Maintaining their genetic distance; limited gene flow between widely hybridising species of *Geum* with contrasting mating systems

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Abstract

Mating system transition from outcrossing to selfing frequently gives rise to sister lineages with contrasting outcrossing rates. The evolutionary fate of such lineages depends on the extent to which they exchange genes. We measured gene flow between outcrossing *Geum rivale* and selfing *G. urbanum*, two sister species derived by mating system transition, which frequently hybridise. A draft genome was generated for *G. urbanum* and used to develop dd-RAD data scorable in both species. Coalescent analysis of RAD data from allopatric populations indicated that the two species diverged 2-3 Mya, and that long term gene flow between them has been very low (M=0.04). *G. rivale* showed greater genetic diversity in sympatry than allopatry, but genetic divergence between species was no lower in sympatry than allopatry, providing little evidence for recent introgression. Clustering of genotypes revealed that, apart from four early generation hybrids, individuals in sympatric populations fell into two genetically distinct groups with <1% admixture that corresponded exactly to their morphological species classification. Although our data suggest limited gene flow, we observed joint segregation of two putatively introgressed SNPs in *G. urbanum* populations that was associated with significant morphological variation; this provides tentative evidence for rare introduction of novel genetic diversity by interspecific gene flow. Our results indicate that despite frequent hybridisation, genetic exchange between *G. rivale* and *G. urbanum* has been very limited throughout their evolutionary history.

Introduction

A key factor influencing the evolutionary trajectory of lineages is their level of gene exchange with related taxa (Abbott *et al.* 2016). A reduction in gene flow facilitates speciation (Coyne & Orr 2004). Limited gene flow from a related taxon may increase the genetic variability of a species and enable acquisition of novel traits that enhance its evolutionary potential, while maintaining its distinctness (Paoletti *et al.* 2006). However, if gene flow is more extensive, species may fuse (Carney *et al.* 2000). Thus, quantifying gene exchange among lineages is crucial for understanding the birth, continued evolution and possible extinction of species.

Quantifying gene flow between lineages is particularly valuable across lineage pairs that share an evolutionary transition, allowing patterns of genetic exchange associated with that evolutionary transition to be recognised. In many plant genera speciation is associated with a mating system transition from outcrossing to selfing (Stebbins 1957; Igic *et al.* 2006; Wright *et al.* 2013; Barrett *et al.* 2014). Although sister species with contrasting mating systems are typically ecologically distinct, hybridisation often occurs between them where habitat isolation breaks down or the ranges of the two taxa meet (e.g. *Mimulus* (Vickery, 1978; Brandvain *et al.* 2014; Kenny & Sweigart 2016), *Rhinanthus* (Ducarme *et al.* 2010), *Centaurium* (Brys *et al.* 2013)).

The transition from outcrossing to selfing affects genome evolution per se (Wright *et al.* 2008) and is commonly accompanied by changes in floral display (Sicard & Lenhard 2011). Thus the transition can potentially lead both to post-zygotic barriers to interspecific gene flow, due to interactions between differently evolved outcrossed and selfed genomes, and to pre-zygotic barriers, through its effect on mating opportunities (Fishman & Wyatt, 1999; Hu, 2015). Quantitative measurement of introgression between sister selfing and outcrossing taxa is needed to determine the strength of these effects, and to ascertain whether they prevent integration of novel genetic material into either species. Introgression would be particularly important for highly inbreeding taxa whose adaptive

42 potential may be impaired by low genetic variability, and a reduced efficacy of positive and
43 background selection (Charlesworth *et al.* 1993; Glémin & Ronfort 2013; Arunkumar *et al.* 2015).

44 Here we quantify gene flow between *Geum rivale* and *G. urbanum*, sister species that differ in mating
45 system and associated floral display (Fig. 1). *Geum rivale*, which typically occupies open, moist
46 habitats, is predominantly outcrossed (outcrossing rate t = 0.8) and possesses a pendulous flower
47 adapted to bee pollination. In contrast, *G. urbanum*, a plant of shaded, well drained sites, is
48 predominantly self-fertilised (t = 0.15) and bears erect flowers adapted to fly pollination (Taylor
49 1997a, b; Ruhsam *et al.* 2010).

50 Allopatric populations of both species occur: *G. rivale* in tall herb montane communities in the UK,
51 and at high latitude sites elsewhere in Europe; *G. urbanum* in the extreme south east of the UK,
52 where rainfall is low, and at the southern extremes of its distribution in continental Europe (Taylor,
53 1997a, b). However, over most of their distributions in the UK and continental Europe the taxa are
54 sympatric. Here they can be found as single species populations in "pure" habitats or together in
55 "mixed" habitats. Typical mixed habitats include naturally wet woodlands and artificially disturbed
56 river banks (Waldren *et al.* 1989).

57 In mixed habitats the highly fertile F1 hybrid *G. x intermedium* is widely reported, associated with
58 hybrid swarms (Taylor 1997a). Detailed analysis of a single hybrid swarm indicated the presence of
59 both F1 hybrids and early generation backcrosses to *G. rivale* that produce fertile seeds by both
60 outcrossing and selfing (Ruhsam *et al.* 2011, 2013). The resulting recombinant offspring express no
61 detectable fitness reduction when grown in a benign environment, but some recombinant types are
62 not apparently present in the population of established adults in the hybrid swarm (Ruhsam 2013),
63 perhaps due to selection (Ruhsam et al. 2013). Thus, the mating events occurring in mixed habitats
64 are common and favourable for introgression, particularly via backcrossing to *G. rivale*. However, so

far there has been no assessment of whether this has led to significant gene exchange between the two species.

To quantify gene exchange between the two *Geum* taxa we adopt a comparative genomic approach. We first generate a draft genome of the inbreeding species, *G. urbanum*, which we use to develop a set of SNP markers common to the two species. These SNPs are then scored in allopatric populations of both species likely to have been geographically isolated for the past 5ky. We analyse these data in a coalescent framework to estimate the age of the two taxa and measure interspecific gene flow from the time at which the species formed to the time when allopatric populations became isolated. We also use the allopatric samples to identify species-specific diagnostic SNPs.

To measure recent gene flow between the *Geum* taxa, in the period since the isolation of the allopatric populations, we analyse an additional set of samples taken from a broad area of sympatry. We look for signals of recent genome-wide introgression by comparing nucleotide diversity and genetic differentiation between the allopatric and sympatric samples. If introgression has occurred, significant increase in genetic diversity within and reduction in genetic differentiation between species are expected in sympatric compared to allopatric samples. We applied a clustering approach to the SNP data to determine the extent of admixture of *G. rivale* and *G. urbanum* genomes within individuals. Finally, we score the sympatric sample for previously identified species-specific diagnostic SNPs to quantify hybridisation and introgression at the level of individual genotypes. We relate these estimates of the hybridity of individuals, based on molecular data, to the morphology of these individuals measured in a common environment.

MATERIALS AND METHODS

Sampling

i. Sampling of Allopatric Populations

Four *G. rivale* individuals were sampled from each of three high elevation tall herb communities located on three distinct Scottish mountain ranges above the altitudinal limits of *G. urbanum* (Table S1; Taylor 1997b). One individual was also sampled from a single population in each of northern Sweden and Iceland where *G. urbanum* is absent (Table S1; http://linnaeus.nrm.se/flora).

Two *G. urbanum* plants were sampled from each of 10 populations in south-east England (Table S1), a region where *G. rivale* is absent due to lack of suitable habitats (Preston *et al.* 2002). Single individuals of *G. urbanum* were also sampled from two areas in Europe where *G. rivale* does not occur, namely Portugal and south-west France (Table S1; http://linnaeus.nrm.se/flora). 0.5 grms young leaf tissue was removed from sampled plants and stored dry in silica gel prior to DNA extraction.

ii. Sampling of Sympatric Populations

The Botanical Society of Britain and Ireland (BSBI) database was searched for the location of sites containing *G. rivale* alone (15), *G. urbanum* alone (14), or both taxa (10) in eight contiguous 10km x 10km squares within vice county 81 in the British Isles (Berwickshire) (Fig. 1, Table S2) (http://bsbidb.org.uk/maps/). At each site a single cutting was collected in August 2013 from each of two plants more than 5m apart, for each of the *Geum* taxa present. Field identification employed floral, fruit and vegetative characters. Newly emerging leaves (0.1 g.) were taken from each plant and frozen (-80°C) prior to DNA extraction. Cuttings were rooted in 20cm diameter pots containing

commercial potting compost. Ninety six of the 98 cuttings were rooted successfully. Plants were overwintered outside in a randomised array in Edinburgh to induce flowering in spring 2014.

Development of SNP markers via ddRAD

i. DNA extraction

DNA was extracted from dried samples using a modification of Doyle & Doyle (1990)'s CTAB protocol, while fresh and frozen material was extracted using the DNeasy plant mini prep kit (Qiagen) following the manufacturer's protocol.

ii. Development of draft Genome

We developed a draft genome for the inbreeder, *G. urbanum* to facilitate SNP genotyping with double-digest Restriction Associated DNA sequencing markers (ddRAD; Peterson *et al.* 2012). We identified the individual within our Punnetts Town sample (Table S1) that was most homozygous at a set of 10 microsatellite loci (Arens *et al.* 2004). Using this sample we extracted 17µg of DNA from 150mg of young leaves. Details of the genome sequencing and assembly, which was conducted by Edinburgh Genomics, are given in the Supplementary Materials.

To identify SNPs within the draft genome and estimate coverage per scaffold, trimmed reads were mapped back to assembled scaffolds using bwa mem version 0.7.5a with -M option (Li & Durbin 2009). 94.0% of reads were aligned to scaffolds. Samtools (Li *et al.* 2009) version 0.1.18 was used to call variants.

iii. ddRAD library prep and sequencing

DNA quality was evaluated using the E-Gel Precast Agarose Electrophoresis System (ThermoFisher Scientific), and samples quantified using the Qubit dsDNA BR Assay Kit, (ThermoFisher Scientific).

Sample preparation, library construction and PCR amplification for double digest RAD sequencing was modified from Peterson *et al.* (2012) (for full details see Supplementary Materials). Superpools of PCR products were sequenced either on an Illumina HiSeq 2500 using 125 based paired-end reads in high output mode (v4 chemistry) or an Illumina MiSeq using 150 base paired-end reads (v2 chemistry).

iv. Quality filtering and preparation of reads from ddRAD

The bioinformatics and analysis pipeline for all ddRAD data is summarized in Figure S1. To match the read lengths produced by MiSeq and HiSeq Illumina technologies for ddRAD analyses, we used fastx trimmer (http://hannonlab.cshl.edu/fastx_toolkit/) to trim MiSeq reads to 125bp (STACKS requires reads of equal length). We then de-multiplexed and filtered reads for quality using process_radtags (STACKS v 1.21; Catchen *et al*. 2011, 2013). This process removed reads with an uncalled base, and those with an average quality score below 20 over a sliding window comprising 15% of a read. Finally, we again trimmed reverse reads to 117bp with fastX trimmer to produce forward and reverse reads of equivalent length after the 8bp barcode was removed from the forward reads.

v. Aligning reads to draft genome

Both *Geum* species are ancient hexaploids (2N = 42), with disomic inheritance (Smedmark *et al.* 2003; Vandepitte *et al.* 2007; Ruhsam *et al.* 2010). Mapping ddRAD sequences from the two *Geum* species must account for divergence between species to allow reads from both species to map to the *G. urbanum* draft genome. However, allowing an excessive number of mismatches can cause reads to map to multiple loci in ancient polyploid taxa, like *Geum*. We explored the effect of varying the number of allowed mismatches (M) on alignments for one allopatric individual from each species (Punnetts Town, *G. urbanum*; Ben Lui, *G. rivale*; both sequenced with Illumina HiSeq).

For a range of M values, we used GSnap (version 2014-12-16; Wu & Watanabe 2005) to map reads to the draft genome. We required that reads map uniquely, specified an indel penalty of two, and did not allow terminal alignments. For the *G. urbanum* sample, between 64.86% to 67.74% of paired-end (PE) ddRAD reads mapped as proper pairs when we allowed one to nine mismatches (M=1 to 9); however the percentage of reads that aligned jumped to 82.84% for M=10. Similarly, the percentage of *G. rivale* PE reads that aligned in proper pairs increased gradually from 39.21% to 58.16% over M=2 to M=9, with a large increase to 79.93% for M=10. Therefore, the structure of the *Geum* genome (e.g., due to polyploidy and/or repetitive sequences) appears to present different environments for aligning ddRAD data at a threshold genetic diversity of ~8.55% (i.e., 10/117 * 100, where 117 equals the read length). Consequently, we performed all subsequent alignments as above, setting M=6 to avoid this threshold but also allow many reads from *G. rivale* to map to the heterospecific draft genome.

vi. Assembling radtags and calling SNPs

We used STACKS' ref_map pipeline (v 1.21; Catchen *et al.* 2011, 2013; Hohenlohe *et al.* 2010) to assemble the aligned reads into radtags. Next, we used STACKS' rxstacks module to correct genotype calls based on population-level genotype information using a bounded (error rate) model with the parameters conf_lim and bound_high equal to 0.25 and 0.1, respectively (see http://catchenlab.life.illinois.edu/stacks/manual/).

vii. Identifying and filtering for paralogs

Ancient hexaploidy complicates analyses of *Geum* because reads from paralogous genome regions could map to identical sites. As a strict filter for identifying genome regions that potentially attract paralogous reads, we used STACKS' populations module to calculate observed heterozygosity and F_{IS} at each identified SNP. Thereafter custom scripts were used to collate data and restrict further analysis to scaffolds free of SNPs that exhibited either excess heterozygosity (>0.5) or negative F_{IS}.

