field.

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26 Supplemental Information

27 **Table S1.** Germination proportion of seeds derived from crosses within and among ploidy levels and
28 taxa in *Mimulus spp.* Only crosses that yielded more than 10 seeds per fruit were included in this
29 experiment. For each replicate (N) we tested between 10 and 110 seeds (average = 69 seeds). Taxa:
30 gut-2x = *M. guttatus* (diploid); gut-4x = *M. guttatus* (neo-tetraploid); lut-4x = *M. luteus* (ancient
31 tetraploid); per-6x = *M. peregrinus* (neo-allohexaploid). Inter-ploidy and Inter-species, denote
32 whether the cross was among individuals of different ploidy levels or belonging to different species
33 (*M. guttatus*, *M. luteus* and *M. peregrinus*), respectively; 1 = Yes, 0 = No.

Cross Type	Inter-ploidy	Inter-species	Mean ± SE	N
gut-2x X gut-4x	1	0	0.040	1
gut-2x X lut-4x	1	1	0.655 ± 0.015	2
gut-2x X per-6x	1	1	0	2
gut-4x X gut-2x	1	0	0.005 ± 0.005	4
gut-4x X per-6x	1	1	0.563 ± 0.285	3
lut-4x X gut-2x	1	1	0.085 ± 0.085	2
lut-4x X per-6x	1	1	0.015 ± 0.015	4
per-6x X gut-2x	1	1	0.035 ± 0.022	4
per-6x X gut-4x	1	1	0.470 ± 0.096	3
per-6x X lut-4x	1	1	0.020 ± 0.020	4
gut-2x X gut-2x	0	0	0.670 ± 0.040	2
gut-4x X gut-4x	0	0	0.847 ± 0.068	3
gut-4x X lut-4x	0	1	0.003 ± 0.003	3
lut-4x X gut-4x	0	1	0.460 ± 0.260	3
lut-4x X lut-4x	0	0	0.665 ± 0.073	4
per-6x X per-6x	0	0	0.950 ± 0.013	4
Total				48

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35 **Table S2:** Populations, population codes, life history, origin, and degree of inbreeding of the plants
36 used to generate synthetic hybrids.

Population code	Population	Species	Country	Inbred/field collected	Life history
COL	Coldstream, Scottish Borders	<i>M. guttatus</i>	UK	Field collected	Perennial
DBL	Dunblane, Perthshire	<i>M. guttatus</i>	UK	1 generation inbred	Perennial
DUN	Dunes, Oregon	<i>M. guttatus</i>	USA	9 generations inbred	Perennial
HOU	Houghton Lodge, Hampshire	<i>M. guttatus</i>	UK	Field collected	Perennial
LMC	Lower Mendocino County, CA	<i>M. guttatus</i>	USA	Field collected	Annual
COL	Coldstream, Scottish Borders	<i>M. luteus</i>	UK	3 generations inbred	Perennial
EVI	Evie, Orkney	<i>M. luteus</i>	UK	1 generation inbred	Perennial
EY	El Yeso	<i>M. luteus var. luteus</i>	Chile	5 generations inbred	Perennial
EYxRC	Artificial hybrid	<i>M. luteus var. luteus</i> x <i>M. luteus var. variegatus</i>	-	1 generation inbred	Perennial
RC	Rio Cipreses	<i>M. luteus var. variegatus</i>	Chile	5 generations inbred	Perennial

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39 **Table S3.** Individuals and families used in the common garden experiment in the glasshouse. All
40 individuals were grown from seed.

