Rapid adaptation follows experimental assisted gene flow in subset of annual monkeyflower populations

Authors: Donna M. Hinrichs[1], Courtney M. Patterson[1], Andrea Turcu[1], Stacy D. Holt Jr.[1], Simon G. Innes[1], Nicholas J. Kooyers[1]*

Affiliations:
[1]Department of Biology, University of Louisiana, Lafayette; Lafayette, LA 70503 USA

[2]Corresponding author. Email: Nicholas.kooyers@louisiana.edu, Phone : +1(337)-482-1258, Address : 410 E. St. Mary Blvd.; Billeaud Hall, Rm 108; Lafayette, LA 70503 USA

ORCID
DMH: 0009-0009-9100-1204
CMP: 0000-0002-8304-5276
AT: 0000-0002-1899-4969
SDH: 0000-0001-7684-024X
SGI: 0000-0003-3859-1400
NJK: 0000-0003-3398-7377

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N.J.K. conceived and designed the experiments. DMH, CMP, AT, SDH, SGI, and NJK collected data and performed the experiments. DMH and NJK analyzed the data. DMH and NJK wrote the original draft of the manuscript, and all authors reviewed and edited the manuscript. All authors approved of the final submission.

Data Availability Statement
Datasets containing field fitness data and phenotypic data from the resurrection experiment will be deposited on Dryad. Sequence data will be uploaded to NSBI SRA as .fastq files.

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Abstract

Assisted gene flow, the human-facilitated movement of species within its range, has the potential to increase genetic diversity and facilitate adaptation for climate-threatened populations. However, concerns regarding outbreeding depression and gene swamping have limited the application of assisted gene flow, and experimental tests have been largely confined to simulations and laboratories. We conducted a landscape-scale manipulation, planting seeds and seedlings from historically hotter and drier source populations into climate-threatened populations of the common yellow monkeyflower (*Mimulus guttatus*) and tracked change in genomes, phenotypes, and fitness. Within the first three generations, source alleles introgressed into half of the target populations. Assisted gene flow increased fitness in one experimental block of populations and slightly decreased fitness in a second block. Fitness increases were associated with flowering earlier and producing fewer trichomes. Greater fitness changes occurred in populations where seeds rather than seedlings were introduced. Both the amount of introgression and fitness changes were heterogeneous between years, suggesting that temporal fluctuations in climate likely impact initial introgression and success of assisted gene flow. Initial increases in ancestry from source populations was low across the genome, limiting concerns of gene swamping. Our results are generally consistent with theoretical models, and provide cautious optimism for an often-maligned conservation strategy.

Significance Statement

Scientists must validate potential management strategies for climate-threatened species. Assisted gene flow – human-assisted movement of individuals within a species range – is a controversial strategy that theoretically results in increased fitness in threatened populations. In a landscape-level test of assisted gene flow in an annual monkeyflower, we find evidence of both increased and decreased fitness due to introgression from source populations following assisted gene flow. The amount of genome-wide introgression depended on the type of germplasm introduced and declined over time. The direction of fitness changes was likely based on the differences in ecologically-important phenotypes in source vs. target populations. Our results suggest that assisted gene flow performs largely as predicted from theoretical models and is a viable conservation strategy.

Keywords: Evolutionary Rescue, Genetic Rescue, Introgression, Adaptation, Conservation Genomics, Restoration, Applied Management**,** Assisted Migration, *Mimulus guttatus* (syn. *Erythranthe guttata*)

Main Text

Increasing patterns of maladaptation and declining population sizes indicate that there is a substantial threat of climate change to many organisms (1–5). The potential risk of species extinction (5–9) and loss of key ecosystem services (10–14) suggest that conservation action is necessary with stipulation that actions will not further endanger species. An increasingly popular intervention is assisted gene flow (AGF) - the facilitated movement of individuals within a species range to increase the overall genetic diversity of a target population or facilitate adaptation of a target population to changing conditions (15). Simulation and experimental evolution studies suggest that AGF could be highly successful at increasing the long-term fitness of populations (16, 17), with the caveat that AGF is largely untested in natural populations (18). Based on these potential benefits, assisted gene flow has been conducted in several plant and animal populations that are at risk of extirpation (18–21).

However, AGF is highly controversial within the conservation community due to both perceived risks and lack of experimental data within natural populations. Two common perceived risks are outbreeding depression and gene swamping (22–24). Introducing novel alleles into a population via outcrossing and subsequent recombination could add potentially maladapted alleles into a locally adapted population or break-up co-adaptive gene complexes (25, 25–32). Adding non-local alleles that are much more adaptive than local alleles could cause the loss of local genetic diversity (33, 34). Theoretical models of evolutionary rescue suggest that some maladaptation is likely to occur following introduction of new variation (35–38), but evidence of outbreeding depression is mixed with most research emphasizing that the effects of outbreeding are temporary (30, 39, 40). Instead, simulation studies suggest that the benefits of AGF can be rapid (<10 generations) when fitness traits are controlled by few large-effect loci (41). Landscape-scale tests of AGF in natural populations are necessary to evaluate potential benefits and risks.

Because AGF has historically been reserved for highly threatened species, there has been limited opportunity to dissect the consequences of AGF through controlled experiments (but see 18, 42, 43). The impact of AGF should be proportional to the number of individual introduced within natural populations and the life stage of the introduced organisms (21, 41). For instance, in plant populations, it may be more useful to introduce seedlings rather than seeds, as seedlings do not need to meet local germination requirements. Alternatively, introducing seeds may buffer against extreme years and allow germination within more benign conditions (44). The amount and location of introgression of non-local alleles into target population genomes is key for assessing potential for gene swamping, outbreeding depression, and hybrid vigor. Spatial heterogeneity between different target populations could create variation in the success of AGF (43). Both the level of introgression and intensity of selection could fluctuate between years. Non-local alleles also have the potential to move via gene flow to geographically proximate populations (e.g. transgenes in wild populations; 45). Evaluating the uncertainty and understanding the mechanisms underlying success or failure of AGF provides more confidence (and less legal risk) for undertaking AGF for threatened or endangered species.

Here, we evaluate short-term fitness consequences after conducting a landscape-scale assisted gene flow experiment in climate-threatened subalpine populations of the common yellow monkeyflower, *Mimulus guttatus* (syn. *Erythranthe guttata*). While *M. guttatus* is widespread across western North America and invasive in the U.K. and New Zealand (46, 47), annual populations in the central Oregon Cascades are arguably climate-threatened (44, 48, 49). Annual populations of *M. guttatus* are characterized by highly variable growing seasons that

depend on ephemeral water availability – growing seasons begin with winter/spring rain or snowmelt and end with terminal droughts (50). Annual populations in the central Oregon Cascades exhibit an adaptation lag, with populations from warmer and drier climates with earlier growing seasons (i.e. populations from the foothills of the Sierra Nevada in California) possessing higher fitness than native populations (48, 51). Short heatwaves in the last decade have had severe demographic impacts on these populations (50.4% average mortality in one heatwave), with some populations exhibiting near or complete mortality (44, 49).