176 The heterozygosity and F_{IS} calculations *for this purpose only* considered UK allopatric *Geum* samples
177 that comprised four individuals from each of three Scottish 'allopatric' *G. rivale* populations (12 *G.*
178 *rivale* individuals), and one randomly chosen individual from each of the 10 Southern England *G.*
179 *urbanum* populations (Table S1). Nucleotide-level heterozygosity and F_{IS} were calculated separately
180 for *G. rivale* and *G. urbanum*; we assumed each set of samples was from a single, large UK
181 population, despite population structure being evident in both species (see Results). Population
182 structure reduces the number of heterozygotes compared to a single panmictic population:
183 therefore, our filters should preferentially remove genomic regions with excess heterozygosity due to
184 paralogy.

185

186 ***Population Genetic Analysis of Allopatric Populations***

187 *i. Patterns of Polymorphism within and between species*

188 We characterized polymorphism within and between the *Geum* species using the 12 allopatric UK *G.*
189 *rivale* samples plus one from each of Iceland and Sweden, and 10 UK *G. urbanum* samples (one from
190 each allopatric UK population) plus one each from France and Portugal (Table S1). We used STACKS'
191 populations module to identify SNPs that are polymorphic within at least one species or show a fixed
192 difference between species. This analysis only considered radtags present in all 26 individuals
193 described above.

194 *ii. Inbreeding coefficients and population differentiation*

195 We applied the STACKS' populations module to estimate the inbreeding coefficients within each
196 population (F_{IS}) and measure differentiation among populations (F_{ST}) for each species using data from
197 the three allopatric *G. rivale* populations in Scotland (4 individuals/pop) and the ten allopatric *G.*

198 *urbanum* populations in England (2 individuals/pop). Estimates of F_{IS} and F_{ST} only considered radtags
199 present in both species, and that were present in all individuals of a given analysis.

200 *iii. Identification of species-specific SNPs*

201 We used alternately fixed SNPs in allopatric populations of the two taxa (identified in (i.) above) to
202 provide an initial list of species-specific SNPs. To minimize linkage between diagnostic SNPs and
203 obtain an estimate of introgression across the whole genome, we selected one (the first) alternately
204 fixed SNP per scaffold for the introgression analysis. However, we note that by using the first SNP per
205 scaffold, we bias our data against (larger) well-assembled scaffolds. Therefore, we repeated our
206 analyses of introgression using all available species-diagnostics SNPs to test for an effect of such bias,
207 and obtained qualitatively identical results (not shown).

208 *iv. Coalescent analysis of gene flow during lineage divergence*

209 We used an analytic likelihood framework to assess the support for alternative models of divergence
210 between *G. rivale* and *G. urbanum* with and without gene flow. The method is described in Lohse *et*
211 *al.* (2016). Briefly, the analysis is based on a single diploid individual for each species and considers
212 the blockwise site frequency spectrum, i.e. the joint frequencies of four polymorphism types (as in
213 "*Patterns of Polymorphism within and between species*", above) in short blocks of sequence: i)
214 heterozygous sites exclusive to *G. rivale*, ii) heterozygous sites exclusive to *G. urbanum*, iii)
215 heterozygous sites shared by both species and iv) fixed differences between species. We counted
216 these site types within 117bp radtags (block), and treated each radtag as an independent block. For
217 randomly mating populations, the polymorphisms at each block represent an independent outcome
218 of the coalescent process, which is a function of the species' history. Since STACKs ignores RAD tags
219 that are monomorphic, we conditioned the likelihood on only observing variable blocks. To do this,
220 we normalized the probability of each blockwise mutational configuration by 1-p_IBS, where p_IBS is
221 the probability of identity in state for a block.

222

223 We primarily wish to test for historical introgression between *G. rivale* and *G. urbanum*. Therefore,
224 we compared three models: (1; "div_2") species diverged at time *T* with no introgression, (2; "$IM_{u\rightarrow r}$") a
225 history of divergence with gene flow from *G. urbanum* to *G. rivale* and (3; "$IM_{r\rightarrow u}$") divergence with
226 gene flow from *G. rivale* to *G. urbanum* (4 parameters). All models assume instantaneous species
227 divergence and a constant N_e within taxa. We also constrained the effective size of the ancestor to
228 equal that of *G. rivale*. To capture the decrease of N_e expected to result from the transition to
229 inbreeding (Charlesworth & Wright 2001), we allowed N_e to differ between *G. urbanum* and the
230 other taxa (the ancestor of *G. rivale* and *G. urbanum* is most likely outcrossing given the rarity of
231 transitions from self-compatibility to self-incompatibility; Igic et al. 2006). Migration was modelled
232 as a constant rate $M = 4N_{anc} \times m$ individuals per generation. We converted estimates of T into years
233 using $t = T \times 2N \times g$ where g is generation time and $N = \theta/4\mu$. We assumed a mutation rate estimated
234 for *Arabidopsis thaliana*, $\mu = 7 \times 10^{-9}$ (Ossowski *et al.* 2010), and an average generation time across
235 the two species of 3 years (Taylor 1997a, b) (generations are likely longer in *G. rivale* than in *G.*
236 *urbanum*).

237 Inbreeding complicates this analysis because it reduces genetic diversity within individuals relative to
238 the population level. To minimize the confounding effect of inbreeding on the analyses, we initially
239 chose the most outbred individuals for analysis. We used a recently outcrossed *G. urbanum*
240 individual (see Results; population Mill Wood), which had genetic diversity ($\pi = 0.0009 \pm 0.0001$)
241 similar to the UK allopatric *G. urbanum* populations as a whole (see Results) and so may adequately
242 represent the diversity of this species in the UK. "Leaky" self-incompatibility also introduces
243 variation in inbreeding among individuals of *G. rivale*. Therefore, we conducted separate analyses
244 that paired the outbred *G. urbanum* Mill Wood individual with 3 allopatric UK samples of *G. rivale*
245 that span a range of heterozygosity to examine how inbreeding in *G. rivale* affects our conclusions.

246

Maximum likelihood estimates under each model were obtained in *Mathematica* v. 10.2 (see File S2 of Lohse *et al.* 2016). To estimate 95% CI for M and T, we obtained discretized marginal support (logarithm of the likelihood) curves for these parameters (maximising the likelihood for all other parameters at each point).

Population Genetic Comparison of Allopatric and Sympatric Populations

i. Genetic diversity in allopatric and sympatric populations

To determine whether potential genetic exchange in sympatric populations resulted in an increase in genetic diversity, we estimated genetic diversity (π) in UK allopatric and Berwickshire samples (pooling Berwickshire's 'pure' and 'mixed' populations) of each species using STACKS' populations module (providing mean and standard error, S.E.). The analysis considered radtags present in at least half of individuals for each species/population type (allo- vs sympatric) combination; we obtained qualitatively similar results when we restricted the analysis to radtags present in all individuals (not shown). The analysis excluded four individuals in the Berwickshire sample identified as obvious early generation hybrids or backcrosses (see Results). This analysis therefore involved 12 allopatric *G. rivale*, 20 allopatric *G. urbanum*, 48 sympatric *G. rivale* and 45 sympatric *G. urbanum* samples.

ii. Genetic differentiation between taxa in allopatry and sympatry

To test whether potential gene exchange resulted in less differentiation between the species in sympatric populations, we calculated genetic differentiation $d_{xy} = \Sigma[(PX * (1-PY)) + ((1-PX) * PY)] / n$ between the two species, separately for allopatric and sympatric samples: P represents the frequency of a focal allele in the sample of species X and Y, and n is the sequence length (Nei & Li 1979; Cruickshank & Hahn 2014). These calculations considered the same samples as used to calculate genetic diversity (π), described above. We estimated d_{xy} for each scaffold, which we

assume to be independent with respect to linkage and determined the mean d_{xy} and its S.E. across scaffolds. All analyzed radtags were present in at least 12 individuals for each species/population type combination, and the same scaffolds were analyzed in allopatric and sympatric estimates of d_{xy} (n=418 scaffolds).

Genetic Analysis of Hybrids and Introgression in Sympatric Populations

i. Cluster analysis using fastSTRUCTURE

As a first approach to analyzing introgression between the two *Geum* taxa in sympatry, we used genotypic clustering implemented in fastSTRUCTURE (Raj *et al.* 2014). Our analysis assumed that samples derived from two populations (i.e., K=2) corresponding to the two *Geum* species, with the possibility of genetic admixture of individuals. The SNP data was derived from a STACKS analysis of all 92 successfully genotyped individuals in the sympatric Berwickshire sample together with data from the British and European allopatric populations of the two species. We analyzed two datasets that differed in degree of filtering: (1) SNPs were present in any fraction of individuals, which led to greater numbers of SNPs but missing values for some individuals, and (2) all SNPs were present in all individuals. Both analyses used SNPs that had been filtered for paralogs, and considered only a single SNP per scaffold to minimize the effect of linkage between SNPs.

ii. Identifying hybrids and introgressed individuals using species-specific SNPs

In our second approach to analyzing recent introgression we used custom scripts and the species-specific SNPs identified in (iii.) (above) to estimate the fraction of alleles in each individual within the sympatric Berwickshire sample that is *G. rivale* in origin (Hybrid Index). SNPs with either un-callable genotypes (based on STACKS' likelihood algorithm) or third alleles (e.g., due to sequencing or alignment error) were excluded from the calculations. We simultaneously tabulated the frequency of SNPs that were heterozygous for the species-diagnostic alleles in each individual.

294 In general, our analyses did not specify minimum coverage because STACKS accounts for coverage
295 when calling genotypes (or leaving a genotype uncalled) (Hohenlohe *et al.* 2010; Catchen *et al.* 2013).
296 However, to check whether specifying minimum coverage would alter our results, we repeated our
297 analyses, requiring a minimum coverage of 20 in all analysed individuals when running
298 fastSTRUCTURE and creating the panel of species-diagnostic SNPs. This quartered the number of
299 species-diagnostic SNPs, but yielded qualitatively identical results (not shown).

300 ***Analysis of Morphological Variation in Sympatric Populations***

301 *i. Measurement of morphological variation in sympatric populations*

302 From the beginning of April 2014 plants grown from cuttings from sympatric populations were
303 monitored weekly for flowering. From each plant a newly opened flower and the stipule located on
304 the flowering stem immediately below the flower were sampled. The following characters, which
305 discriminate between *G. rivale* and *G. urbanum*, were measured: angle at which flowers are held
306 (degrees from vertical) (FA), petal length (PL) (mm), petal width (PW) (mm), petal shape (PS)
307 (proportional height of widest part of petal)), sepal length (SL) (mm), stamen number (SN), stipule
308 length (STL) (mm) and stipule width (STW) (mm) (Ruhsam *et al.* 2011, 2013).

309 *ii. Statistical analysis of morphological data*

310 Principal Component Analysis (PCA) was conducted on the total sympatric sample using data on all
311 eight morphological characters measured. Morphologically distinct groupings were recognised on
312 scatterplots of the first two principal component scores and related to parental species and hybrid
313 classes defined genetically by species-specific SNPs. PCA based on the same characters was also used
314 to summarise morphological variation separately within *G. urbanum.* GLMs were used to test for the
315 effect of two SNP variants, putatively jointly introgressed from *G. rivale* (see results), on the first two
316 principal component scores for *G. urbanum*. All statistical analyses were conducted in Minitab 16.

RESULTS

Development of SNP markers via ddRAD

i. Draft Genome

The genome assembly contained a total of 170,030 scaffolds, with an N50 of 24.6Kb and total assembly size of 1.2Gb. In order to identify signs that scaffolds represent multiple copies of the genome, the distributions of coverage per scaffold and percent variant bases per scaffold was assessed. Scaffolds of length less than 10Kb were excluded, leaving 32,182 scaffolds (with a total length of 909 Mb). The distribution of percent variant bases per scaffold is shown in Figure S2, and the distribution of coverage per scaffold is shown in Figure S3. Coverage per scaffold follows a roughly normal distribution, which would be expected if the scaffolds mostly represented the same number of copies of the genome.

Core genic regions were well assembled, and appeared to be present in approximately three (haploid) copies as expected for an ancient hexaploid. We searched our assembled genome for 248 ultra-conserved eukaryotic genes (CEGs), listed by Parra *et al.* (2007), using their Core Eukaryotic Genes Mapping Approach. We identified 93% and 97% of the core genes that were complete or partially complete, respectively. On average, complete and partially complete CEGs were represented by 3.39 and 3.82 orthologs per CEG, respectively, with at least 90% of CEGs represented by more than one ortholog.

ii. ddRAD tags

Following quality filtering, $2.7 * 10^7$ reads remained in the MiSeq data, and between $6.0 * 10^7$ and $7.3 * 10^7$ reads remained that derived from the five HiSeq libraries. Following alignments, assembling radtags with STACKS, and applying corrections with rxstacks, our dataset included 230,356 radtags

for *G. rivale* and *G. urbanum*, collectively. However, coverage was highly stochastic. For example, only ~2% (4524) of radtags were represented in more than one half of our samples.

iii. Identifying and filtering for paralogs

When the STACKS' populations module was used to analyse the raw SNP data from the allopatric populations of the inbreeding taxon *G. urbanum*, F_{IS} estimates were low and sometimes negative, which is unexpected for a highly inbreeding species. This suggests that paralogous reads have mapped to identical locations and thereby increased individual heterozygosity (see Table S4). We therefore applied our filter for paralogy (rejecting 1344 scaffolds with SNPs that exhibit either excess heterozygosity (>0.5) or negative F_{IS}) and, unless specifically noted, we henceforth only present results from paralogy-filtered SNPs.