Taxon	Number of seed families	Number of individuals	Number of flowers
<i>M. guttatus</i>	7	113	236
<i>M. luteus</i>	5	200	99
<i>M. × robertsii</i> (synthetic)	26	255	582
<i>M. peregrinus</i>	17	303	618
(synthetic)			
<i>M. peregrinus</i>	4	73	160
(natural)			
Total	59	843	1,796

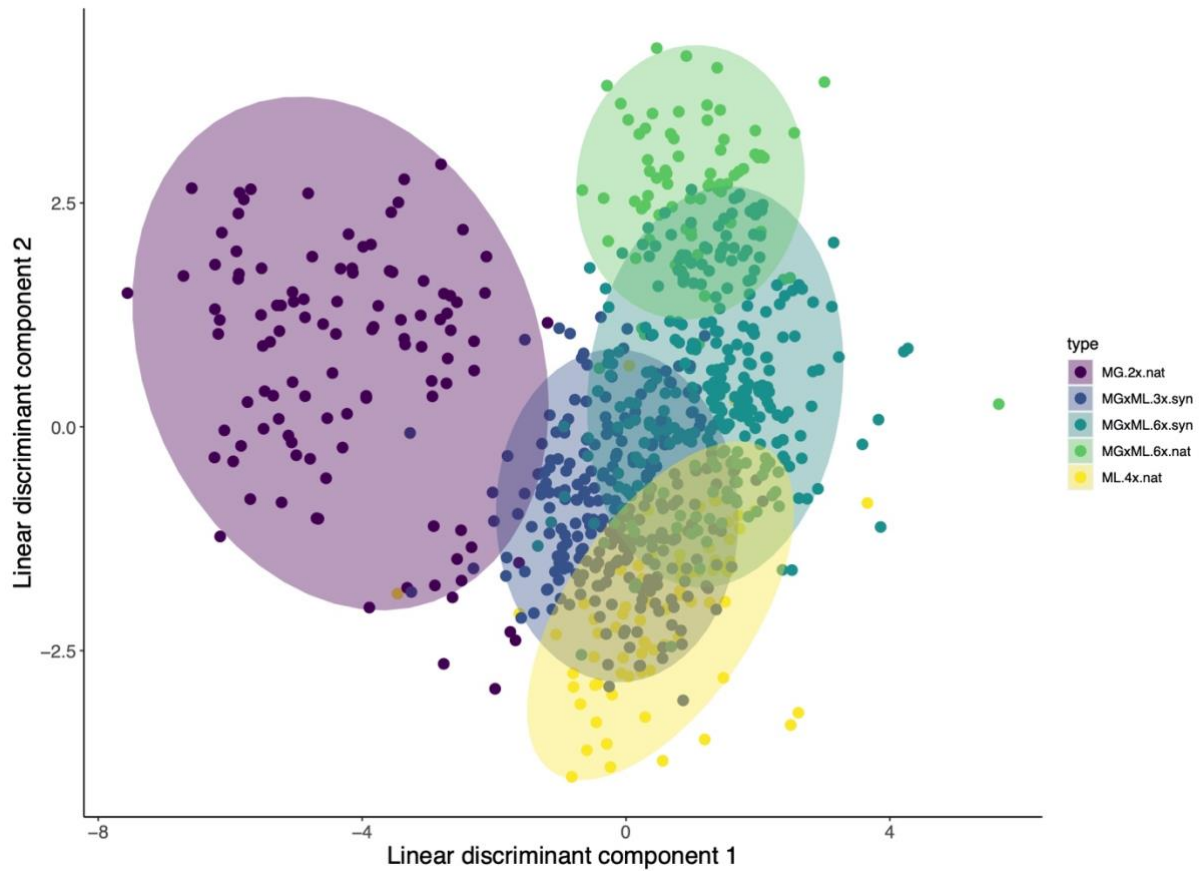
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42 **Table S4.** Number of genotypes and clonal replicates (in parenthesis) used in the field experiment in Leadhills, Scotland (2015-2016) to assess survival of
43 *Mimulus spp.* with different ploidy levels. *Natural* refers to individuals obtained from field populations, while *synthetic* indicates inter-specific hybrid
44 genotypes produced through artificial crossing as well as synthetic polyploids derived from colchicine treatment (see Methods).