We leverage twelve central Oregon Cascade populations with long-term demographic data to conduct our landscape-scale AGF experiment (Fig. 1, Table S1). Populations were clustered geographically into four blocks of three populations with all populations contained within a 25km^2 region. Baseline data collection began in 2018 with yearly surveys of phenotypes and fecundity in each population. The assisted gene flow experiment began in 2021 with the introduction of individuals from a cross between two maternal lines, one from a low elevation population in the Sierra Nevada and one from a high elevation population in the Sierra Nevada. These populations were chosen because they had high relative fitness in a nearby Oregon common garden over multiple years (44, 48, 51). Within each of our experimental blocks, we assigned populations to one of three experimental treatments: control, seeds, or seedlings. In the control populations, we dispersed 200 seeds native to that population. These seeds were pooled from as many different maternal lines as possible from the baseline collections (2018-2020) described above. In the seed treatment populations, we dispersed 200 seeds of the F_1 source individuals uniformly across the population. In the seedling treatment populations, we planted 19-20 seedlings of the F_1 source individuals along a transect extending across the center of target populations. We use field surveys and collections before and after assisted gene flow to evaluate the fitness consequences and underlying genetic and phenotypic mechanisms of AGF.

Fitness consequences of assisted gene flow

Assisted gene flow was associated with modest differences in fitness across the target populations, but the effects differed in direction and magnitude when evaluating different fitness metrics and different life stages of the introduced individuals. Floral abundance increased by 20.4% in populations where CA seeds were introduced, and 8.5% in populations where CA seedlings were introduced, relative to control populations (AGF:treatment, $X^2 = 9.58$, $p = 0.008$; Fig. 2A, Table S2-3). Seed production did not change in target populations relative to control populations after conducting assisted gene flow (AGF:treatment, $X^2 = 3.0$, $p = 0.22$). There were moderate increases in seed production on average across all populations post-AGF (28-43%; Fig. 2B). Introduction of novel alleles may also be expected to increase variation in fitness within target populations. Standard deviation in floral abundance increased by 26.4% in populations where CA seeds were dispersed and by 3.1% in populations where CA seedlings were planted (Fig. S2A). The standard deviation in seed production decreased by 19.3% in populations where CA seeds were dispersed and increased by 43.0% in populations where CA seedlings were planted (Fig. S2B). These results suggest that assisted gene flow is either weakly effective across all populations or only effective in some of the target populations.

Target populations exhibited substantial heterogeneity in their responses to AGF. Assisted gene flow-related increases in flower abundance were primarily driven by two of the four experimental blocks (HOV/HDM/FSM and SMG/RRM/OWC; Fig. 2C, Table S4-5). Conducting AGF via seeds increased floral abundance by 24.8% (HDM) and 62.7% (RRM), and conducting AGF via

seedlings increased floral abundance by 23.7% (FSM) and 39.7% (OWC) (HOV/HDM/FSM: AGF:treatment, $X^2 = 8.4$, $p = 0.014$; SMG/RRM/OWC: AGF:treatment, $X^2 = 66.8$, $p = 3.1 \times 10^{-15}$). Similar trends were observed in these experimental blocks regarding seed production (HOV/HDM/FSM: AGF:treatment, $X^2 = 19.1$, $p = 6.8 \times 10^{-5}$; SMG/RRM/OWC: AGF:treatment, $X^2 = 13.4$, $p = 0.001$; Fig. 2D). However, AGF resulted in decreases in both floral abundance and seed production in a third block relative to control populations (EVS/MTD/BMP; floral abundance: AGF:treatment, $X^2 = 11.2$, $p = 0.004$, seed production: AGF:treatment, $X^2 = 12.5$, $p = 0.002$). Differences in responses across populations likely depends both on stochastic (e.g., gene flow between plants within populations) and deterministic factors (e.g., seed inviability and the magnitude of early season selection on hybrid individuals).

We observed changes in both the magnitude and direction of fitness responses to AGF in the years following introduction (Fig. S3-S4, Table S6-7). There was a statistically significant increase in floral abundance in AGF treatments in the year following AGF implementation (2022: AGF:treatment, $X^2 = 9.9$, $p = 0.007$) but not in 2023. Seed production exhibited similar heterogeneity between years with greater increases in populations receiving AGF in 2022 and increases in control populations in 2023. There were also differences between populations in the degree of temporal heterogeneity in fitness. For instance, introducing CA seeds increased floral abundance in RRM most prominently in 2022, but introducing seedlings into the nearby OWC populations increased floral abundance more prominently in 2023. These results highlight that temporal heterogeneity in selection will have a strong impact on AGF results, but also that some populations exhibit consistent increases in fitness across multiple years.

Genetic evidence for introgression into AGF-targeted populations.

To validate whether the fitness consequences above were consistent with introgression of California alleles into target populations, we grew seeds from each year and population in the UL-Lafayette greenhouse and sequenced whole genomes to an average of 10.9x coverage for 872 individuals. Each population and year combination was represented by 5-27 individuals (Total N = 872; mean = 14.53; SD = 5.2; Table S7). We also sequenced 7 F_1 lines and 20 plants from the low elevation source population. We examined two lines of evidence with this dataset for evidence of introgression: introgression of private alleles from California and whole genome shifts toward California-like ancestry.

We find evidence of introgression of private alleles into two of the four experimental blocks. In the experimental block with the greatest fitness increases after conducting AGF (SMG/RRM/OWC), we observed that AGF increased the number of CA private alleles that were successfully incorporated relative to the control population (treatment: $X^2 = 616.7$, $p = 2.2 \times 10^{-16}$; Fig. 3A, Table S8). Likewise, there is an increase in the number of CA private alleles in the experimental block with the greatest fitness decreases after conducting assisted gene flow (EVS/MTD/BMP: treatment: $X^2 = 76.151$, $p = 2.2 \times 10^{-16}$). However, there is no evidence of introgression from private alleles in the other two experiment blocks – including a block that had increased fitness in AGF treatment relative to control populations. The lack of evidence for genome-wide introgression does not necessarily mean that there were not source alleles introduced, but does indicate that introgression was, at best, rare. In populations with evidence of introgression from source populations, there was no obvious pattern in the distribution of introgression in private alleles across the genome and all private alleles were found at relatively low frequencies in both 2021 and 2022 (Fig. 3B, Fig. S5). Notably, introgression was higher in 2021 than in 2022 and different private alleles introgressed in 2021 and 2022 as only 5.86% of