Population Genetic Analysis of Allopatric Populations

i. Patterns of polymorphism within and between species

The majority of SNPs in the dataset were polymorphic in *G. rivale* but invariant in *G. urbanum*. This type of polymorphic site occurred approximately four times more frequently than the reverse case (Table 1). 22% of SNPs were alternately fixed between the species (Table 1) and only 1.5% of SNPs were shared polymorphism (Table 1).

ii. Inbreeding coefficients and population differentiation

For the allopatric populations sampled in Britain we obtained F_{IS} estimates close to 0.25 for *G. rivale*, consistent with leaky self-incompatibility (Ruhsam *et al.* 2010), and F_{IS} greater than 0.9, consistent with very high selfing rates, for eight of the ten UK 'pure' *G. urbanum* populations (Table 2). Among the two *G. urbanum* populations with low F_{IS}, the first population (Mill Wood F_{IS} = 0.0845), included one (of the two) sample(s) that was heterozygous at 92% of the 71 polymorphic SNPs analysed,

363 suggesting that this sample was derived from a recent outcrossing event between two diverged
364 inbred lines. In the second population, Selwyn Wood, only one SNP was recorded as polymorphic,
365 suggesting that this population may have been founded by few (possibly a single) highly selfing
366 individuals.

367 Allopatric populations of *G. rivale* in Britain exhibited less population differentiation than *G. urbanum*
368 populations (mean pairwise F_{ST} 0.13 and 0.38 respectively), as expected from their contrasting
369 breeding systems.

370 *iii. Coalescent analysis of gene flow during lineage divergence*

371 Table S5 summarizes the numbers of each polymorphism type and blocks analysed for the *G.*
372 *urbanum* - *G.rivale* sample pairs. All three pairs had approximately 1400 SNPs distributed among ca.
373 660 blocks (i.e., radtags). Model comparisons for all three sample pairs reject a model of strict
374 divergence, and suggest that introgression has occurred between the *Geum* species (Table 3), but at
375 a very low rate (see below). For two sample pairs (involving *G. rivale* samples Ben Lui 1 and Ben
376 Lawers 5), the model of gene flow from *G. rivale* to *G. urbanum* (i.e., $IM_{r\rightarrow u}$) fit the data significantly
377 better than the model of strict divergence (i.e., div_2), whereas the model $IM_{u\rightarrow r}$ does not fit
378 significantly better than div_2 (Table 3). Models that include gene flow ($IM_{r\rightarrow u}$ and $IM_{u\rightarrow r}$) also fit the
379 data significantly better than the div_2 model for the third pair (involving sample Ben Lui 4), but $IM_{r\rightarrow u}$
380 and $IM_{u\rightarrow r}$ have effectively equal support (Table 3). Results from this latter pair likely differ from the
381 former pairs because it includes a single block with a shared heterozygous site, while the other
382 sample pairs lack shared heterozygous sites (Tables S5, S6). As Ben Lui 4 is likely the most
383 heterozygous *G. rivale* sample (Table S5), we focus on this pair, but note that parameter estimates
384 (Table S6) and general conclusions (i.e., support for very low introgression rates) are similar for all
385 three sample pairs.

386

The three models (div_2, $IM_{r\rightarrow u}$, $IM_{u\rightarrow r}$) yield similar estimates of Ne and divergence time (Ben Lui 4 pair: Table 4; all three sample pairs: Table S6). In general, Ne of *G. urbanum* is a half to a quarter of that of *G. rivale* (and their common ancestor), and all three models suggest that the species diverged approximately 2 to 3 million years ago (Table 4; Table S6). Models $IM_{u\rightarrow r}$ and $IM_{r\rightarrow u}$ both suggest a low but significant long-term rate of effective gene flow ($M \approx 0.04$), of approximately one migrant every 25 generations (Table 4; see Table S6 for M for additional sample pairs).

Population Genetic Comparison of Allopatric and Sympatric Populations

i. Genetic diversity in allopatric and sympatric populations

Estimates of genetic diversity (π) were based on 826 radtags, each 117 base pairs in length, totaling 96 642 base-pairs; as these radtags were the same as those used to develop species-diagnostic SNPs, they occur in both species. Genetic diversity was greater in *G. rivale* (mean $\pi \pm$ SE: 'allopatric' 0.0044 ± 0.0001; Berwickshire 0.0048 ± 0.0001) than *G. urbanum* ('allopatric' 0.0011 ± 0.0001; Berwickshire 0.0011 ± 0.0001), and was similar between population types for *G. urbanum*. However *G. rivale* genetic diversity was slightly greater for sympatric than allopatric samples. Since STACKS ignores monomorphic radtags, we note that these estimates of π should be interpreted in terms of relative, but not absolute diversity (see also Arnold *et al*. 2013).

ii. Genetic differentiation between taxa in allopatry and sympatry

Genetic differentiation between *G. rivale* and *G. urbanum* is similar for UK allopatric and sympatric samples (mean d_{xy} (+/- SE) equals 0.0115 +/- 0.0005 and 0.0112 +/- 0.0005, respectively). Again, we note that, due to the fact that STACKS' ignores monomorphic radtags, these estimates should be viewed as relative measures (and upper limits) of d_{xy}, and not as absolute estimates.

410 ***Hybrids and Introgression in Sympatric Populations***

411 *i. Cluster analysis using fastSTRUCTURE*

412 *fastSTRUCTURE* analysis that considered SNPs present in either all (188 SNPs) or a proportion of (492
413 SNPs; results not shown) individuals analysed in the combined allopatric and sympatric populations
414 yielded highly consistent results. Figure 2 illustrates fastSTRUCTURE results for analyses based on
415 188 SNPs. All except one of the 36 individuals in the allopatric populations, and all except 4 of the 96
416 individuals in the sympatric populations, show less than 1% admixture. In the sympatric population Q
417 values (proportion of *G. rivale* genome) of the four individuals with substantial admixture are 0.496,
418 0.517, 0.663 and 0.935. On the basis of these Q values there is no evidence for significant
419 introgression from *G. rivale* into *G. urbanum*. The substantially admixed individuals present are likely
420 to be recently formed F1 or F2 hybrids, and different generations of backcrosses to *G. rivale*.

421 *ii. Identifying hybrids and introgressed individuals using species-specific SNPs*

422 To initially assess the ability of the species-specific SNPs to discriminate the species, we genotyped
423 the 10 British allopatric *G. urbanum* samples that had not been used to generate species-diagnostic
424 SNPs. 215 out of 220 diagnostic SNPs were homozygous for the *G. urbanum* allele in all individuals.
425 Five SNPs were homozygous for the putative *G. rivale* allele in one or more of the 10 *G. urbanum*
426 individuals, indicating that these SNPs were not fixed between species. They were removed from
427 subsequent analysis of 96 individuals of *Geum* from the 39 sites sampled within the sympatric
428 Berwickshire population. The mean number of SNPs successfully scored per individual was 207.3
429 (range 161 – 215 Figure S5). The variation in hybrid index (HI) in the Berwickshire population is
430 illustrated in Figure 3.

431 The vast majority of individuals in the total sympatric sample (92 out of 96) have genotypes in which
432 more than 97% of the species-specific SNPs are derived from only one of the two *Geum* species.
433 Individuals possessing >97% *G. rivale* variants are hereafter regarded as *G. rivale*, while plants with

>97% *G. urbanum* variants are classified as *G. urbanum*. The four remaining individuals with substantial proportions of variants from both species correspond to the four early generation hybrids identified in the fastSTRUCTURE clustering analysis. The correlation between the Q values calculated by fastStructure and the hybrid index calculated here is high (r=0.99) (see Figure S6 for comparison using 188 SNPs), in part because some SNPs (i.e., 61 of the 492 SNPs available for fastSTRUCTURE analysis) are common to both analyses. Early generation hybrids were significantly associated with 'mixed' rather than 'pure' sites within Berwickshire (Fisher's exact test, P=0.044).

Neither of the two individuals possessing a hybrid index close to 0.5 are heterozygous at all SNPs, the situation anticipated if they were simple F1 hybrids (Table 3). Instead they are homozygous at a substantial fraction of the species-specific SNPs (15% and 18%), suggesting that they may have been derived by selfing of F1 hybrids. Two further individuals contain alleles derived predominantly from *G. rivale*, but additionally possess a substantial complement of *G. urbanum* alleles (29% and 12% respectively) (Table 5). On the basis of their complement of alleles alone, these plants are most likely to represent first and second generation backcrosses to *G. rivale* respectively. However, their origin must again be more complex, possibly involving selfing, because a substantial fraction of the *G. urbanum* alleles they possess (10% and 33% respectively) are present in homozygous form, whereas if they had been simple backcrosses, all *G. urbanum* alleles would have been present as heterozygotes.

Among the 92 plants from Berwickshire possessing a preponderance (>97%) of species-specific alleles from one taxon, 33 of the 47 individuals assigned to *G. rivale* and 26 of the 45 plants assigned to *G. urbanum* possessed from 1 to 10 alleles classified as specific to the alternate species (Fig. S7). These could represent alleles that have introgressed, or alternatively alleles present at low frequency in the focal species that have not been detected in the limited sample of allopatric genotypes used to identify species-specific SNPs. The maximum frequencies of putatively introgressed alleles are low

458 (9.6% and 14.4% in *G. rivale* and *G. urbanum* respectively). Furthermore 76% are polymorphic in *G.*
459 *rivale* only, 20% are polymorphic in *G. urbanum* only, and 4% are polymorphic in both species. These
460 proportions are not significantly different from those found in the total sample of SNPs scored
461 (P>0.05, Table 1). Therefore, misclassification of species-specific SNPs rather than introgression is the
462 most likely explanation for the apparent presence of up to 3% admixture in the *Geum* genomes from
463 sympatric populations.

464 With one exception, the putatively introgressed alleles discussed above were randomly associated
465 with each other within each of the sympatric *G. rivale* and *G. urbanum* populations. However, two
466 SNPs at radtags 20454 and 24791 respectively, and originally classified as specific to *G. rivale*,
467 showed complete association in genotypic state within sympatric *G. urbanum*. The alleles were
468 present at a mean frequency of 14.4% and distributed across six sites. In six individuals both alleles
469 were present in homozygous form, while a seventh individual was heterozygous at both radtags.
470 Since the SNPs involved are located on different scaffolds, these results may indicate the presence of
471 a section of genome spanning the two scaffolds, possibly derived from *G. rivale*, which is segregating
472 in the *G. urbanum* population.

473 ***Analysis of Morphological Variation in Sympatric Populations***

474 *i. Correspondence between Phenotypic and Genotypic classification*

475 Morphological measurements of eight floral and vegetative characters commonly used to distinguish
476 *G. rivale* and *G. urbanum* were obtained from 87 of the 96 individuals from Berwickshire that had
477 been scored for species-specific SNPs. The results of Principal Component Analysis based on these
478 morphological data are illustrated in Figure 4. The first two principal components, accounting for
479 82.4% and 6.7% of the variation respectively, distinguish two major groups, with two individuals
480 falling between these on the first PCA axis. Individuals classified as *G. rivale* (N=43) and *G. urbanum*
481 (N=40) on the basis of species-specific SNPs fall clearly into the two major groups with +ve and –ve

PCA1 values, respectively. The two individuals with intermediate PCA1 scores correspond to the early generation hybrids identified with clustering and species-specific SNPs. The two genotypes identified as complex backcrosses to *G. rivale*, on the basis of species-specific SNPs, group morphologically with individuals classified as *G. rivale* on the PCA plot.

ii. Morphological Variation and Putative Introgression

To determine whether presence of putatively introgressed genetic material marked jointly by alleles at radtags 20454 and 24792 affected the morphology of *G. urbanum* (see above), data on the eight floral and vegetative characters measured in the sympatric *G. urbanum* population were used to generate the first two principal component scores describing morphological variation. Analysis of variance showed no significant effect of joint presence/absence of the alleles on PCA1, but a significant effect on PCA2 score ($F_{1,38} = 8.67$, P=0.005; Fig.5). Individuals of *G. urbanum* jointly possessing these alleles showed a significantly larger flower angle than those lacking them (91.4 vs. 60.8 degrees, p=0.018). Greater flower angle is a feature characteristic of *G. rivale* and is most heavily weighted in PCA2.

499

500 **DISCUSSION**

501 We have developed a set of sequence based markers in two closely related non-model plant species
502 with contrasting mating systems that share a polyploid history. Analysis of the markers across
503 allopatric, and pure and mixed sympatric samples of *G. rivale* and *G. urbanum* indicate that the
504 species are 2-3MY old, and that levels of introgression between the species, both before and after
505 the period of recent sympatry, have been very small. While hybrid genotypes continue to be
506 produced in contemporary populations, the proportion of introgressed loci across the genomes of
507 the vast majority of individuals is much lower than 3%.

508 The development of sequence based markers, using next generation sequencing, poses technical
509 problems in our *Geum* species. Their genomes are large (1.2 and 1.6 Gb in *G. urbanum* and *G. rivale*
510 respectively) and although inheritance is disomic the species are ancient hexaploids. This means that
511 there will generally be three closely related copies of every sequence, creating significant difficulties
512 in distinguishing homologs from paralogs (Smedmark *et al.* 2003). We adopted the ddRAD technique
513 that allows modulation of the number of RAD tags generated per genome to ensure adequate depth
514 of coverage (Peterson *et al.* 2012). To maximize the chance of distinguishing homologs and paralogs
515 we used paired end sequencing yielding long reads, and generated a draft genome of the largely
516 homozygous inbreeding species against which to map our ddRADtag sequences. During the data
517 analysis phase we employed very rigorous filtering of scaffolds and SNPs to exclude paralogs, which
518 removed about three-quarters of scaffolds from our dataset. This filtering contributed to the
519 development of a modest number of SNPs compared to comparable studies in other genera (e.g.
520 *Populus*, Christe *et al.* 2016; *Betula*, Zohren *et al.* 2016), but that was nevertheless orders of
521 magnitude higher than for previous microsatellite based studies; moreover, we developed sufficient
522 SNPs to yield high precision estimates of introgression (e.g., 200 SNPs can estimate introgression of

523 0.5%). Furthermore our RAD data gave estimates of both inbreeding coefficients and population
524 genetic differentiation that are congruent with results from microsatellite markers, providing
525 confidence in the conclusions drawn from their analysis (Ruhsam *et al.* 2010, 2013).

