

Title

Potential limits to the benefits of admixture during biological invasion

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Abstract

Species introductions often bring together genetically divergent source populations, resulting in genetic admixture. This geographic reshuffling of diversity has the potential to generate favorable new genetic combinations, facilitating the establishment and invasive spread of introduced populations. Observational support for the superior performance of admixed introductions has been mixed, however, and the broad importance of admixture to invasion questioned. Under most underlying mechanisms, admixture's benefits should be expected to increase with greater divergence among and lower genetic diversity within source populations, though these effects have not been quantified in invaders. We experimentally crossed source populations differing in divergence in the invasive plant *Centaurea solstitialis*. Crosses resulted in many positive (heterotic) interactions, but fitness benefits declined and were ultimately negative at high source divergence, with patterns suggesting cyto-nuclear epistasis. We explored the literature to assess whether such negative epistatic interactions might be impeding admixture at high source population divergence. Admixed introductions reported for plants came from sources with a wide range of genetic variation, but were disproportionately absent where there was high genetic divergence among native populations. We conclude that while admixture is common in species introductions and often happens under conditions expected to be beneficial to invaders, these conditions may be constrained by predictable negative genetic interactions, potentially explaining conflicting evidence for admixture's benefits to invasion.

70 **Introduction**

Introduced non-native species are now a common feature of ecosystems on Earth, and they are recognized as being one of the dominant sources of biodiversity change in the Anthropocene (Ellis *et al.* 2012; Vellend *et al.* 2013). While many factors will shape the success of species introductions and their ecological interactions with recipient environments (Sakai *et al.* 2001),
 75 there is increasing evidence that genetic factors can play a role in this process (Baker & Stebbins 1965; Ellstrand & Schierenbeck 2000; Lee 2002; Cox 2004; Colautti & Barrett 2013; Rius & Darling 2014; Whitney & Gering 2015; Mesgaran *et al.* 2016). Understanding when, where, and how genetic changes influence the outcomes of colonization is likely to be crucial to resolving broader questions about when species introductions will lead to establishment and invasive
 80 spread (Hufbauer 2008, 2017; Lee & Gelembiuk 2008; Forsman 2014; Rius & Darling 2014; Szűcs *et al.* 2014, 2017a; Bock *et al.* 2015; Colautti & Lau 2015; Dlugosch *et al.* 2015a; Williams *et al.* 2016; Ochocki & Miller 2017; Weiss-Lehman *et al.* 2017).

In particular, successful introductions often result from admixture between divergent genotypes
 85 originating from different source populations (Dlugosch & Parker 2008a; Uller & Leimu 2011; Dlugosch *et al.* 2015a). Admixture has the potential to facilitate species invasions by creating unique opportunities for positive genetic interactions among previously isolated alleles and for adaptive evolution of novel genotypes, which could increase the fitness of admixed populations (Kolbe *et al.* 2004; Lavergne & Molofsky 2007; Keller & Taylor 2010; Wagner *et al.* 2017).
 90 These same mechanisms are known to have contributed directly to non-native species establishment and the rise of particularly invasive novel genotypes in cases involving hybridization between species, and the potential for similar benefits of admixture within species appears widespread (Ellstrand & Schierenbeck 2000; Drake 2006; Hovick & Whitney 2014). Consequently, there is intensifying interest in the potential for genetic admixture to provide a
 95 general mechanism by which many non-native species are able to establish and develop into invaders (Frankham 2005; Hufbauer 2008, 2017; Verhoeven *et al.* 2011; Molofsky *et al.* 2014; Rius & Darling 2014; Bock *et al.* 2015; Dlugosch *et al.* 2015a).

Given that introduced species are often derived from multiple source populations, genetic
 100 admixture could be a frequent path to the evolution of invasiveness, but only if its fitness effects are typically positive under conditions commonly experienced during introductions. Positive correlations between fitness traits and evidence of admixture have been identified in some invasions (Keller *et al.* 2014; Rius & Darling 2014), and experimental admixtures have performed better in several studies (Turgeon *et al.* 2011; van Kleunen *et al.* 2015; Wagner *et al.*
 105 2017). On the other hand, studies that have found no association between admixture and increased invasiveness have called into question whether mixing of divergent populations can realistically be expected to contribute to increased fitness and introduction success across many invaders (Wolfe *et al.* 2007; Dutech *et al.* 2012; Chapple *et al.* 2013). These conflicting results are not necessarily surprising given that studies of native species have long demonstrated that
 110 mating across different populations can have fitness effects ranging from positive to detrimental, depending upon the mechanisms underlying the interactions between genotypes and their fitness effects (Price & Waser 1979; Lynch 1991; Edmands 1999; Keller & Waller 2002; Reed & Frankham 2003; Birchler *et al.* 2010; Frankham *et al.* 2011; Chen 2013).