223 loci have CA alleles present in both years (Table S9). These results confirm that introgression of
224 CA alleles did occur in multiple experimental blocks, and non-local alleles remain at relatively
225 low frequencies in the first years following introduction.
226
227 Introgression of CA alleles should also cause AGF target populations to become more
228 genetically similar to California parents and F_1 lines (Fig. S6). Genetic principal component
229 analysis conducted with 4-fold degenerate sites suggest that there is substantial genetic
230 divergence between source populations (CA parents/F_1) and native Oregon populations,
231 represented by PC1 (48.5% of variation). Only one experimental block (SMG/RRM/OWC) has
232 evidence of a shift toward CA/F_1 plants along PC1 (AGF:treatment, $X^2 = 18.3$, $p = 0.0001$). Both
233 introducing seeds and introducing seedlings were effective in increasing CA ancestry in this
234 block. However, the magnitude of the shift toward CA ancestry is small and all plants are much
235 closer to Oregon ancestry than California ancestry. This suggests that very low genome-wide
236 levels of introgression can create substantial fitness impacts.
237
238 **Phenotype mechanisms underlying AGF fitness responses**
239 Changes in fitness due to the introgression of California alleles suggests that there should be
240 some underlying change in phenotype that links genetic variation to population performance.
241 We conducted a greenhouse resurrection experiment growing maternal lines collected from all
242 years (2018-2022; Table S10) and populations to determine how phenological, morphological,
243 and physiological phenotypes changed in populations that received AGF relative to control
244 populations. Both flowering time and number of trichomes decreased across populations with
245 introduced CA F_1 individuals relative to control populations (flowering time: $X^2 = 6.74$, $p = 0.03$;
246 trichomes: $X^2 = 6.8$, $p = 0.03$; Fig. 4AB, Table S11). AGF-driven phenotypic differences were
247 stronger for populations receiving seeds rather than seedlings. Despite this general pattern,
248 there was heterogeneity in phenotype responses across the four experimental blocks. In the
249 population block with evidence of introgression and fitness increases (SMG/RRM/OWC),
250 flowering time decreased by 6.8 and 3.1 days on average in treatments introducing seeds and
251 seedlings compared to an increase of 0.88 days in the control treatment (Fig. 4C). Trichomes
252 decrease in a similar fashion (Fig. 4D). However, in the experimental block with evidence of
253 introgression and decreased fitness (EVS/MTD/BMP), flowering time increased by 4.3 and 0.8
254 days on average in treatments introducing seeds and seedlings (Fig. 4E). Trichomes were not
255 significantly altered in this block (Fig. 4F). Taken together, these results indicate that the
256 introgression of CA alleles altered flowering time and created variation in fitness following AGF.
257
258 **Discussion**
259 We find that assisted gene flow has the potential to increase fitness of target populations within
260 only a few generations. Theoretical models simulating genetic or evolutionary rescue indicate
261 that introduction of foreign alleles often lead to initial maladaptation due to outbreeding
262 depression followed by increases in fitness as adaptive alleles are selected and maladaptive
263 alleles purged from the population (36, 37). We find two blocks that had evidence of
264 introgression and that also exhibited changes in fitness post AGF. Both target populations in
265 one block with introgression exhibited slight evidence of maladaptation or outbreeding
266 depression relative to control populations in the first three generations, consistent with
267 theoretical expectations (Fig. 2). Surveys over the next decade will determine whether
268 introgression eventually increases population fitness. However, target populations in the other
269 experimental block with introgression had substantial fitness gains within these first generations
270 (Fig. 2). There are multiple non-mutually exclusive hypotheses explaining this rapid fitness

271 increase. One explanation for fitness gains is hybrid vigor or heterosis, where outbreeding leads
272 to increased heterozygosity and an increase in fitness (i.e. flour beetle). However, since allele
273 frequencies associated with the introgression of private alleles are low (Fig. 3), this is unlikely to
274 be the driving mechanism. Also unlikely is a scenario where the influx of genetic diversity
275 rescues the population from inbreeding or high genetic load as target populations have high
276 levels of genetic diversity (52). The most likely possibility is that target populations were poorly
277 adapted to their local conditions and AGF introduced novel genetic variants that shifted
278 phenotype means and allowed transgressive phenotypes or novel phenotypic combinations to
279 emerge. While our greenhouse work establishes post-AGF shifts toward earlier flowering and
280 lower trichomes relative to control populations, field common gardens with manipulations to key
281 climate variables are necessary to dissect the agents and targets of selection underlying fitness
282 gains.
283
284 **Climatic variation impacts introgression and fitness gains associated with AGF**
285 Our results provide key insights into the expectations for assisted gene flow within complex
286 environments. We find variation in the success of assisted gene flow across both space and
287 time (Fig. 2, Fig S4). Our finding that two experimental blocks had evidence of introgression
288 from assisted gene flow while two other blocks did not could be explained by both deterministic
289 and stochastic factors. Lack of introgression could stem from stochastic factors. For instance,
290 relatively few seeds or seedlings were introduced and introgression is dependent on pollination
291 between native and introduced individuals.
292
293 However, success of introgression and associated fitness differences were likely impacted by
294 the climatic differences between populations. While populations within each experimental block
295 were located at the same elevation, experimental blocks were located over an elevation
296 gradient of ~500m – creating substantial differences in growing season timing and both abiotic
297 and biotic conditions during the growing season. The lowest elevation experimental block was
298 the block with evidence of introgression and fitness gains associated with earlier flowering and
299 extended growth. These populations have an earlier growing season start date and a longer
300 growth season than higher elevation populations – conditions that are most similar to the
301 historical conditions with the source California populations (50). Notably, low elevation
302 populations also had more delayed times to flowering than high elevation populations at the
303 beginning of the experiment. An infusion of novel alleles promoting earlier flowering, such as
304 those from the early flowering California source plants, would be most likely to be effective in
305 these populations and could be detrimental in target populations that already flower earlier than
306 the source populations. Conditions for germination of seeds may also have differed between
307 populations and this is likely a key barrier for the success of assisted gene flow (53).
308
309 The difference in introgression and fitness in different years following AGF also suggests that
310 climate abnormalities in the initial years of AGF act as an important filter for AGF success.
311 Patterns of introgression differ substantially between years. Most introgression took place during
312 the year that seeds and seedling were introduced (2021). This should be expected as seeds
313 germinate readily in *M. guttatus* and we planted a number of seedlings that were ready to flower
314 (Fig. S1). However, there was also a substantial heat dome event during the 2021 growing
315 season (54) that had devastating consequences on many target populations (49) and prevented
316 many of the source seedlings from producing seed (Table S12). We also expected evidence of
317 introgression in subsequent years from seeds that did not germinate the first year and from
318 seeds which were generated as hybrids between source and target populations. There was far
319 more limited evidence of introgression of private alleles relative to control populations in 2022

(Fig. 3). It is notable that we judge levels of introgression relative to control populations as the likely pollinators (bees) are capable of flying between control and treatment populations within an experimental block (1-2km; 55). Indeed, alleles that we inferred as private to source populations appear in every control population (Fig. 3). This is primarily due to our inference of private alleles as we likely did not detect some rare alleles in Oregon in the sequencing of 2018-20 Oregon populations. However, tracking source population alleles that escape treatment populations is important for determining off-target impacts of AGF (56).