526 Coalescent analyses of RAD data from allopatric populations, which contrast the fit of simple models
527 of the species' histories to polymorphism data, gave species' age estimates of between 2 and 3
528 million years. This is considerably older than some estimates of time of divergence for other sister
529 taxa that possess contrasting outcrossing and selfing mating systems. These range from 50 - 100kya
530 for *Capsella grandiflora* and *C. rubella* (Brandvain *et al.* 2013) through 500-1100kya for *Mimulus*
531 *guttatus* and *M. nasutus* (Aeschbacher *et al.* 2016) to 1Mya for *Arabidopsis lyrata* and *A. thaliana*
532 (Tang *et al.* 2007). Our estimated age of the *Geum* species is consistent with the numerous
533 morphological and physiological differences that are exhibited between them (Taylor 1997a, b).
534 However despite their age, *G. rivale* and *G. urbanum* apparently exhibit few intrinsic hybrid
535 incompatibilities (Ruhsam et al. 2013); these observations parallel those from *Drosophila* species,
536 which show few post-zygotic incompatibilities for species of a similar age (Coyne & Orr 1989, 1997).
537
538 Coalescent analyses also show that low levels of effective introgression are likely to have occurred
539 between the *Geum* species up to the time of isolation of allopatric populations, but there is no strong
540 signal of introgression in a particular direction. For clarity, M measures the long-term effective
541 migration, and accounts for reduced gene migration due to selection against hybrids (see below).
542 Our estimate of the introgression rate between the *Geum* species (M=0.04 diploid genomes per
543 generation) is an order of magnitude lower than that estimated for ancient introgression from
544 inbreeding *M. nasutus* into outcrossing *M. guttatus* (M=0.1-1; Aeschbacher *et al.* 2016) and between
545 outcrossing and selfing subspecies of *Clarkia* in their secondary contact zone (M=0.897-0.169;
546 Pettergill & Moeller 2012). It is many orders of magnitude below contemporary estimates of

547 unidirectional gene migration from the inbreeding rice *Oryza nivaria* to its outcrossing relative *O.*
548 *rufipogon* (M=2.3 x 10^5; Zhou *et al.* 2008).
549
550 Our coalescent analyses necessarily rely on simplifying assumptions. First, the blockwise site-
551 frequency spectrum approach we adopted assumes a constant N_e and μ across blocks and so ignores
552 effects of heterogeneity in mutation rate and the effects of background selection on linked sites,
553 which could lead to a spurious signal of introgression (Ewing & Jensen 2016). Second, our models are
554 drastic simplifications of the history of these species and, in particular, assume constant N_e in *G.*
555 *rivale* and the ancestral population, which is unrealistic given the climatic fluctuations and the
556 associated range shifts of European taxa during the Pleistocene. Following species divergence, either
557 a change in N_e or introgression can alter the joint site-frequency spectrum (Chen 2012); by assuming
558 $N_{anc} = N_{riv}$, we potentially conflate changes in N_e with introgression, which, again, could lead to a
559 spurious signal of introgression. Given these issues and the low estimates of introgression, we do not
560 interpret the support of IM models over the div_2 model as strong evidence for ancient long-term
561 gene flow between the *Geum* species.
562
563 As a first test of more recent introgression in *Geum* we conducted comparisons of genetic diversity
564 and differentiation between species in allopatry vs. sympatry. Introgression, which is only possible in
565 sympatric populations, can potentially increase genetic diversity of the recipient species, and erode
566 genetic differences between species. Hence, our comparisons of π and dxy between sympatric and
567 allopatric populations can provide evidence for or against introgression. Relative genetic diversity
568 was greater for *G*. *rivale* than *G. urbanum*, as expected based on their different mating systems
569 (Charlesworth 2003). In addition, genetic diversity was approximately 9% higher for *G. rivale* in
570 sympatry than in allopatry, but was equal between population types for *G. urbanum*. While this is
571 consistent with introgression into *G. rivale*, but not *G. urbanum*, other factors could account for this

result, including a greater effective population size of *G. rivale* in sympatry than in allopatry. Furthermore, genetic differentiation between species (dxy) was similar in sympatry and allopatry, providing no evidence for introgression.

As a second test of recent introgression we analysed the genomic composition of individuals from a region of sympatry in Berwickshire and compared them with samples from isolated, reference populations. The cluster analysis performed by fastSTRUCTURE (which did not rely exclusively on species-diagnostic SNPs), and the analysis based on a limited subset of species-diagnostic SNPs identified the same four individuals as early hybrids and backcrosses, and yielded similar genomic composition estimates that were congruent with morphological classifications. Critically, they suggest that the upper limit for the proportion of introgressed genome present in Berwickshire plants is ~3%. However, the real value for introgression is likely to be lower because the assumption that all our species diagnostic SNPs are alternately fixed between the species is unlikely to be true.

Although our overall results indicate very little recent genetic exchange, one intriguing result provides tentative evidence for introgression from *G. rivale* to *G. urbanum*. Two SNP variants located on different scaffolds that were classified as specific to *G. rivale* in allopatry show co-segregation within the sympatric *G. urbanum* population. Moreover, *G. urbanum* individuals possessing these alleles show a significantly greater flower angle, a characteristic of *G. rivale*. A possible explanation is introgression of a chromosome segment from *G. rivale* into *G. urbanum* that is now polymorphic within the sympatric population. Given the absence of evidence for backcrossing of hybrids to *G. urbanum* (Ruhsam *et al.* 2011, 2013), such introgression could only arise through the establishment, following hybridisation, of a self-fertilising lineage that largely retains genetic material from *G. urbanum*. Previous work has shown that F1 hybrids are self-compatible and a proportion may be capable of autopollination, so this scenario, though unlikely, is not impossible (Ruhsam *et al.* 2013).

597

598 Despite the reproductive barrier that adoption of a selfing mating system naturally imposes (Martin
599 & Willis 2007; Briscoe Runquist *et al.* 2014; Brys *et al.* 2014), the widespread occurrence of the F1
600 hybrid *G. intermedium* throughout the area of sympatry of *G. rivale* and *G. urbanum* in Berwickshire
601 and elsewhere (Preston *et al.* 2002), and our own detection of early generation hybrids, indicates
602 that low levels of introgression are not caused by lack of opportunity for hybridisation. Previous
603 studies have shown that where hybridisation occurs, a wide array of fertile recombinant offspring are
604 produced both by backcrossing with *G. rivale* and by self-fertilisation and that under benign
605 conditions these show little evidence of intrinsic genetic incompatibility (Ruhsam *et al.* 2011, 2013).
606 Thus *G. rivale* and *G. urbanum* join a growing list of sister species which readily produce F1 hybrids
607 and viable recombinant offspring, yet show little or no signal of introgression (e.g. *Encelia* (Kyhos *et*
608 *al.* 1981); *Quercus*, *Arctostaphylos*, *Iris* (Nason *et al.* 1992); *Rhododendron* (Milne *et al.* 2003);
609 *Begonia* (Twyford *et al.* 2015); *Populus* (Christe *et al.* 2016); *Bombina* (Nürnberger *et al.* 2016).
610
611 In many of the above examples ecological selection against recombinants has been invoked to
612 explain the maintenance of species distinctions. There are good grounds for proposing the same
613 explanation to account for lack of introgression in *Geum*. Since their initial divergence the *Geum*
614 species have evolved marked differences in pollination syndrome, phenology, vegetative morphology
615 and many aspects of physiology including tolerance of shade and waterlogging (Waldren *et al.* 1988;
616 Taylor 1997a, b). Therefore multiple ecologically important characters could potentially be the
617 targets of selection against recombinant individuals in natural populations (Nosil *et al.* 2009).
618 Selection may be particularly effective in the self-fertilising recombinants that are required to allow
619 introgression from *G. rivale* into *G. urbanum*. Here reduced effective recombination rates will lead to
620 correlated selective effects across the genome, and deleterious recessive mutations present as
621 genetic load in genome segments derived from outcrossing *G. rivale* will be exposed (Hu 2015). Given

our ability readily to artificially cross the *Geum* species, and produce large numbers of later generation hybrids, there is now the potential experimentally to test the hypothesis that strong ecological selection maintains species integrity using appropriate field experiments.

626 **Author contributions**

627 C.Y.J, K.G. and R.A.E. conceived the study; C.Y.J., F.T., M.T. and R.A.E. gathered data; C.Y.J., K.L., F.T.
628 and R.A.E. analysed data; C.Y. J., K.L. and R.A.E. wrote the manuscript with input and revisions from
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630

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635 **Data accessibility**

636 DNA sequences: will be made available on European Nucleotide Archive before publication

637 Assembled genome: will be made available (University of Edinburgh link) before publication

638 Morphological data: will be made available on Dryad before publication

639 Scripts: will be made available on github.com before publication

640

641 **Conflict of interest:** None declared.

642

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800

801

Polymorphism Type	Count
Alternately fixed SNPs	488
SNP polymorphic in *G. rivale* but one allele fixed in *G. urbanum* (e.g., *G. rivale*: A, T; *G. urbanum*: T)	1334
SNP polymorphic in *G. urbanum* but one allele fixed in *G. rivale*	338
G. rivale and *G. urbanum* share polymorphism	34
Total SNPs analysed	2194

802

803 Table 1: Frequency of polymorphism of different forms within and between *Geum* species. Analysis
804 allows multiple SNPs per scaffold.

805

806

Population by species	Number of SNPs	F_{IS}
G. rivale		
Ben Lawers	309	0.24
Coire Garblach	347	0.26
Ben Lui	341	0.24
G. urbanum		
Priory Wood	67	0.98
Mill Wood	71	0.08
Burgh Wood	76	0.89
Hoades Wood	47	0.95
Punnetts Town	61	0.85
Frith Wood	69	0.96
Stanford Bridge	68	0.97
Combe Wood	62	0.99
Copperhurst	67	0.99
Selwyn Wood	Only 1 polymorphic locus	

807

808 Table 2. F_{IS} estimates for 'allopatric' UK *G. rivale* and *G. urbanum* populations using data filtered for
809 paralogs.

810

811

812

Sample	div_2	$IM_{u \rightarrow r}$	$IM_{r \rightarrow u}$
Ben Lui 1	-12.9	-12.5	0
Ben Lui 4	-9.66	0	-0.721
Ben Lawers 5	-6.67	-6.51	0

813

814 Table 3. Difference in log Likelihoods between the "best" and alternate models. "Sample" refers to
815 which *G. rivale* individual is paired with the single *G. urbanum* individual.

816

817

818

	Model		
	div_2	$IM_{r \rightarrow u}$	$IM_{u \rightarrow r}$
$N_{anc} = N_{rivb}$	$1.30 * 10^5$	$1.24 * 10^5$	$1.11 * 10^5$
N_{urb}	$3.77 * 10^4$	$3.09 * 10^4$	$3.38 * 10^4$
t (years)	$2.22 * 10^6$ ($2.01 * 10^6 – 2.43 * 10^6$)	$2.35 * 10^6$	$2.48 * 10^6$
M (mean, 95% CI's)		0.04 (0.005 - 0.191)	0.04 (0.007 - 0.101)

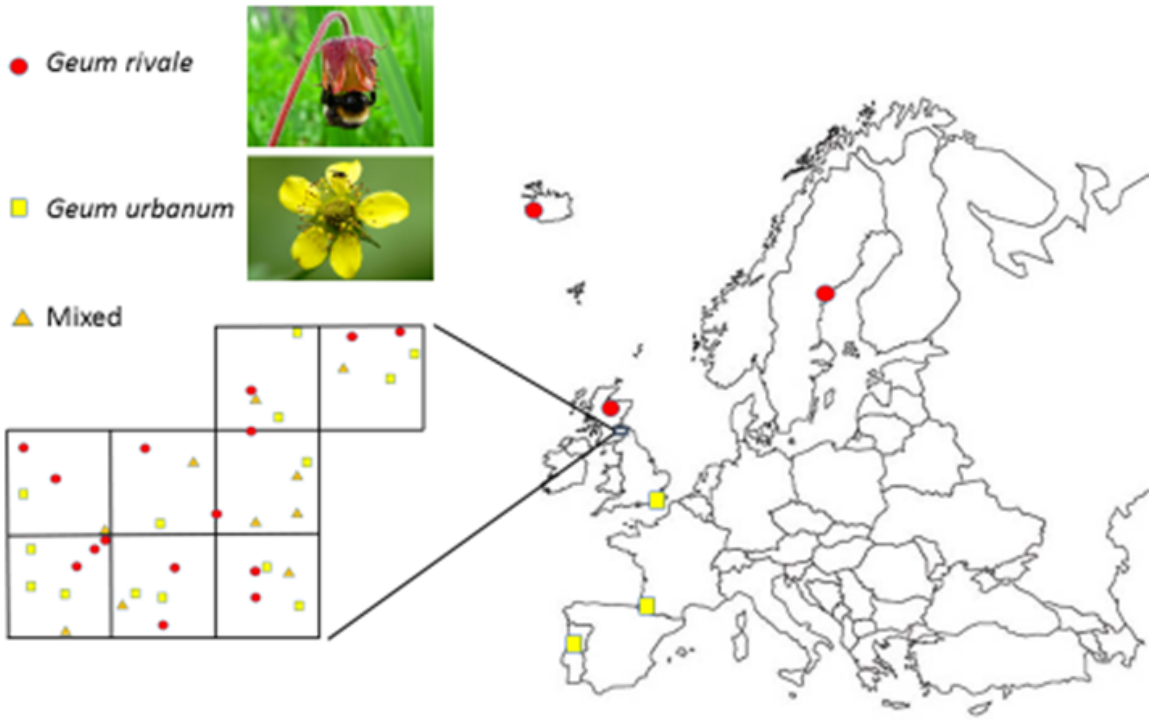
819

820 Table 4. Parameter estimates under the models for *G. rivale* sample, Ben Lui 4. 95% Confidence
821 intervals provided in parentheses. See Table S6 for parameter estimates for all three sample pairs.