Species	2x Natural	3x Natural	3x Synthetic	4x Natural	4x Synthetic	6x Natural	6x Synthetic	8x Synthetic	Total
<i>M. guttatus</i>	34 (87)	-	-	-	15 (29)	-	-	-	49 (116)
<i>M. luteus</i>	-	-	-	27 (71)	-	-	-	5 (15)	32 (86)
<i>M. peregrinus</i>	-	-	-	-	-	73 (212)	56 (160)	-	129 (372)
<i>M. × robertsii</i>	-	19 (57)	69 (197)	-	-	-	-	-	88 (254)
Total									298 (828)

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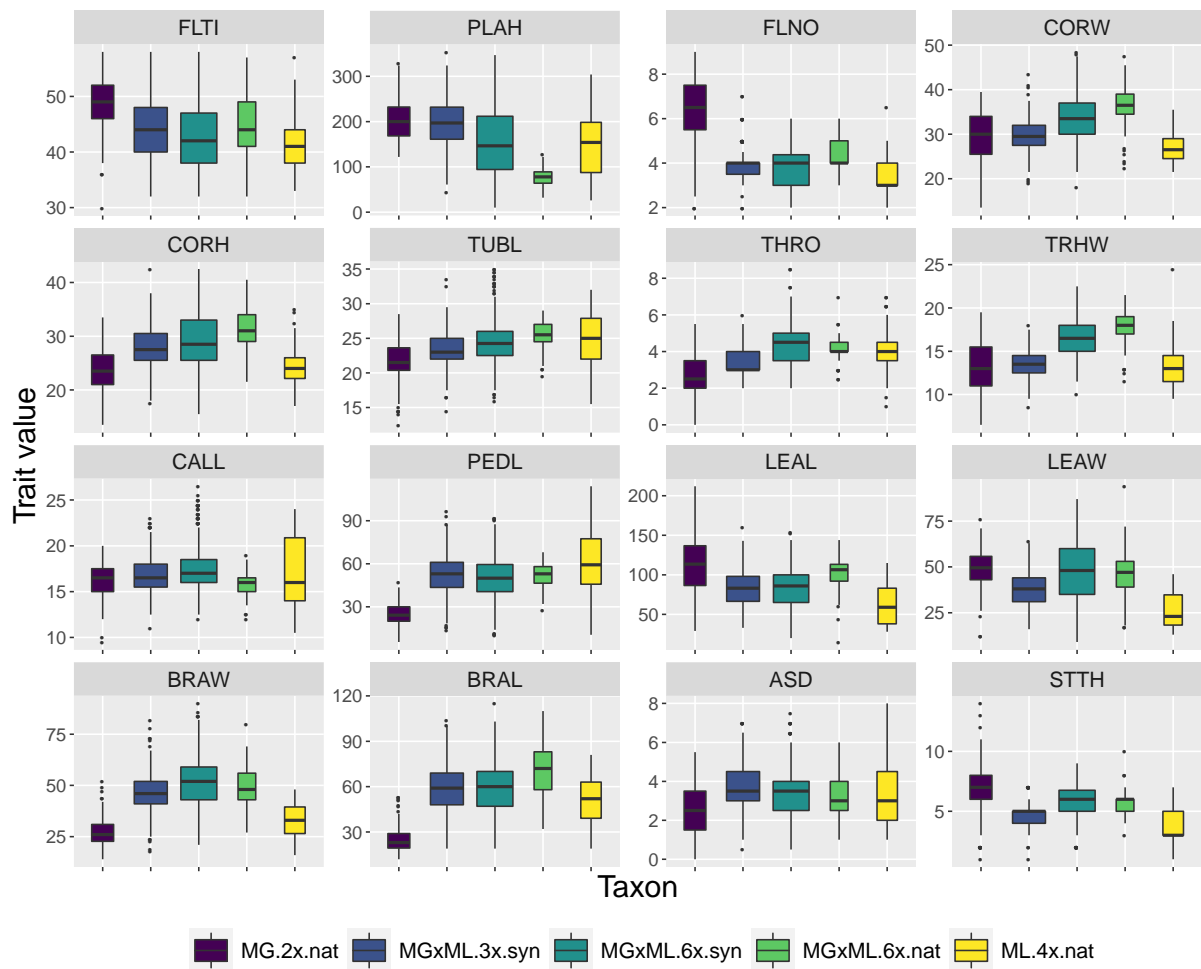