Limited introgression of private alleles genome-wide does not exclude the possibility that there may be introgression in key genomic regions that could have impacted phenotypes. On the contrary, some populations with relatively limited introgression of private alleles in 2022 had strongly increased floral abundance relative to control populations in the same year (e.g. RRM). Future studies scanning the genome for allele frequency change will detect the key regions underlying shifts in fitness. However, temporal variance in relative fitness in AGF populations relative to control populations likely indicates that temporal variation in climate plays a key role in the outcome of AGF. High elevation *M. guttatus* populations in the central Oregon Cascades are known for strong fluctuating selection maintaining high genetic and phenotypic diversity within populations (52, 57–59). Notably, one of the key phenotypes undergoing fluctuating selection is flowering time with year-to-year variation driven by differences in growing season start date and duration (58). An important consideration for AGF is that introduced alleles may be adaptive in some years but not in others. For instance, we worry that introduced alleles that increase in frequency during the relatively benign years (i.e. 2022 growing season) may be maladaptive and cause populations to be less resilient during future heatwaves. Moreover, strong fluctuations during the first years after AGF could either enhance or prevent source population alleles from incorporating into target populations depending on whether the fluctuation makes the environment more similar to the historic source environment. The relative impacts of fluctuating selection during AGF require additional theoretical work.

Assessing key choices for implementing AGF

Our results suggest that the logistics of assisted gene flow are key to the end result. Current recommendations suggest introducing 5-20% of the target population census size from source populations (21, 41). Our experiment introduced << 5% of the census size regardless of whether we introduce seeds or seedlings. Population census sizes can differ dramatically between years, with limited individuals flowering during years with early growing season start dates and abnormally hot conditions (44, 49). This decision likely limited the initial amount of introgression in target populations. Notably, even with a limited number of individuals introduced, we observed both introgression and fitness impacts within half of our target populations. Despite this success, we suggest introducing higher numbers of individuals to ensure more rapid incorporation into the genome (i.e., 10-20% of census size).

Another key choice is deciding how to introduce source populations. Our experiment examines a single introduction of seeds vs. seedlings. In experimental blocks with clear evidence of introgression, both seed and seedling treatments had effects, but the introduction of seeds increased introgression and led to larger magnitude fitness effects. The introduction of seeds was likely more effective for three reasons: first, we introduced more seeds than planted seedlings; second, 77% of seedlings died following introduction due to a substantial heatwave and the rest produced limited fruits (1.17 ± 0.99 SD fruits); third, seeds may not have germinated within the first year and could have provided a reservoir capable of introducing alleles in multiple growing seasons. Thus, we do not observe the potential downside of

introducing seeds – that they must overcome seed dormancy requirements and novel establishment conditions associated with non-native sites (53). Our results suggest that single introductions are less likely to be effective than multiple introductions and that introducing seeds may have longer lasting effects in unpredictable climates.

Our experiment also included a non-standard choice for applied assisted gene flow efforts. We introduced germplasm resulting from a cross of two maternal lines sourced from two populations. Our choice was justified by the success of these two lines and populations in a nearby common garden experiment (44, 48), evidence of inbreeding depression in nearby natural populations (60), and the need for a genetically-tractable system. Yet, most species with the need for AGF will not have the large history of evolutionary ecology and genetics studies that our study system possesses. Source populations for AGF are typically selected via climate transfer zones or seedlot selection tools based on historical climates and contemporary conditions (61). Experimental and simulation studies are needed to better assess how to choose source populations – phenotypic and genomic data could provide rapid assessment of potential source populations that could complement or even replace climate data. In the absence of data, including both native and climate-adapted germplasm in introductions either via seed mixes or through hybridization of populations can allow natural selection to incorporate ecologically-important variants.

Conclusions

Our study provides cautious support for utilizing assisted gene flow to preserve natural populations experiencing the detrimental effects of climate change, consistent with the growing recommendations for conducting AGF in a variety of maladapted populations (16, 17, 62, 63). We find evidence of the beginning stages of evolutionary rescue despite relatively limited genome-wide patterns of introgression, limiting potential concerns regarding outbreeding depression or gene swamping. However, attention to organismal biology of the threatened species and logistical details is clearly key to the success or failure of AGF and we encourage responsible evidence-based decision making.

Materials and Methods

We conducted a landscape-scale manipulative experiment involving 12 target populations within a 25km^2 region of the Central Oregon Cascade Mountains. Populations were geographically clustered into four groups of three populations ('blocks'). Elevations were similar within blocks but varied between blocks (1087-1567 m; Table S1). We identified two source populations from the foothills of the Sierra Nevada in central California (Fig. 1; Table S1). These populations were chosen because they have higher fitness than the native populations in a nearby common garden (48, 64). A single maternal line from two source populations (BEL, SHL) were crossed together in bulk to produce the F_1 seeds used for AGF. In each block, populations were designated as control, seed or seedling treatment. In the control treatment populations, 200 seeds previously collected from the native population were uniformly sown throughout the population (20+ maternal lines). In the seed treatment populations, we dispersed 200 F_1 source seeds uniformly across the population. In the seedling treatment populations, we planted 19-20 14-day old seedlings grown from the F_1 seeds along a transect extending across the center of target populations. For seedling growth conditions prior to planting, see extended methods. We recorded survival of translocated seedlings weekly, and counted number of fruits produced after senescence in order to assess the potential for introgression of new alleles in these populations; only 23% of seedlings survived to flower, and those individuals that produced an average of 1.17 ± 0.99 SD fruits (Table S11).