822

Sample and classification	% '*rivale*' in genome	Homozygous '*rivale*' allele	Heterozygous	Homozygous '*urbanum*' allele	Total Loci scored
29A (H)	47.2	9	166	20	195
32C (H)	45.3	7	132	22	161
26B (BCR)	87.9	138	28	7	173
27B (BCR)	70.8	95	113	6	214

Table 5 Genotypic composition at species-specific ddRAD loci for individuals classified as early generation hybrids (H) and backcrosses to *G. rivale* (BCR).

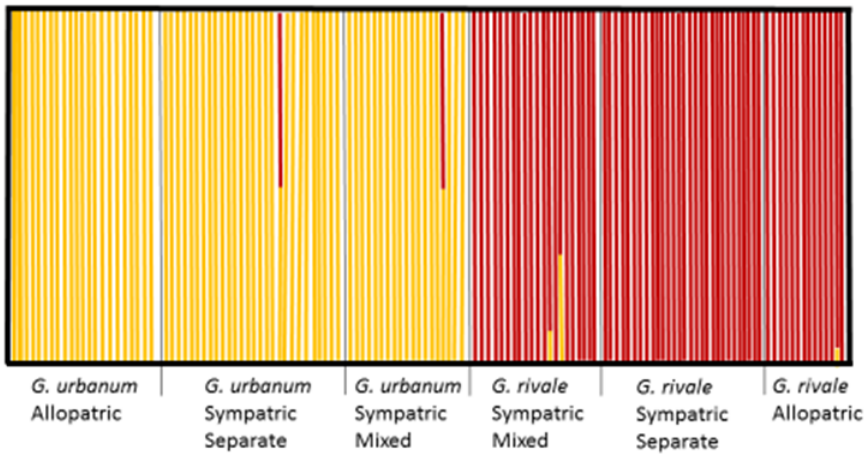


832

833

834 Figure 1. Locations of allopatric populations (large map) of *Geum rivale* and *G. urbanum* and detailed
835 distribution of 39 sympatric populations in eight 10km x 10km squares (inset).

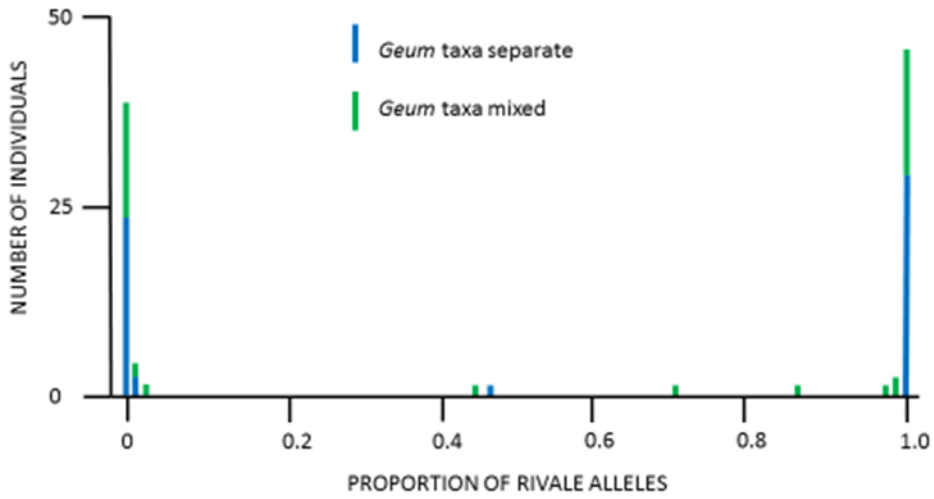
836



837

838 Figure 2. Results of fastSTRUCTURE (Raj et al. 2014) cluster analysis for K=2 based on 188 SNP loci
839 present in all 133 *Geum* individuals scored in the allopatric and sympatric populations. Each bar
840 represents one individual and shows the proportion of the genome from *G. rivale* (red) and *G.*
841 *urbanum* (yellow).

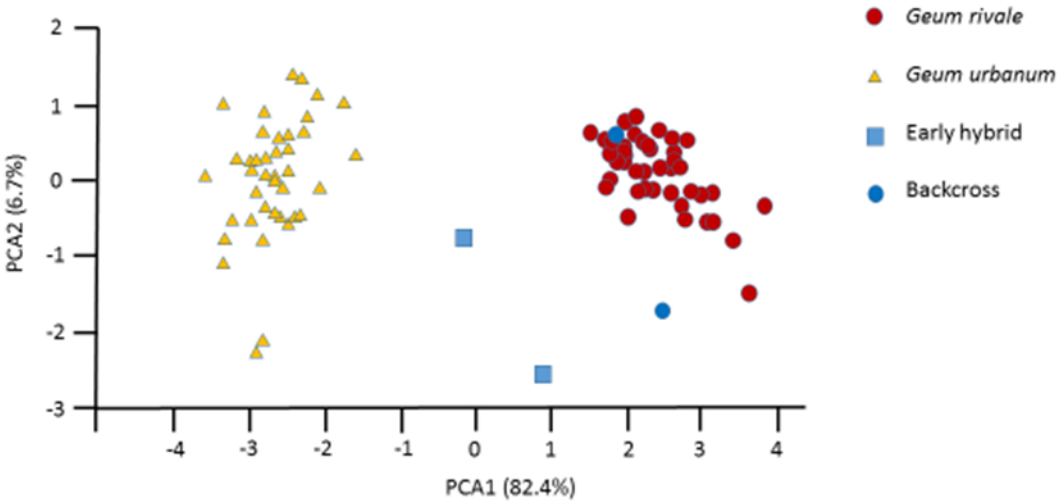
842



843

844 Figure 3. Distribution of hybrid index (proportion of *G. rivale* species-specific alleles) for 96
845 individuals within 39 sympatric populations where *G. rivale* and *G. urbanum* were either found alone
846 (blue bars, 'pure population') or together (green bars, 'mixed population').

847



848

849 Figure 4. PCA analysis based on eight morphological characters measured in a common environment
850 for a sample of 87 individuals of *Geum* sampled from sympatric populations. Genetic class (from
851 Hybrid Index) of each individual (*G. rivale*, *G. urbanum*, early hybrid, or backcross to *G. rivale*) derived
852 from an analysis of 215 species-specific markers is shown.

853

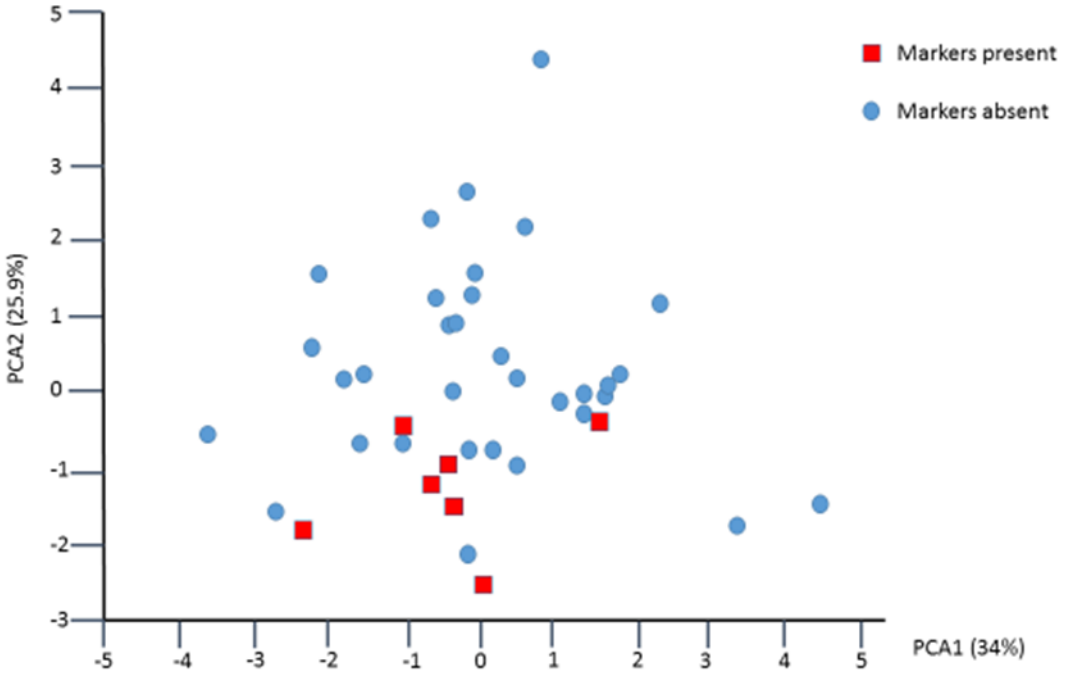


Figure 5. PCA analysis of 40 individuals of *G. urbanum* sampled from sympatric populations based on variation for eight morphological characters measured in a common environment. Red, square individuals jointly possess species-specific SNPs from *G. rivale* at radtags 20454 and 24791.

SUPPLEMENTARY MATERIAL

i. Development of Draft Genome for *G. urbanum*

Reads were generated from three Illumina libraries: a 151 paired end library with a mean insert size of 250 (sequenced on Hi seq 2500 (v1 chemistry) to give 144 million read pairs), a 151 paired end library with a mean insert size of 450 (sequenced on Hi seq to give 134 million read pairs), and a 251 paired end library with a mean insert size of 450 (sequenced on Illumina Mi seq (v2 chemistry) to give 11.9 million read pairs). Illumina adapters were removed and reads quality trimmed using cutadapt version 1.3 (Marcel, 2011) with the option "-q 30". Trimmed reads were assembled into contigs and scaffolds using MaSuRCA version 2.0.3.1; MaSuRCA (Zimin et al., 2013) was run using the command line options:

GRAPH_KMER_SIZE=auto, USE_LINKING_MATES=1, LIMIT_JUMP_COVERAGE = 60, ovlMerSize=30, cgwErrorRate=0.25, ovlMemory=4GB, KMER_COUNT_THRESHOLD = 1, NUM_THREADS= 64,JF_SIZE=23000000000, DO_HOMOPOLYMER_TRIM=0.

ii. Sample Preparation, Library Preparation, and PCR Amplification for ddRAD

For each individual sample, 35ul of genomic DNA (250ung) was digested in a total volume of 50 ul, comprising 1.0 ul SphI-HF (20u), 1.0 ul EcoRI-HF (20u), 5.0 ul 10x Cutsmart buffer (New England Biolabs, UK) and 8.0 ul dH2O for a total of 3 hours, at 37°C. The reaction was purified by the addition of Ampure XP beads at a ratio 1.8 x beads: 1x DNA rather than by heat denaturation, to maintain the integrity of the EcoRI generated overhangs.

Samples were eluted in 25ul EB (Qiagen). Unique barcoded EcoRI P1 adapters (Peterson et al. 2012) and a generic SphI P2 adapter (Table S3) were ligated to the complementary overhangs of the DNA fragments generated by the EcoRI:SphI double digest reaction. Each ligation reaction was carried out in a total volume of 40ul, consisting of 25ul DNA, 2.0 ul P1 adapter (4uM), 2.0 ul P2 adapter (4uM), 4.0 ul 10x T4 Ligase buffer (New England Biolabs, UK) 1ul Quick Ligase (New England Biolabs, UK), and the reaction was incubated at 23°C for 30 min followed by heat denaturation at 65 °C for 15 min.

After ligation the reactions were pooled into libraries containing a maximum of 24 samples, each barcoded with a unique EcoRI PI adapter sequence. The pooled libraries were then subjected to two rounds of Ampure XP bead clean up, at a ratio of 1x Ampure XP beads: 1 x DNA and eluted in a volume of 30ul EB (Qiagen). Individual libraries were size selected by BluePippin Size selection system, 2% Precast Agarose gel cassette (sage science) using the 'Tight' collection mode set to a target size of 300bp. The success of size selection was assessed by Bioanalyzer DNA HS chip (Agilent Technologies).

For each ddRAD library, PCR amplification was carried out in 2 independent 50 µL reactions consisting of 20 ul size selected DNA, 25ul 2× Phusion Master Mix (New England Biolabs, UK), 2.5 ul DMSO, and 1ul ddRAD P1 PCR primer (10 nm), and a barcoded P2 primer (10 nm) that introduced a 8bp unique index during amplification (Table S3), using the following cycling parameters: 98 °C for 30 s followed by 12 cycles of 98 °C for 10 s and 72 °C for 60 s.

PCR products were then pooled, and purified by Ampure XP beads at a ratio of 0.8 x beads: 1x DNA, and the mean insert size of the individual libraries was assessed by Bioanalyzer DNA HS chip, (Agilent Technologies), and were quantified by qPCR on an Illumina Eco instrument using the Kapa Library Quantification Kit.mA final super pool was created consisting of equimolar individual library PCR reactions and the quantification of the pool was measured by qPCR.