There are several non-mutually-exclusive mechanisms that could generate positive fitness effects as a consequence of either the genetic interactions that can result from bringing together new combinations of alleles within individuals or the increase in genetic diversity that should result from combining divergent populations (Lynch 1991; Dlugosch *et al.* 2015a; Hufbauer 2017):

1. *Genetic Rescue*. Also known as ‘directional dominance’ (Birchler *et al.* 2010; Chen 2013), genetic rescue refers to the rescue of deleterious inbred (homozygous) loci by outbreeding with a divergent population (Tallmon *et al.* 2004). Homozygous loci in introduced populations can be derived from both historic genetic load already present in native source population, and additional fixation of deleterious variants during founding events (Excoffier *et al.* 2009). Multiple introductions from divergent sources can therefore provide genetic rescue to an establishing population by contributing/restoring superior alleles that increase the fitness of introduced genotypes, potentially resulting in superior genotypes that transgress the fitness of both parental populations (Ellstrand & Schierenbeck 2000; Hufbauer 2008; Keller & Taylor 2010; Rius & Darling 2014; van Kleunen *et al.* 2015). Genetic rescue should benefit invasions when diversity within founding populations is low and there is significant genetic load to rescue (Lynch *et al.* 1995; Lohr & Haag 2015). The fitness benefits should occur in the first generation of admixture and scale positively with divergence between source populations, until all loci are rescued and fitness gains plateau (Lynch 1991).
2. *Overdominance*. Also known as ‘heterozygote advantage’, overdominance occurs when heterozygous allele combinations have higher fitness than any homozygous genotype (Birchler *et al.* 2010; Chen 2013). These effects are expected to manifest primarily in the first (F1) generation of an admixture event, but decay quickly due to the increase in homozygosity that occurs over subsequent generations, unless heterozygosity can be preserved by asexual propagation, polyploidy, or other means (Ellstrand & Schierenbeck 2000; Drake 2006; Facon *et al.* 2008). These effects should be strongest when opportunities for novel heterozygosity in admixed progeny are highest, i.e. when there are greater numbers of fixed differences between source populations.
3. *Epistasis*. Epistasis occurs when alleles at different loci interact (Phillips 2008). Epistatic interactions that arise from admixture are predicted to have increasing effects on fitness as divergence among source populations increases, due to natural selection for locally co-adapted allele combinations and/or to the build up of Bateson-Dobzhansky-Muller incompatibilities from genetic drift, acting separately in each source population (Lynch & Walsh 1998; Moyle & Nakazato 2010). The fitness effects of epistatic interactions in the first generation can be positive (heterotic) but will become increasingly negative with greater genetic distance between parents, particularly in later generations when co-adapted multi-locus genotypes are broken apart by recombination (i.e. ‘hybrid breakdown’ and ‘outbreeding depression’; Lynch 1991; Orr & Turelli 2001; Bomblies *et al.* 2007; Frankham *et al.* 2011). Negative epistatic effects are thought to be one of the most important paths to reproductive isolation and speciation, and could impede admixture of divergent sources during multiple introductions, though the influence of

these effects on species invasions is rarely discussed and largely unknown (Carroll *et al.* 2003; Dlugosch *et al.* 2015a).

4. *Complementarity*. As diversity increases within a population, different genotypes may occupy somewhat different and complementary niches, sometimes increasing the mean fitness across the population as a whole (Crawford & Whitney 2010; Chen *et al.* 2015). Complementarity will occur immediately under multiple introductions, even before interbreeding. It should be most beneficial at low genetic diversity within a focal population, where niche diversity among genotypes is low, and become increasingly likely as divergence between admixing populations increases and greater numbers of genotypes are combined (Wang *et al.* 2012; Le Roux *et al.* 2014), though this effect may plateau with diversity after niches are exhausted (Ellers *et al.* 2011).
5. *Evolutionary Rescue*. Finally, across multiple generations and longer timescales, populations that will go extinct or fail to spread because they lack adaptation to local conditions could be rescued by inputs of additional genetic variation, a scenario known as ‘evolutionary rescue’ (Carlson *et al.* 2014). For introduced species in particular, additional inputs of genetic diversity could facilitate both adaptation to the novel environment of introduction and adaptation in traits that facilitate colonization in general, such as increased dispersal ability (Thompson 1998; Cox 2004; Holt *et al.* 2005; Phillips *et al.* 2006; Prentis *et al.* 2008; Weiss-Lehman *et al.* 2017). Evolutionary rescue should be most likely in populations with low genetic diversity (such that adaptive variation is limiting), and increasingly impactful as the divergence between admixing populations increases and combines a greater numbers of unique alleles (Rieseberg *et al.* 2007; Wagner *et al.* 2017; Ochocki & Miller 2017; Szűcs *et al.* 2017).