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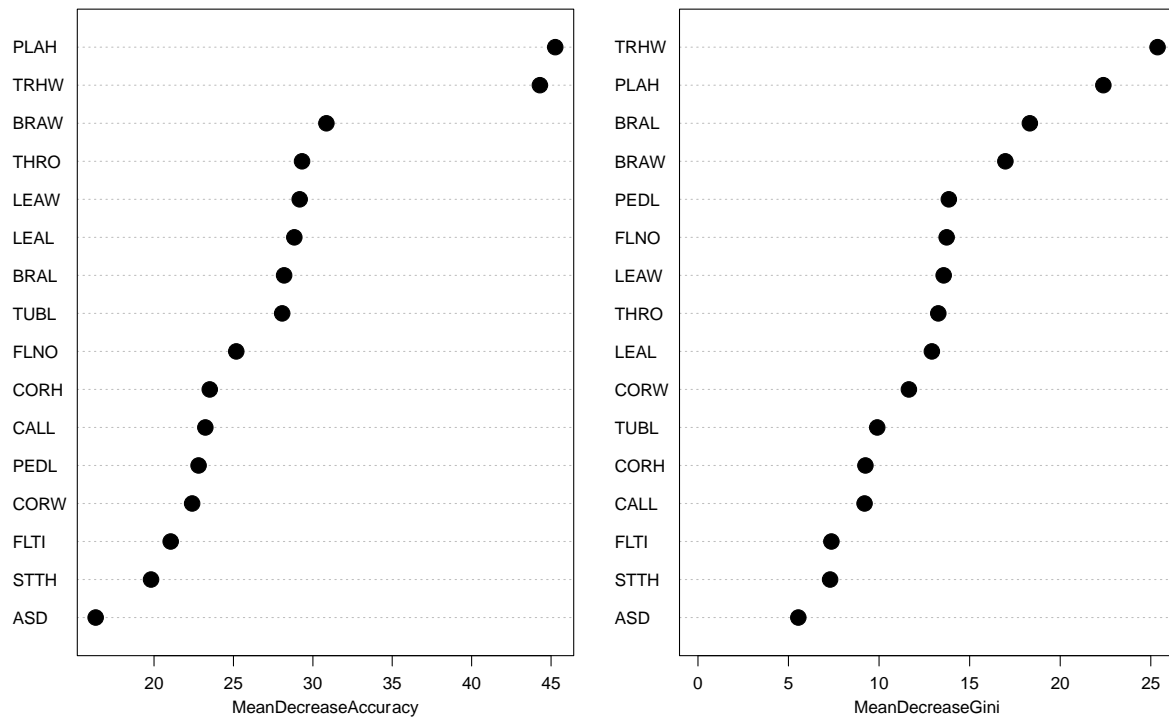
48 **Figure S1.** Linear discriminant analysis of 16 phenotypic traits in monkeyflowers (*Mimulus spp.*)
49 measured in a common garden in the glasshouse. MG.2x.nat = *M. guttatus*, diploid; MGxML.3x.syn =
50 synthetic *M. × robertsii*, triploid; MGxML.6x.syn = synthetic *M. peregrinus*, allohexaploid; ML.4x.nat
51 = *M. luteus*, tetraploid.