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916 SUPPLEMENTARY TABLES

917

Site	Coordinates	Taxon
Ben Lawers, UK	56°32’23.46”N/ 004°17’27.58”W	R (4)
Coire Garblach, UK	57°01’38.29”N/ 003°50’08.17”W	R (4)
Ben Lui, UK	56°23’38.87”N/ 004°49’28.02”W	R (4)
Iceland	64°07'N / 21°50'W	R (1)
Sweden	63°17'27.00"N/ 18°42'54.00"E	R (1)
Selwyn Wood, UK	50°57’43.96”N/ 000°12’40.52”E	U (2)
Combe Wood, UK	51°02’05.03”N/ 000°18’31.39”E	U (2)
Burgh Wood, UK	51°01’21.30”N/ 000°27’13.26”E	U (2)
Hoads Wood, UK	51°08’47.89”N/ 000°47’27.02”E	U (2)
Stanford Bridge, UK	51°09’00.56”N/ 000°45’33.55”E	U (2)
Frith Wood, UK	51°10’22.20”N/ 000°42’59.47”E	U (2)
Priory Wood, UK	51°05’07.33”N/ 000°54’02.91”E	U (2)
Copperhurst, UK	51°04’55.62”N/ 000°56’53.11”E	U (2)
Mill Wood, UK	50°58’47.12”N/ 000°24’56.60”E	U (2)
Punnets Town, UK	50°58’20.12”N/ 000°18’43.66”E	U (2)
Portugal	40°22'43.00"N/ 008°22'11.00"E	U (1)
France	43°16'59.57"N/ 001°28'56.59"E	U (1)

918

919 Table S1. Sampling locations for allopatric populations of *Geum rivale* (R) and *G. urbanum* (U).
920 Numbers in brackets = sample size.

921

Site Number	Elevation(m)	Coordinates	Taxa
1	331	55°49'26.54"N/ 002°46'46.78"W	R (2)
2	271	55°46'34.73"N/ 002°47'05.62"W	U (2)
3	360	55°47'24.57"N/ 002°43'31.04"W	R (2)
4	300	55°42'00.02"N/ 002°45'29.21"W	U (2)
5	232	55°44'28.64"N/ 002°38'33.57"W	R (2)
6	180	55°39'10.43"N/ 002°41'50.99"W	R (2) + U (2)
7	100	55°41'36.40"N/ 002°42'32.59"W	U (2)
8	200	55°43'09.71"N/ 002°41'37.32"W	R (2)
9	230	55°44'09.73"N/ 002°40'25.34"W	R (2)
10	285	55°44'42.61"N/ 002°38'33.91"W	R (2) + U (2)
11	195	55°43'59.74"N/ 002°46'10.80"W	U (2)
12	230	55°45'01.53"N/ 002°33'59.10"W	U (2)
13	207	55°45'32.21"N/ 002°28'43.98"W	R (2)
14	300	55°48'48.07"N/ 002°35'30.28"W	R (2)
15	208	55°48'23.28"N/ 002°30'06.09"W	R (2) + U (2)
16	225	55°40'44.39"N/ 002°37'27.14"W	R (2) + U (2)
17	140	55°39'59.73"N/ 002°33'15.31"W	R (2)
18	175	55°41'09.26"N/ 002°33'42.29"W	U (2)
19	180	55°42'51.82"N/ 002°32'54.37"W	R (2)
20	190	55°42'05.55"N/ 002°35'57.70"W	U (2)
21	247	55°52'05.45"N/ 002°25'54.64"W	R (2)
22	213	55°49'57.38"N/ 002°26'06.32"W	R (2)
23	180	55°50'21.93"N/ 002°22'53.91"W	U (2)
24	236	55°51'59.51"N/ 002°25'42.03"W	R (2) + U (2)
25	120	55°55'13.74"N/ 002°20'45.68"W	U (2)
26	180	55°45'10.70"N/ 002°24'30.61"W	R (2) + U(2)
27	100	55°45'38.64"N/ 002°21'06.79"W	R (2) + U (2)
28	150	55°47'19.70"N/ 002°21'12.24"W	R (2) + U (2)
29	90	55°48'12.97"N/ 002°20'30.05"W	U (2)

30	135	55°41’34.10”N/ 002°25’04.03”W	R (2)
31	140	55°42’46.67”N/ 002°23’30.15”W	U (2)
32	100	55°42’41.00”N/ 002°21’59.39”W	R (2) + U (2)
33	60	55°40’58.23”N/ 002°21’03.75”W	U (2)
34	130	55°42’46.72”N/ 002°25’01.36”W	R (2)
35	220	55°55’03.85”N/ 002°15’55.46”W	R (2)
36	140	55°53’14.06”N/ 002°17’24.90”W	R (2) + U (2)
37	130	55°52’48.31”N/ 002°12’25.08”W	U (2)
38	150	55°55’13.92”N/ 002°11’29.87”W	R (2)
39	150	55°54’23.37”N/ 002°10’22.08”W	U (2)

Table S2. Sampling locations for pure and mixed populations of *Geum rivale* (R) and *G. rivale* (U) within an area of geographic sympatry in Scotland. Numbers in brackets = sample size.

926 Table S3. EcoRI P1 adapters and generic SphI P2 adapter; P1 and P2 primers used in ddRAD
927 amplification

Oligo ID	EcoRI Adapter P1.1_Oligo sequence EcoRI Adapter P1.2_Oligo sequence
ACACGACA_EcoRI_P1.Top	5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTACACGACA-3'
ACACGACA_EcoRI_P1.Bottom	5'-Phos-AATTTGTCGTGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3'
ACGTAGCA_EcoRI_P1.Top	5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTACGTAGCA-3'
ACGTAGCA_EcoRI_Bottom	5'-Phos-AATTTGCTACGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3'
ACTGCTCA_EcoRI_P1. Top	5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTACTGCTCA-3'
ACTGCTCA_EcoRI_P1.Bottom	5'-Phos-AATTTGAGCAGTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3'
AGCTGTGA_EcoRI_P1. Top	5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGCTGTGA-3'
AGCTGTGA_EcoRI_P1.Bottom	5'-Phos-AATTTCACAGCTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3'
AGTCACGA_EcoRI_P1. Top	5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTAGTCACGA-3'
AGTCACGA_EcoRI_P1.Bottom	5'-Phos-AATTTCGTGACTAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3'
ATATCATA_EcoRI_P1. Top	5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTATATCATA-3'
ATATCATA_EcoRI_P1.Bottom	5'-Phos-AATTTATGATATAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3'
CACACAGT_EcoRI_P1. Top	5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTCACACAGT-3'
CACACAGT_EcoRI_P1.Bottom	5'-Phos-AATTACTGTGTGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3'
CATGATCA_EcoRI_P1. Top	5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATACTA-3'
CATGATCA_EcoRI_P1.Bottom	5'-Phos-AATTTGATCATGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3'
CGCGCATA_EcoRI_P1. Top	5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTCGCGCATA-3'

928

CGCGCATA_EcoRI_P1.Bottom	5'-Phos-AATTTATGCGCGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
CTAGTGTC_EcoRI_P1.Top	5' -ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTAGTGTC-3’
CTAGTGTC_EcoRI_P1.Bottom	5'-Phos-AATTGACACTAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
CTCTGCTC_EcoRI_P1. Top	5' -ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTCTGCTC-3’
CTCTGCTC_EcoRI_P1.Bottom	5'-Phos-AATTGAGCAGAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
CTGATGCT_EcoRI_P1. Top	5' -ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTGATGCT-3’
CTGATGCT_EcoRIP1.Bottom	5'-Phos-AATTAGCATCAGAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
GACTGCAG_EcoRI_P1. Top	5' -ACACTCTTTCCCTACACGACGCTCTTCCGATCTGACTGCAG-3’
GACTGCAG_EcoRI_P1.Bottom	5'-Phos-AATTCTGCAGTCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
GATCGTGA_EcoRI_P1. Top	5' -ACACTCTTTCCCTACACGACGCTCTTCCGATCTGATCGTGA-3’
GATCGTGA_EcoRI_P1.Bottom	5'-Phos-AATTTCACGATCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
GCATGTGC_EcoRI_P1. Top	5' -ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCATGTGC-3’
GCATGTGC_EcoRI_P1.Bottom	5'-Phos-AATTGCACATGCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
GCTACAGC_EcoRI_P1 Top	5' -ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTACAGC-3’
GCTACAGC__EcoRI_P1.Bottom	5'-Phos-AATTGCTGTAGCAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
GTACATCA_EcoRI_P1. Top	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTACATCA-3’
GTACATCA_EcoRI_P1.Bottom	5'-Phos-AATTTGATGTACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
GTGTACTG_EcoRI_P1 Top	5' -ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTGTACTG-3’
GTGTACTG_EcoRI_P1.Bottom	5'-Phos-AATTCAGTACACAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’

929

TACGATAT_EcoRI_P1.Top	5' -ACACTCTTTCCCTACACGACGCTCTTCCGATCTTACGATAT
TACGATAT_EcoRI_P1.Bottom	5'-Phos-AATTATATCGTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
TCAGCATC_EcoRI_P1. Top	5' -ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCAGCATC-3’
TCAGCATC_EcoRI_P1.Bottom	5'-Phos-AATTGATGCTGAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
TCGAGTGA_EcoRI_P1 Top	5' -ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGAGTGA-3’
TCGAGTGA_EcoRI_P1.Bottom	5'-Phos-AATTTCACTCGAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
TCTCTCGA_EcoRI_P1. Top	5' -ACACTCTTTCCCTACACGACGCTCTTCCGATCTTCTCTCGA-3’
TCTCTCGA_EcoRI_P1.Bottom	5'-Phos-AATTTCGAGAGAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
TGCACTAC_EcoRI_P1. Top	5' -ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCACTAC-3’
TGCACTAC_EcoRI_P1.Bottom	5'-Phos-AATTGTAGTGCAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
TGTGACTG_EcoRI_P1. Top	5' -ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGTGACTG-3’
TGTGACTG_EcoRI_P1.Bottom	5'-Phos-AATTCAGTCACAAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3’
Oligo ID	**SphI Adapter P2.1_Oligo sequence** **SphI Adapter P2.2_Oligo sequence**
SphI-P2_Top	5’- Phos-AGATCGGAAGAGCGAGAACAA-3’
SphI-P2_Bottom	5'-GTGACTGGAGTTCAGACGTGTGCT CTTCCGATCTCATG-3'

930

Oligo ID	P1 PCR primer_Oligo sequence
P1 PCR primer	5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG-3'
Oligo ID	**P2 PCR primer_Oligo sequence**
ddRAD_PCR2_Idx_AACGTGAT	5'- CAAGCAGAAGACGGCATACGAGATAACGTGATGTGACTGGAGTTCAGACGTGTGC-3'
ddRAD_PCR2_Idx_CGCTGATC	5'- CAAGCAGAAGACGGCATACGAGATCGCTGATCGTGACTGGAGTTCAGACGTGTGC-3'
ddRAD_PCR2_Idx_ACAAGCTA	5'- CAAGCAGAAGACGGCATACGAGATACAAGCTAGTGACTGGAGTTCAGACGTGTGC-3'
ddRAD_PCR2_Idx_CTGTAGCC	5'- CAAGCAGAAGACGGCATACGAGATCTGTAGCCGTGACTGGAGTTCAGACGTGTGC-3'
ddRAD_PCR2_Idx_ACGCTCGA	5'- CAAGCAGAAGACGGCATACGAGATACGCTCGAGTGACTGGAGTTCAGACGTGTGC-3'
ddRAD_PCR2_Idx_ACGTATCA	5'- CAAGCAGAAGACGGCATACGAGATACGTATCAGTGACTGGAGTTCAGACGTGTGC -3'
ddRAD_PCR2_Idx_ACTATGCA	5'- CAAGCAGAAGACGGCATACGAGATACTATGCAGTGACTGGAGTTCAGACGTGTGC -3'
ddRAD_PCR2_Idx_AGAGTCAA	5'- CAAGCAGAAGACGGCATACGAGATAGAGTCAAGTGACTGGAGTTCAGACGTGTGC -3'
ddRAD_PCR2_Idx_AGATCGCA	5'- CAAGCAGAAGACGGCATACGAGATAGATCGCAGTGACTGGAGTTCAGACGTGTGC-3'
ddRAD_PCR2_Idx_AGCAGGAA	5'- CAAGCAGAAGACGGCATACGAGATAGCAGGAAGTGACTGGAGTTCAGACGTGTGC-3'
ddRAD_PCR2_Idx_AGTCACTA	5'- CAAGCAGAAGACGGCATACGAGATAGTCACTAGTGACTGGAGTTCAGACGTGTGC-3'
ddRAD_PCR2_Idx_ATCCTGTA	5'- CAAGCAGAAGACGGCATACGAGATATCCTGTAGTGACTGGAGTTCAGACGTGTGC-3'
ddRAD_PCR2_Idx_ATTGAGGA	5'- CAAGCAGAAGACGGCATACGAGATATTGAGGAGTGACTGGAGTTCAGACGTGTGC-3'
ddRAD_PCR2_Idx_CACTTCGA	5'- CAAGCAGAAGACGGCATACGAGATCACTTCGAGTGACTGGAGTTCAGACGTGTGC -3'
ddRAD_PCR2_Idx_CAGCGTTA	5'- CAAGCAGAAGACGGCATACGAGATCAGCGTTAGTGACTGGAGTTCAGACGTGTGC -3'
ddRAD_PCR2_Idx_CATACCAA	5'- CAAGCAGAAGACGGCATACGAGATCATACCAAGTGACTGGAGTTCAGACGTGTGC -3'
ddRAD_PCR2_Idx_CCAGTTCA	5'- CAAGCAGAAGACGGCATACGAGATCCAGTTCAGTGACTGGAGTTCAGACGTGTGC -3'
ddRAD_PCR2_Idx_GTGTTCTA	5'- CAAGCAGAAGACGGCATACGAGATGTGTTCTAGTGACTGGAGTTCAGACGTGTGC-3'
ddRAD_PCR2_Idx_GCTCGGTA	5'- CAAGCAGAAGACGGCATACGAGATGCTCGGTAGTGACTGGAGTTCAGACGTGTGC -3'
ddRAD_PCR2_Idx_GGAGAACA	5'- CAAGCAGAAGACGGCATACGAGATGGAGAACAGTGACTGGAGTTCAGACGTGTGC -3'
ddRAD_PCR2_Idx_GTGTTCTA	5'- CAAGCAGAAGACGGCATACGAGATGTGTTCTAGTGACTGGAGTTCAGACGTGTGC -3'
ddRAD_PCR2_Idx_TAGGATGA	5'- CAAGCAGAAGACGGCATACGAGATTAGGATGAGTGACTGGAGTTCAGACGTGTGC -3'
ddRAD_PCR2_Idx_TGGCTTCA	5'- CAAGCAGAAGACGGCATACGAGATTGGCTTCAGTGACTGGAGTTCAGACGTGTGC-3'
ddRAD_PCR2_Idx_TTCACGCA	5'- CAAGCAGAAGACGGCATACGAGATTTCACGCAGTGACTGGAGTTCAGACGTGTGC-3'
ddRAD_PCR2_Idx_GCCAAGAC	5'- CAAGCAGAAGACGGCATACGAGATGCCAAGACGTGACTGGAGTTCAGACGTGTGC-3'

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	Unfiltered for paralogs	
Population by species	Number of SNPs	F_{IS}
G. rivale		
Ben Lawers	2531	-0.0082
Coire Garblach	2605	0.0384
Ben Lui	2664	0.0423
G. urbanum		
Priory Wood	551	0.6216
Mill Wood	531	-0.0697
Burgh Wood	542	0.5913
Hoades Wood	451	0.5100
Punnetts Town	495	0.4636
Frith Wood	507	0.6134
Stanford Bridge	535	0.6112
Combe Wood	536	0.6110
Copperhurst	509	0.5874
Selwyn Wood	166	-0.4759

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935 Table S4. F_{IS} estimates for 'allopatric' UK *G. rivale* and *G. urbanum* populations using data not filtered
936 for paralogs.