418
419 *Evaluating AGF impacts on fitness within natural populations*
420 We assessed fitness at the end of the growing season in each population in each year from
421 2018-2023 (previously reported in (44, 49). Plants were collected along two 7.5 m transects
422 spanning the population. From 2018-2020, one individual was collected every 15 cm along each
423 transect, totaling 100 individuals/population. From 2021-2023, one individual was collected
424 every 30 cm along each transect, totaling 50 individuals/population. We counted all flowers and
425 seeds for each individual as proxies of fitness.
426
427 We evaluated whether AGF increased fecundity of the target populations within a general linear
428 mixed model framework (LMM). We conducted univariate LMM to assess the effects of AGF
429 (pre/post), Introduction treatment (control/seeds/seedlings), and the interaction between
430 AGF:Introduction on both metrics of fitness (flower abundance and seed production). Year and
431 population were random effects in each model. Floral abundance and seed production were
432 log+1-transformed to better fit model assumptions. To determine whether assisted gene flow
433 increases the variance in fitness, we conduced similar univariate LMMs using the standard
434 deviation of floral abundance and seed production between individuals within a population as
435 response variables. Standard deviation of flower abundance was log+1-transformed, but
436 standard deviation of seed production was not log+1-transformed. Analyses were performed
437 using the *lme4* v 1.1-35.5 package in R v 4.4.1 (65). Statistical significance of LMM was
438 assessed via ANOVA with type III sum of squares using the Anova() function in the *car* v 3.1-2
439 package (66). We note that interpretation of models differed for each fitness variable as flower
440 abundances could logistically not be affected by AGF in 2021 as plants sampled along transects
441 were almost certainly native Oregon plants; thus, floral abundance in 2021 is considered pre-
442 AGF in models. However, seed production could have been impacted in 2021 through pollen
443 transfer from introduced CA seeds/seedlings and can be considered post-AGF in models.
444
445 To better dissect the impacts of AGF in the experimental manipulation, we evaluated variation
446 among population blocks and between post-AGF years. We examined differences among
447 different experimental blocks by subsetting the data into the four blocks and repeating the above
448 univariate LMMs for each block. Because each treatment was only represented by one
449 population using this method, population was removed as a random variable for these models.
450 We assessed heterogeneity in AGF-related fitness variation across post-AGF years by
451 subsetting data into each post-AGF year and retaining all baseline years. In these models, year
452 was removed as a random variable.
453
454 *Validating AGF introgression via genomic analysis.*
455 We germinated seeds from 24-30 maternal lines/population ~2 months after collection each
456 year in the UL-Lafayette Greenhouse (*Refresher Generation Growth Conditions* in extended
457 methods). We also included 7 individuals from one parental population (BEL) and 20 F1
458 individuals grown in the 2021 common garden. Seeds from the other parent line (SHL) did not
459 germinate. One individual from each maternal line had buds or leaves collected for genetics and
460 this individual was selfed for use in the resurrection experiment described below. DNA was
461 extracted using a modified CTAB extraction (67) and diluted to 3 ng/μL for library construction.
462 Libraries for whole genome sequencing were constructed using a customized dual-index
463 Nextera library construction protocol (68). Each population and year combination was
464 represented by 5-27 individuals (Total N = 872; mean = 14.53; SD = 5.2; Table S7). Poor fitness
465 in the field that limited collections as well as reduced germination led to the reduced sample size
466 of some populations in some years. Post-barcoding, we pooled either 48 or 96 individual

467 equimolar libraries and sequenced each pool on a single lane on either a HiSeqX or NovoseqX
468 Plus platforms (150bp paired end reads; Novogene, Davis, CA). Batch effects due to either
469 sequencing platform or lane were minimal.
470
471 We constructed a custom bioinformatics pipeline for our population genomics study
472 (https://github.com/donnasaurrus/AGF). Initial read quality was evaluated using fastQC (69).
473 High-quality reads were aligned a *Mimulus guttatus* reference genome from a nearby population
474 (IM62 v3: https://phytozome-next.jgi.doe.gov/info/Mguttatusvar_IM62_v3_1) utilizing bwa-mem
475 with default parameters (70) and converted to bam files using SAMtools (71). Read groups and
476 duplicates were marked using PicardTools, and SNPs were called using bcftools (72). We
477 filtered for variant quality, strand bias, and mapping quality using GATK [QD < 2.0 || FS > 60.0 ||
478 MQ < 40.0 || MQRankSum < -12.5] (73). We used VCFtools (74) to filter retaining biallelic SNPs
479 with minimum quality score of 500, and removing any SNPs with >25% missing data. This SNP
480 dataset included 18,118,076 SNPs. Mean coverage and error rates of our sequence data (.bam
481 files) were assessed using Qualimap (75, 76). Mean coverage depth was 10.9x (SD = 7.7), and
482 mean error rate was 2.7% (SD = 0.39%) (Table S13).
483
484 We first evaluated introgression via AGF into Oregon populations using private alleles. We
485 filtered the SNP dataset to only include sites where both reference and alternative alleles were
486 at a frequency > 0.3 in BEL and F1 individuals *and* were monomorphic in all populations in
487 Oregon prior to conducting AGF (2018-2020). We note that these alleles may not necessarily be
488 truly private as some rare alleles in Oregon populations may escape detection in the three
489 baseline years, either due to the presence of a seed bank or a finite sample size. This dataset
490 consisted of 15,691 SNPs distributed genome-wide. We evaluated whether there was
491 introgression in target populations that received AGF from source populations compared to
492 control populations. We transformed allele frequencies into a binomial variable, where loci with
493 a CA private allele frequency of 0 were coded as 0, and any non-zero allele frequencies were
494 coded as 1. We conducted a generalized linear mixed model with a binomial distribution and a
495 logit link function using the *glmmTMB* v 1.1.9 package in R v 4.4.1 (77), with treatment (levels:
496 Control, Seed, Seedling) included as a fixed factor, and with year and population included as
497 random variables. To assess differential responses between blocks, we repeated this model,
498 subsetting data into individual experimental blocks and removing population as a random
499 variable. Statistical significance was assessed as in above GLMs for fitness.
500
501 We evaluated the extent that introgression shifted genome-wide genetic diversity toward
502 California source populations using a principal component analysis that included Oregon
503 populations from all years as well as individuals from California parental and F_1 lines. We filtered
504 SNPs to include only 4-fold degenerate sites and only used individuals with < 20% missing data
505 (N = 803). This dataset included 1,056,971 SNPs. A PCA was conducted using --pca in *plink*
506 v1.9 (78). PC1 separated Oregon from California populations (Fig. S6). We examined shifts in
507 PC1 toward CA populations following AGF using univariate GLMs identical those used above
508 for fitness except with PC1 and PC2 as response variables. In these models, AGF (pre/post),
509 introduction treatment (control/seeds/seedlings), and the interaction between AGF:Introduction
510 were fixed effects while population and year were included as random effects.
511
512 *Identifying phenotypic shifts following AGF*
513 We conducted a greenhouse resurrection experiment to examine how phenotypes shift
514 following AGF. We germinated seed from 988 maternal lines stemming from every population
515 and year from 2018 - 2022 (mean ± SD: 17.0 ± 6.1 lines per population per year, Table S9).

These seeds were selfed lines from the refresher generation(s) described above. Germination and growth conditions are described in extended methods (*Resurrection Experiment Growth Conditions*). We surveyed for germination and flowering daily and calculated flowering time as the number of days between germination and opening of the first flower. At flowering, we identified the node of the first flower, counted the number of leaves and branches, and measured plant height, corolla width, corolla length, corolla height, and stem diameter. We also collected the larger single 2nd true leaf to measure leaf area, specific leaf area, number of trichomes, and relative water content. Further details in phenotypes can be found in extended methods. There were strong correlations among several of the phenotypes (Table S14) so we choose to only include a subset of phenotypes in analyses including flowering time, plant height, leaf number, corolla width and number of trichomes.