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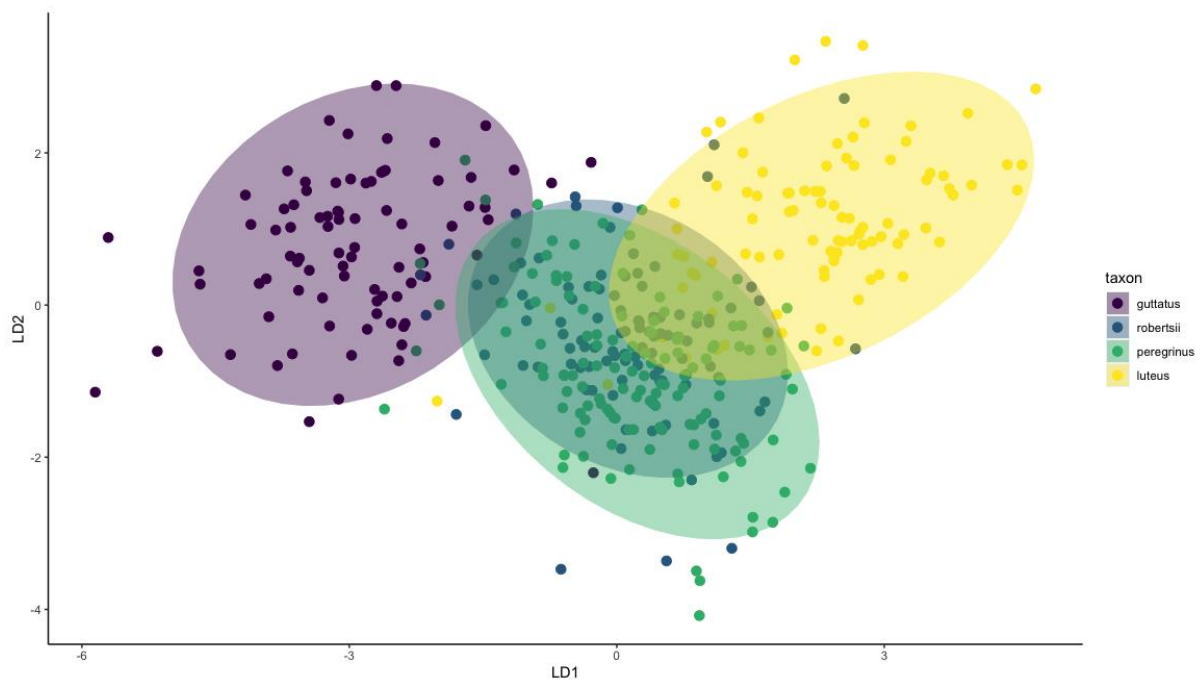
54 **Figure S2.** Comparison of 16 phenotypic traits in five monkeyflowers (*Mimulus spp.*) measured in a
 55 common garden in the glasshouse. MG.2x.nat = *M. guttatus*, diploid; MGxML.3x.syn = synthetic *M. ×*
 56 *robertsii*, triploid; MGxML.6x.syn = synthetic *M. peregrinus*, allohexaploid; MGxML.6x.nat = natural
 57 *M. peregrinus*, allohexaploid; ML.4x.nat = natural *M. luteus*, tetraploid. The traits we measured
 58 were: plant height (PLAH), stem thickness (STTH), leaf width (LEAW) and length (LEAL) of the largest
 59 leaf, bract width (BRAW) and length (BRAL), days to flowering (FLTI), flowering node of first flower
 60 (FLNO) and pedicel length (PEDL), corolla width (CORW), corolla height (CORH), corolla tube length
 61 (TUBL), throat opening (THRO), throat width (TRHW), calyx length (CALL), and anther-stigma
 62 distance (ASD). All units are in mm except FLTI (days) and FLNO (flower number).



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64 **Figure S3.** Variable importance for phenotypic classification as measured by Random Forest analysis
65 in a common garden experiment of four taxa of monkeyflowers grown in the glasshouse. The five
66 taxa included here were: *M. guttatus*, *M. luteus*, synthetically created *M. × robertsii* and *M.*
67 *peregrinus*, and natural *M. peregrinus*.