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Sample	Heterozygosity	No. SNPs private to *G. rivale*	No. SNPs private to *G. urbanum*	No. SNPs shared by both species	No. alternately fixed SNPs	No. DNA blocks
Ben Lui 1		250	88	0	1184	668
Ben Lui 4		299	86	1	1170	672
Ben Lawers 5		179	88	0	1189	649

942

943 Table S5: Polymorphism counts and numbers of DNA blocks for coalescent analysis for three
944 individual *G. rivale* samples from UK allopatric populations.

945

	Model		
	div_2	$IM_{r \rightarrow u}$	$IM_{u \rightarrow r}$
Ben Lui 1			
$N_{anc} = N_{rivb}$	$1.03 * 10^5$	$1.01 * 10^5$	$6.42 * 10^4$
N_{urb}	$3.46 * 10^4$	$3.23 * 10^4$	$3.29 * 10^4$
t (years)	$2.36 * 10^6$	$2.41 * 10^6$	$3.03 * 10^6$
M		0.02 (0.00 – 0.18)	0.08 (0.06 – 0.11)
Ben Lui 4			
$N_{anc} = N_{rivb}$	$1.30 * 10^5$	$1.24 * 10^5$	$1.11 * 10^5$
N_{urb}	$3.77 * 10^4$	$3.09 * 10^4$	$3.38 * 10^4$
t (years)	$2.22 * 10^6$ ($2.01 * 10^6$ – $2.43 * 10^6$)	$2.35 * 10^6$	$2.48 * 10^6$
M (mean, 95% CI's)		0.04 (0.005 - 0.191)	0.04 (0.007 - 0.101)
Ben Lawers 5			
$N_{anc} = N_{rivb}$	$7.41 * 10^4$	$7.34 * 10^4$	$5.11 * 10^4$
N_{urb}	$3.54 * 10^4$	$3.40 * 10^4$	$3.38 * 10^4$
t (years)	$2.60 * 10^6$	$2.64 * 10^6$	$3.10 * 10^6$
M (mean, 95% CI's)		0.01 (0.00 – 0.103)	0.02 (0.002 - 0.052)

946

947 Table S6 Parameter estimates under models analysed for sample pairs involving the samples: Ben Lui
948 1, Ben Lui 4, and Ben Lawers 5. 95% Confidence intervals provided in parentheses. See Figure S4 for
949 plots of 95% CI's.

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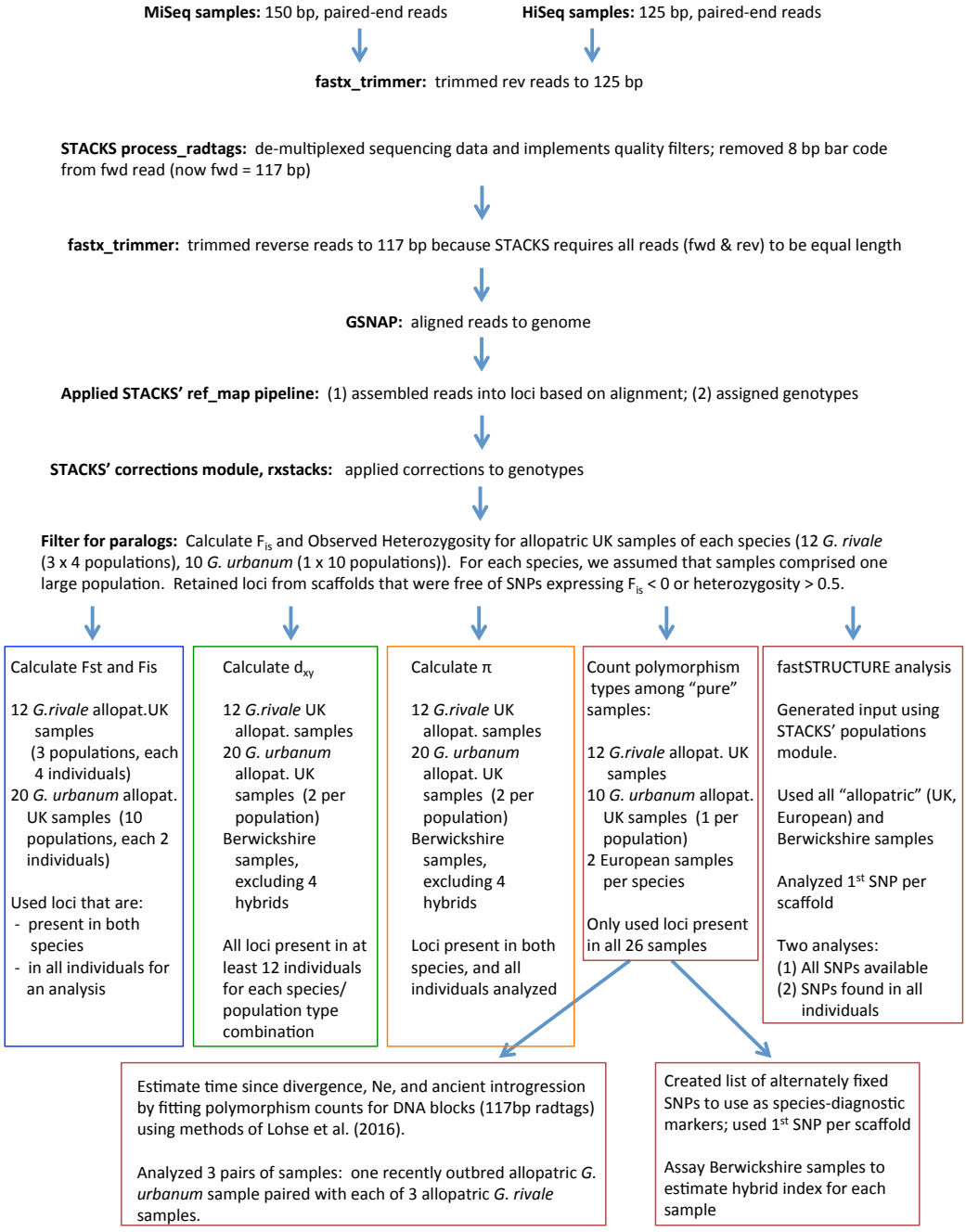
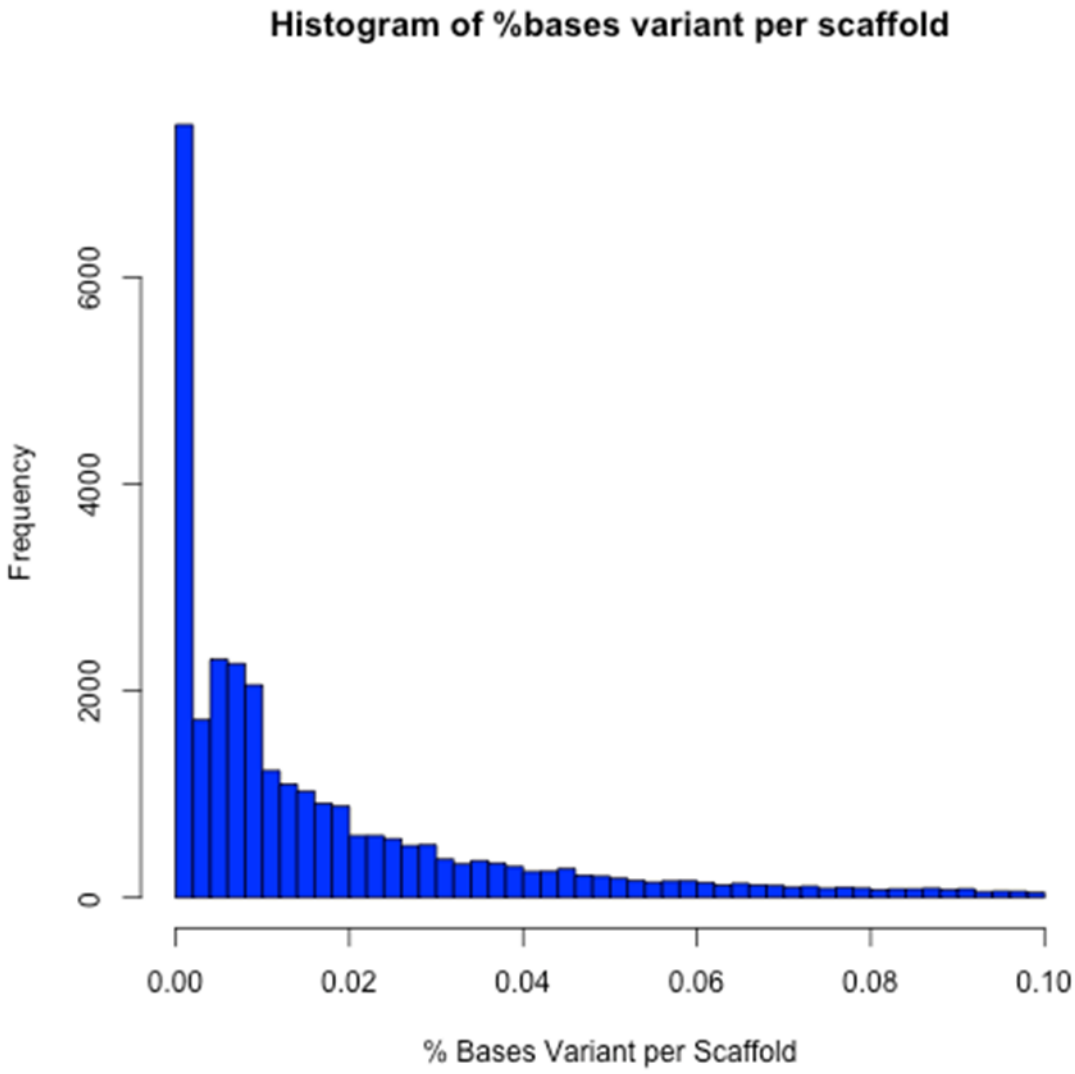
952 SUPPLEMENTARY FIGURES