We used univariate GLMMs to evaluate potential shifts in phenotypes following AGF treatment. As in above GLMMs, AGF (pre/post), introduction treatment (control/seeds/seedlings), and the interaction between AGF:Introduction were fixed effects while population and year were included as random effects. Distributions, links, and transformations were chosen to maximize model fit. The GLMM for flowering time had a Poisson distribution with a log link. All other variables were log-transformed and had Gaussian distributions with identity links. Statistical significance of fixed factors was assessed via ANOVA with type III sum of squares using the Anova() function in the *car* v3.1-2 R package (66). Changes in phenotype due to AGF would create significant AGF:Introduction interactions with shifts likely toward CA phenotypic means. To better examine how phenotypic change was occurring within experimental blocks, we subset the dataset by block and ran identical models for each block without population as a random factor.

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Figures

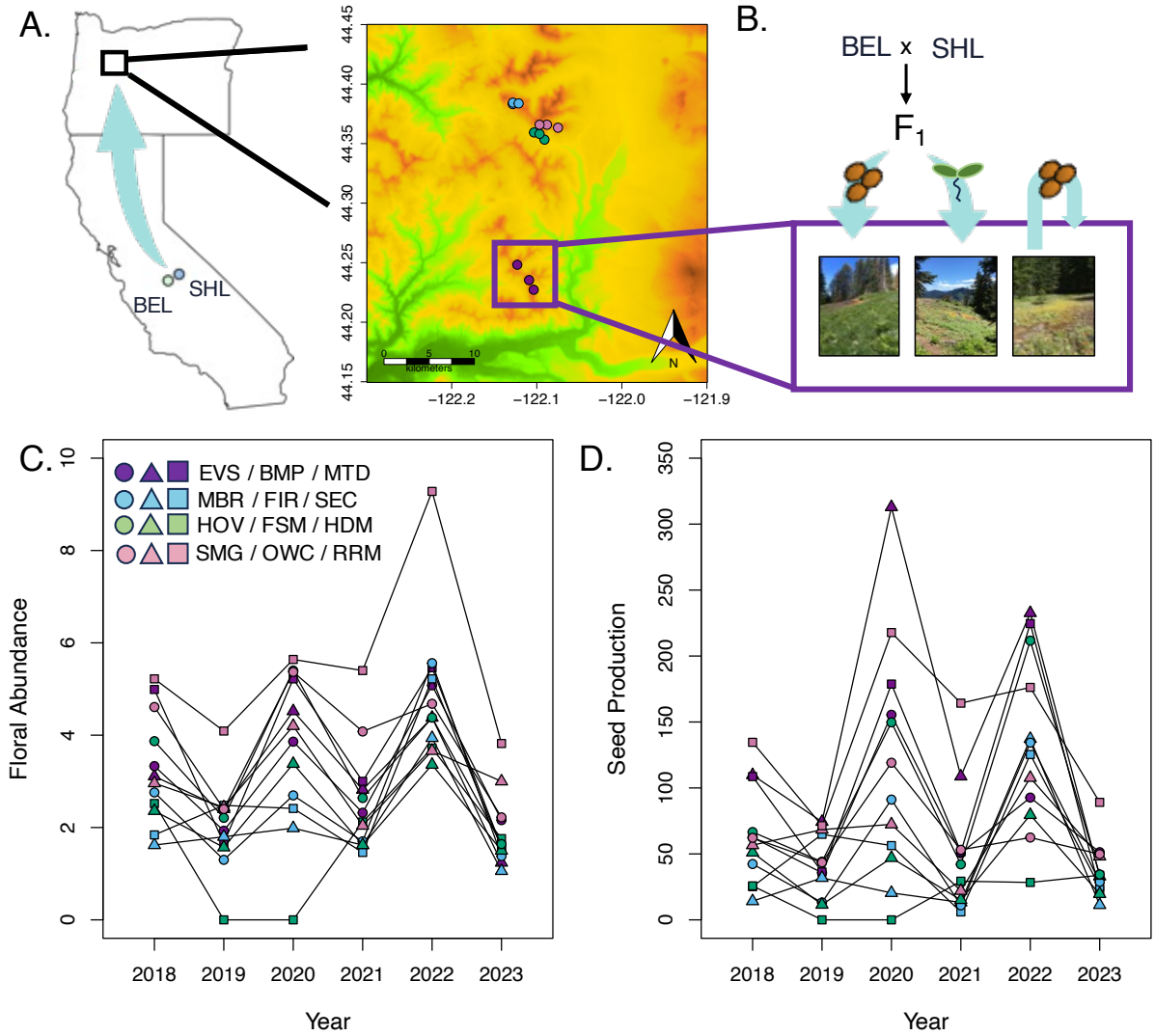

Fig 1: Experimental design and variation in fitness from 2018-2023 across 12 central Oregon Populations. **A.** Map of study area and locations of source populations (BEL and SHL) and target populations for assisted gene flow. Raster depicts elevation with warmer value indicative of higher elevations. **B.** Experimental design for assisted gene flow manipulation into one of the four experimental blocks. Each block consists of three populations, one that receives seed from a cross between source populations (F_1 seed), one that receives seedlings grown from the same cross, and a third that receives seed from the native population. **C-D.** Variation in floral abundance and seed production, respectively, across populations and years included in the experiment. Colors represent different experiment blocks and shapes represent treatment (local seeds, source seeds, source seedlings). Assisted gene flow occurred in 2021.