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70 **Figure S4.** Linear discriminant analysis of 9 phenotypic traits in monkeyflowers (*Mimulus spp.*)

71 measured in a field plot in central Scotland.

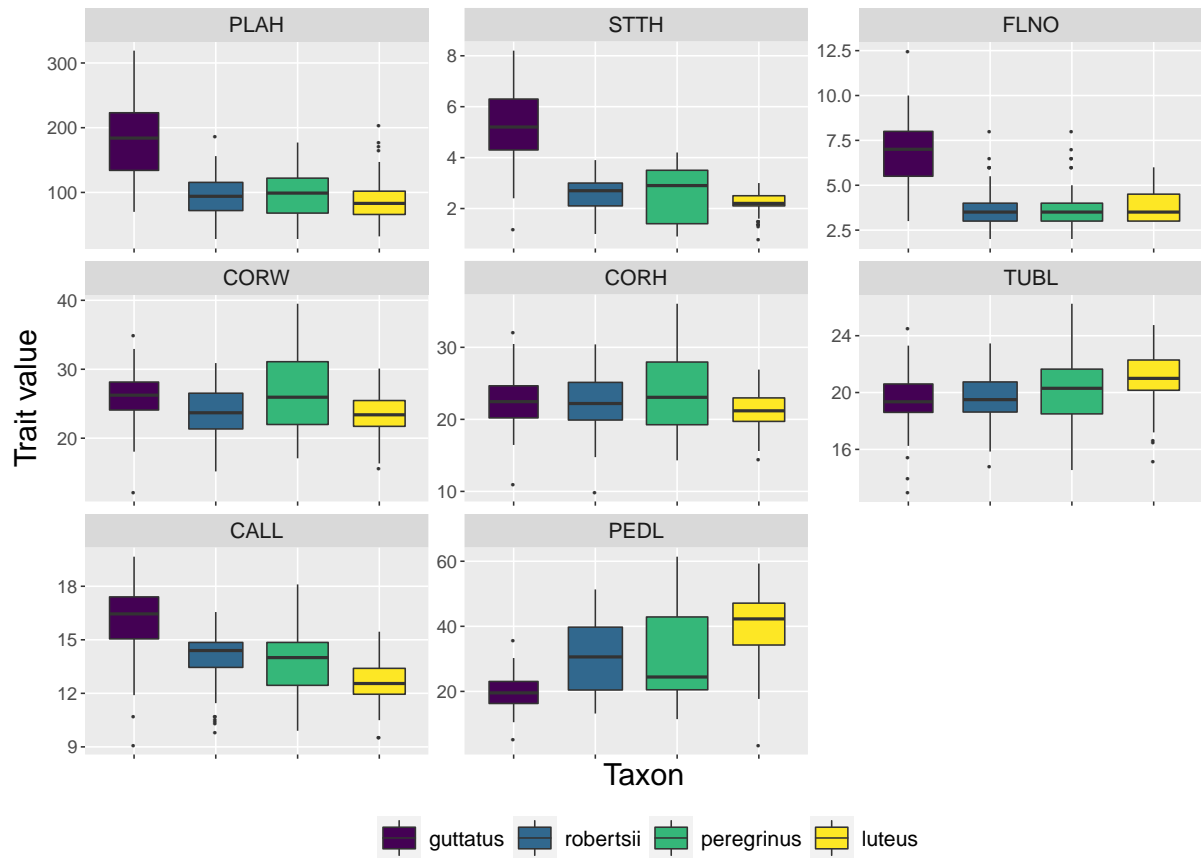


Figure S5. Comparison of eight phenotypic traits among four monkeyflower taxa (*Mimulus spp.*) measured in a field plot in central Scotland. Trait names as in Figure S2. All units are in mm except FLNO (flower number).