953

954 Figure S1: Bioinformatics pipeline for analysis of *Geum* ddRAD sequences.

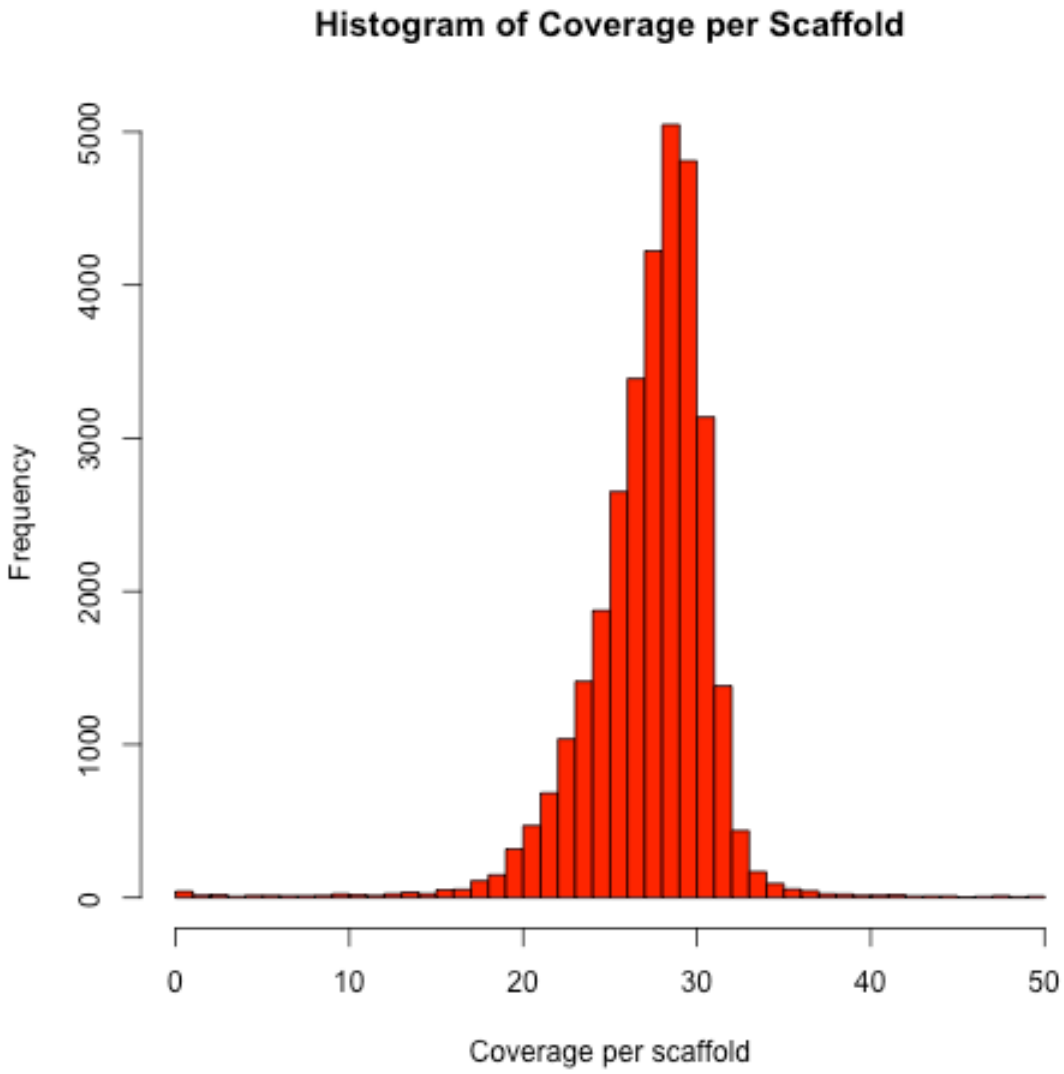


955

956 Figure S2.

957

958



959

960 Figure S3

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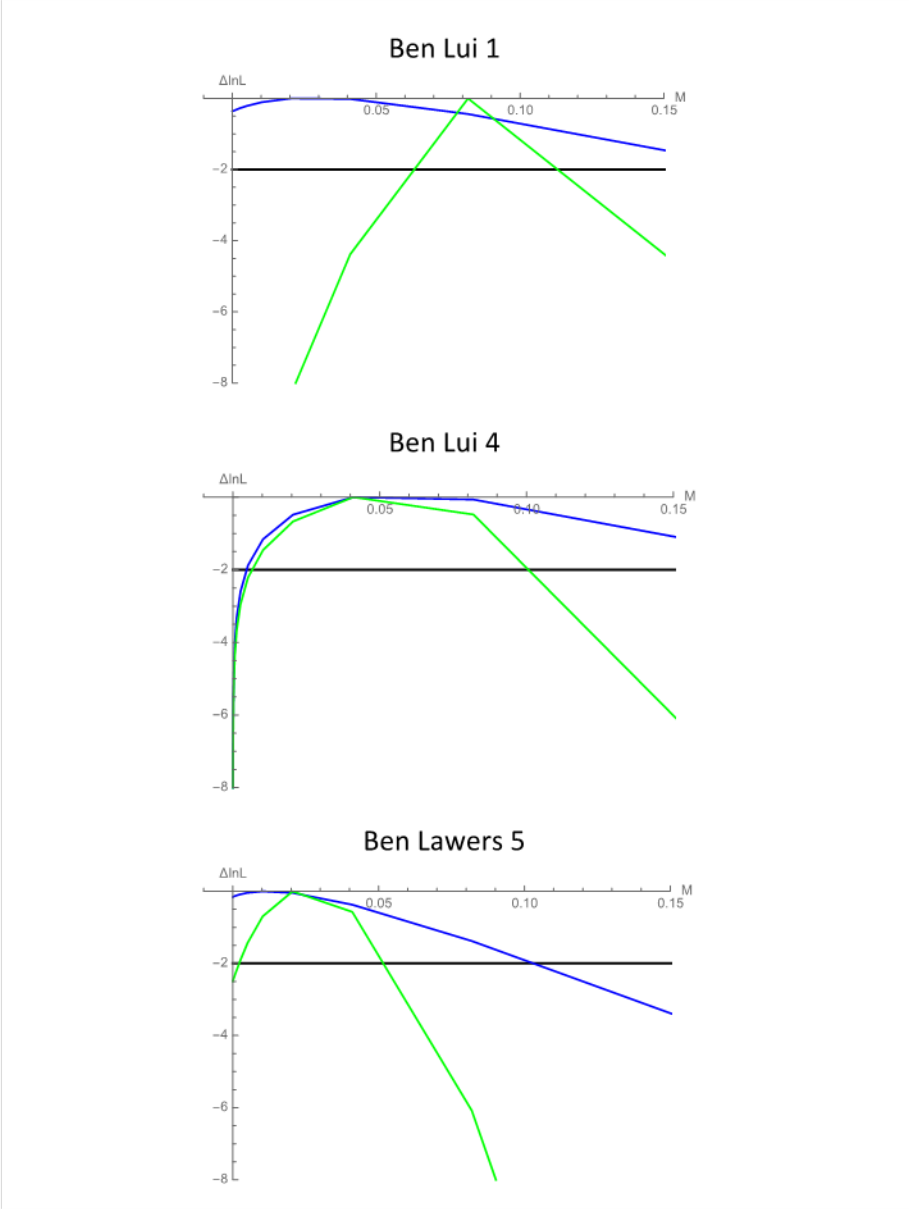

962

963 Figure S4: Log-Likelihood plots for estimates of M for three pairs of *Geum* samples, each involving a
964 different *G. rivale* individual (Ben Lui 1, Ben Lui 4, Ben Lawers 5). Blue and green lines represent log-
965 likelihoods of M for models $IM_{r \rightarrow u}$ and $IM_{u \rightarrow r}$, respectively. 95% CI's are bounded where these lines
966 intersect the dark horizontal line.

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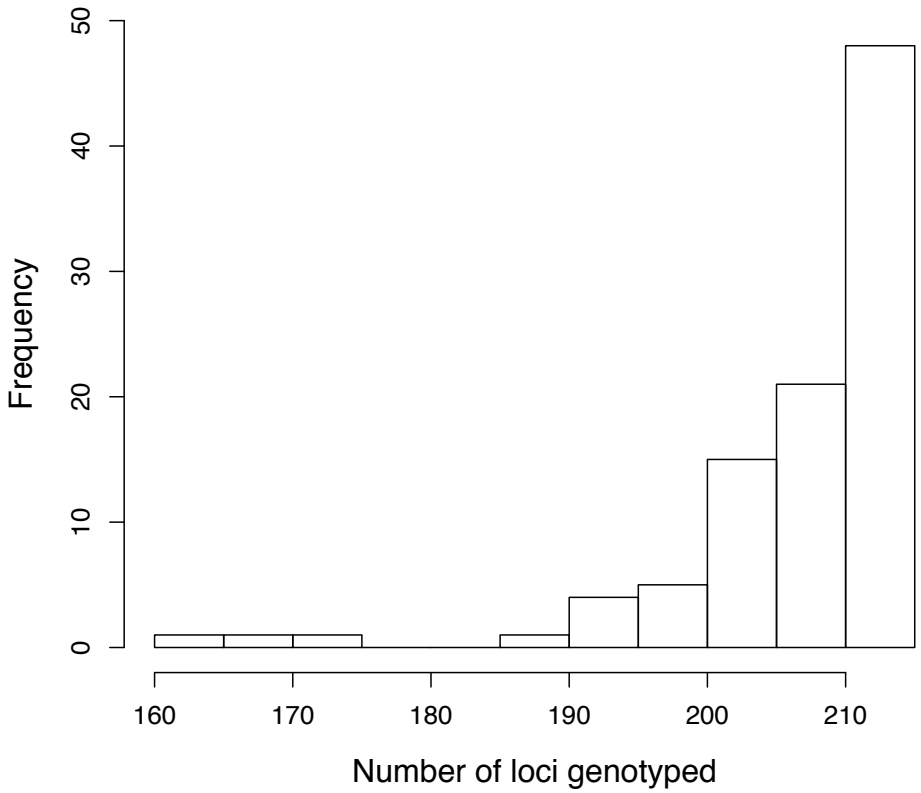
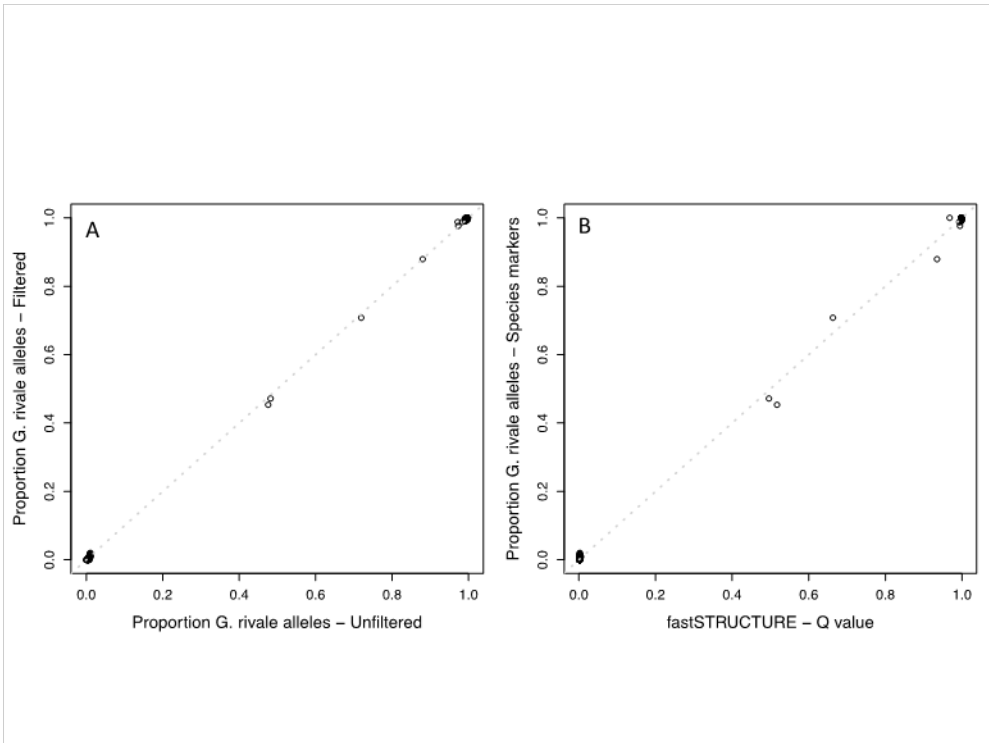
968



969

970 Figure S5. Number of species-specific SNPs scored per individual in a sample of 97 *Geum* plants from
971 39 populations in Berwickshire.

972



973

974 Figure S6. Genomic composition estimates for Berwickshire samples derived from paralog-filtered
975 species-diagnostic SNPs (y-axis in both panels) compared to analogous measures in two alternate
976 analyses: (A) species-diagnostic SNPs from data unfiltered for paralogs, and (B) Admixture
977 proportion (Q value) calculated by fastSTRUCTURE analysis that required loci to be present in all
978 individuals (188 SNPs).

979

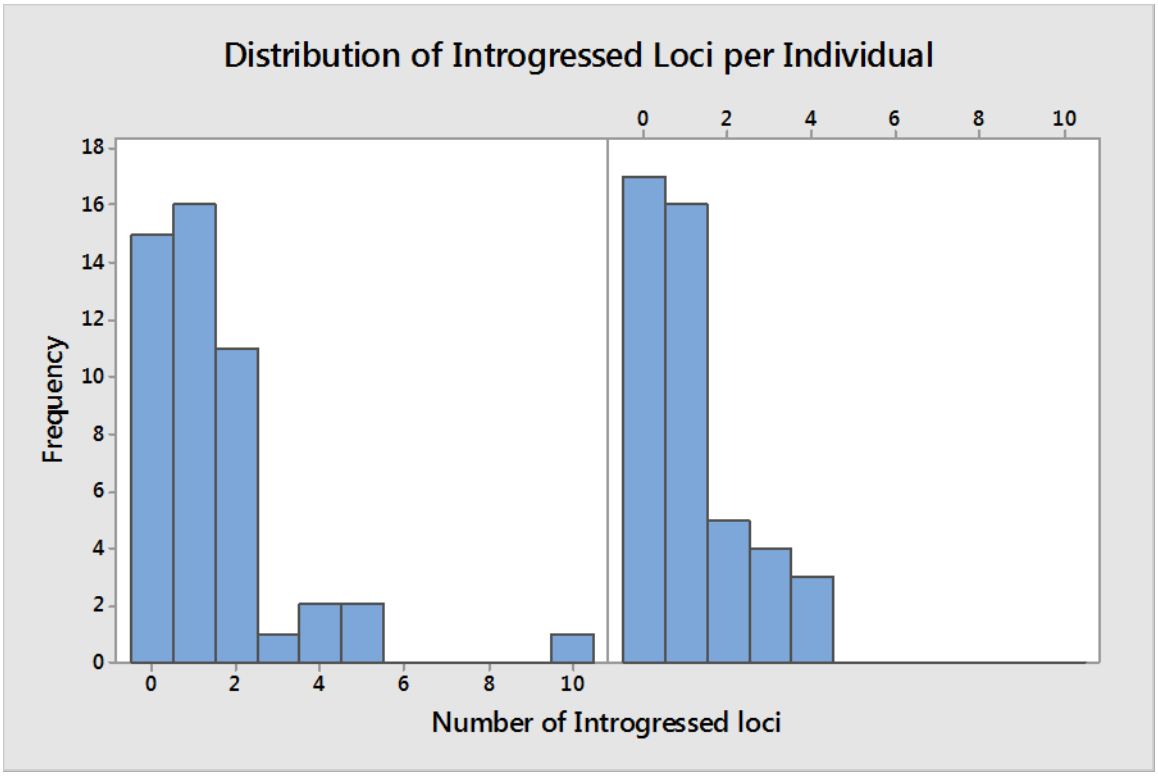


Figure S7. Number of putatively introgressed loci per individual in individuals classified as *G. rivale* (left panel) and *G. urbanum* (right panel) sampled from 39 populations in Berwickshire.