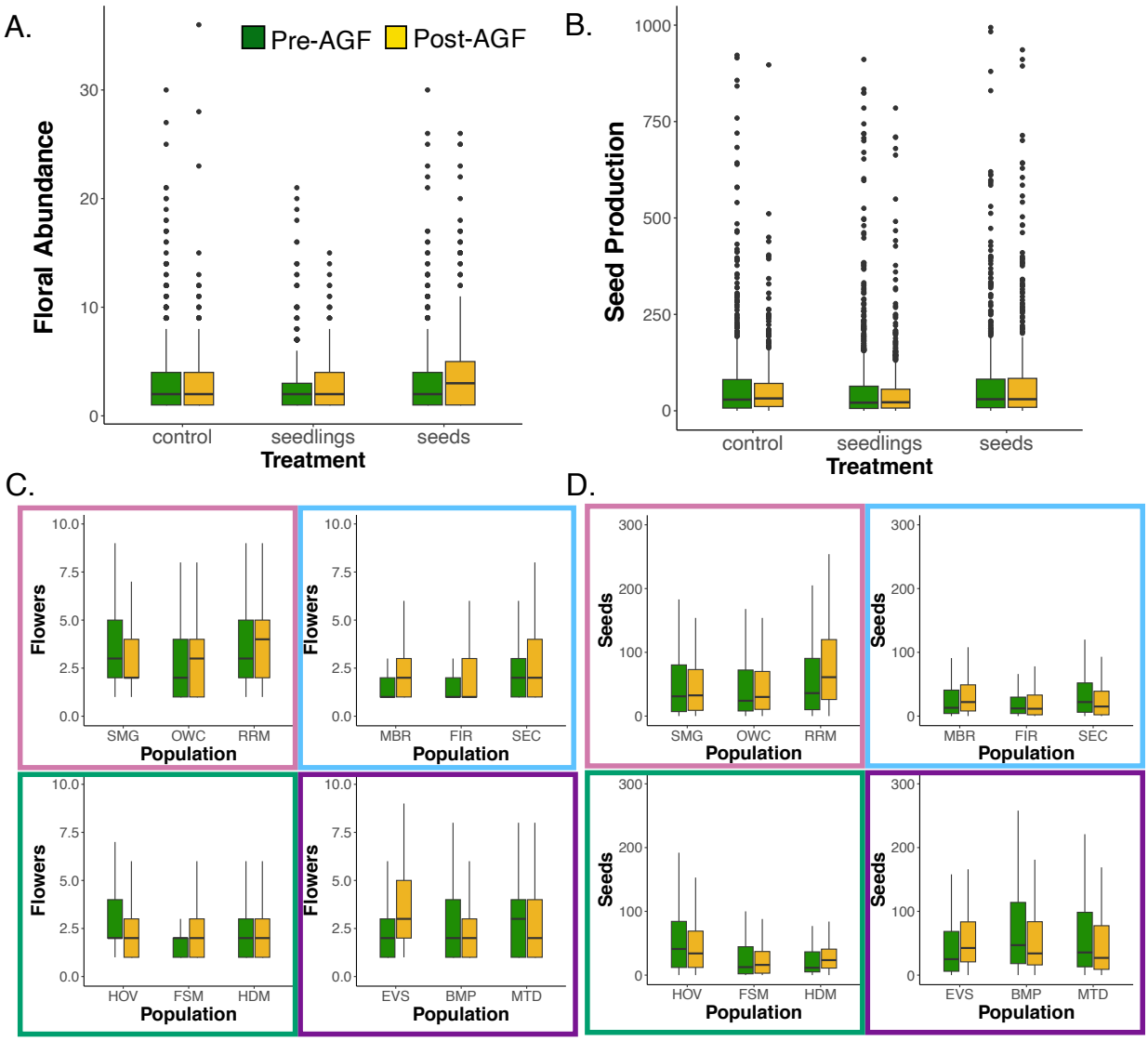

Fig. 2: Assessing changes in fitness following assisted gene flow. **A-B**. Boxplots depict changes in floral abundance and seed production following assisted gene flow across treatments: control (native seed introduced), source seeds introduced (CA F_1), or source seedling introduced (CA F_1). Each point is an individual. Pre-AGF (green boxes) includes 2018-2021 growing seasons for floral abundance and 2018-2020 growing seasons for seed production. **C-D.** Changes in floral abundance and seed production parsed into the four experimental blocks. In each subpanel, left, center and right populations correspond to control, seedlings and seed population treatments, respectively. Border colors on the subpanels correspond to the location of the experimental block on the map in Fig. 1. Edges of boxes in boxplots correspond to the interquartile range, the line inside boxes to medians and the whiskers extend to the most extreme points within 1.5 × IQR from box hinges.

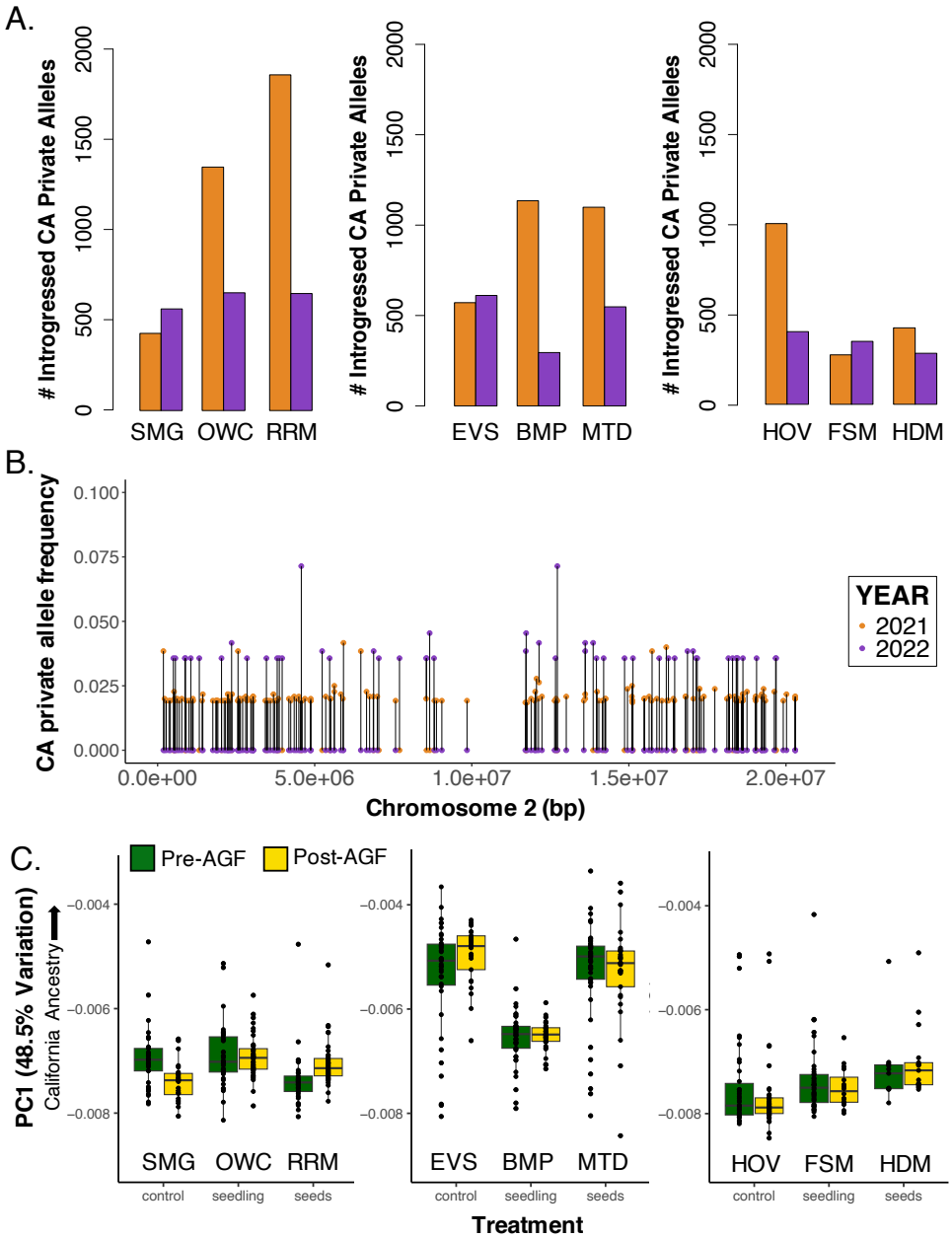

Fig. 3 Patterns of introgression into target populations following assisted gene flow. **A**. Number of loci with evidence of introgression of CA private alleles for three experimental blocks. In each subpanel, left, center and right populations correspond to control, seedling and seed population treatments, respectively. Total number of potential loci with California private alleles was 15,691. **B**. Allele frequencies of California private alleles across a representative chromosome (chromosome 2 in the RRM population – a population with source seeds introduced). Allele frequencies may be higher in 2022 because there was greater sample size (27 vs. 14) in 2021. **C.** Variation in genetic principal component 1 before and after assisted gene flow within three experimental blocks. California populations are distinct from Oregon populations with greater values of PC1 (Fig. S6). Edges of boxes in boxplots correspond to the interquartile range, the line inside boxes to medians and the whiskers extend to the most extreme points within 1.5 × IQR from box hinges.

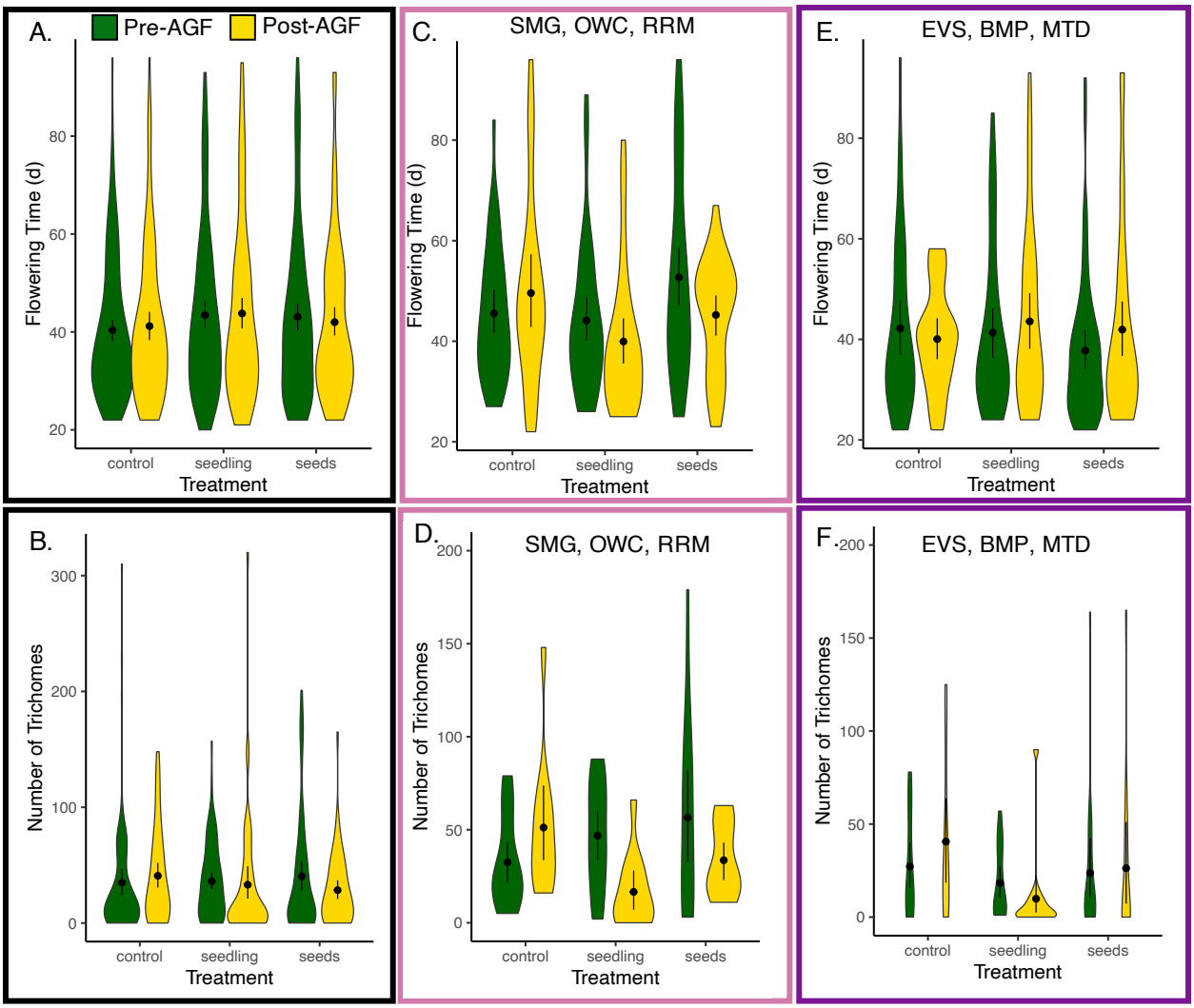

Fig. 4 Shifts in flowering time and number of trichomes accompany assisted gene flow. A-B. Violin plots depict variation in flowering time and number of trichomes before (green) and after (gold) assisted gene flow. Four populations are pooled within each treatment. **C-F.** Variation in flowering time and number of trichomes from the two experimental blocks with genomic evidence of introgression and fitness impacts of AGF. Fitness was elevated following AFG in the SMG, OWC, RRM block, but lower in the EVS, BMP, MTD block (Fig. 2). Border color of panels corresponds to experiment block color in Fig.1. Points and whiskers inside violin plots correspond to mean and standard error, respectively.

Extended Data

Extended Methods

Fig. S1: Seedling transplant into a central Oregon Population (FIR) at different developmental stages.

Fig. S2: Changes in variation in fitness following assisted gene flow.

Fig. S3: Variation in floral abundance and seed production across six growing seasons and twelve populations

Fig. S4: Relative changes in floral abundance and seed production across years following assisted gene flow.

Fig. S5: Limited introgression of California private alleles within target populations.

Fig. S6: Populations structure across source and target monkeyflower populations.

Fig. S7: Variation between pre- and post-assisted gene flow in flowering time within a common garden by experimental block

Fig. S8: Variation between pre- and post-assisted gene flow in number of trichomes within a common garden by experimental block.

Supplemental Tables

Table S1: Population geographic locations and climatic data

Table S2: Model summaries of fitness effects of Assisted Gene Flow within Oregon populations

Table S3: Estimated marginal means and raw means for of fitness effects of assisted gene flow across Oregon populations

Table S4: ANOVA summary of fitness effects of assisted gene flow within each block

Table S5: Estimated marginal means and raw means for of fitness effects of assisted gene flow within individual blocks

Table S6: ANOVA summary of fitness effects in individual years following assisted gene flow

Table S7: Sampling summary for genomic analysis

Table S8: Generalized linear model summaries for private allele analysis

Table S9: Counts of private alleles across target Oregon populations and years

Table S10: Sampling summary from the resurrection experiment

Table S11: Generalized linear modeling results from the resurrection experiment

Table S12: Source seedling performance in target Oregon populations

Table S13: Population summary of coverage and mean depth for genomic analysis

Table S14: Phenotypic correlations in the resurrection experiment