Based on these mechanisms, fitness benefits from admixture should be expected to vary in predictable ways (Fig. 1a,b). As divergence between sources increases, opportunities for rescue of genetic load, the creation of overdominant heterozygotes, epistatic interactions among loci, complementarity, and evolutionary rescue should all increase. These interactions should all be positive for fitness at low divergence, though benefits of most effects should ultimately plateau, and epistatic interactions will become increasingly negative, with increasing divergence (Fig. 1a). Benefits of all mechanisms other than epistasis should also be strongest where founding populations harbor low within-population diversity (Fig. 1b), especially to the extent that this represents fixation of deleterious alleles (Lohr & Haag 2015) [with the caveat that species with a history of inbreeding may have purged genetic load (Crnokrak & Barrett 2002) and therefore stand to benefit only from evolutionary rescue, complementarity, and/or overdominance when at low genetic variation]. Thus the fitness effects of admixture are expected to vary in magnitude under different scenarios, but to be either positive or neutral under most mechanisms other than epistasis (Lynch 1991; Dlugosch *et al.* 2015a; Hufbauer 2017), consistent with the idea that admixture could be almost universally beneficial to invaders (Frankham 2005; Hufbauer 2008, 2017; Verhoeven *et al.* 2011; Rius & Darling 2014).

With these considerations in mind, where do admixture events and their benefits to introduced species fall in the parameter space of divergence among source populations and genetic variation

within founding populations? To date there has been little study of the divergence among potentially admixing populations during species introductions (Dlugosch *et al.* 2015a). The question of how much genetic diversity is available in introduced populations has received considerably more attention. Previous studies have shown that introduced populations generally do not experience large reductions in diversity relative to native populations (though certainly many exceptions exist) and that low levels of marker diversity do not prevent adaptation (Dlugosch & Parker 2008a; b; Uller & Leimu 2011; Szűcs *et al.* 2017a). Nevertheless, a lack of strong founder/bottleneck effects does not preclude the presence of historical genetic load and/or low diversity derived from source populations. Manipulations of genetic diversity have shown a range of effects on the performance of experimental invading populations, and the potential benefits of admixture in this regard remain an active area of research (Crawford & Whitney 2010; Szűcs *et al.* 2014, 2017a; Williams *et al.* 2016; Hufbauer 2017; Wagner *et al.* 2017; Ochocki & Miller 2017; Weiss-Lehman *et al.* 2017).

Species introductions might thus create particularly abundant opportunities for genetic interactions (mechanisms 1-3 above) which can provide immediate fitness benefits in the first generation of admixture and will be especially relevant to establishing admixed populations. In this study, we experimentally test for fitness effects of genetic interactions in controlled crosses of *Centaurea solstitialis* L. ('yellow starthistle'; Asteraceae), a highly invasive annual plant in the Americas. We cross native populations that span a range of genetic divergence and diversity to test for associations between the fitness of admixed progeny and these factors, which are expected to shape the outcome of genetic interactions that will be manifest in early generations of admixed mating. We ask whether fitness benefits increase as expected. We then explore the literature to put our results into a broader context by asking whether reported cases of admixture in introduced plants are being realized under conditions in which we might expect admixture to be favorable for many invaders, and whether there might be limits on these benefits due to negative genetic interactions. We interpret our findings with respect to the likelihood that admixture is a general mechanism promoting the invasiveness of introduced species.

Materials and Methods

Study System

We experimentally tested for genetic interactions in controlled crosses among native populations of *C. solstitialis* spanning a range of genetic diversity and divergence. This species was introduced in large numbers to the Americas as a seed contaminant of alfalfa stock imported from the Old World, where it escaped agricultural fields and became a major pest of grasslands (Gerlach 1997). A lineage in western Europe appears to be the product of ancient admixture between populations from eastern Europe and Asia, and has served as a bridgehead for invasions in the Americas (Barker *et al.* 2017). Several invading populations show evidence of additional recent admixture with other native populations (Dlugosch *et al.* 2013; Eriksen *et al.* 2014; Barker *et al.* 2017). Phenotypic studies have revealed evolutionary increases in plant size during this range expansion, which is associated with higher fitness (Widmer *et al.* 2007; Eriksen *et al.* 2012; Dlugosch *et al.* 2015b). Experimental crosses in *C. solstitialis* provide an opportunity to better understand how admixture might be influencing the success of invading lineages.

Collections

250 Genotypes of *C. solstitialis* were collected from 21 native sites (Supporting Information Table S1) spanning multiple potential source regions across western and eastern Europe, Asia, and southern Greece (Gerlach 1997; Tutin *et al.* 2010; Barker *et al.* 2017). Seeds were collected from wild plants during August–September 2008, from each of 9–22 maternal plants located at least 1 m apart along a linear transect at each site. In Asia, seeds of mothers at each site were
 255 combined into bulk collections by site. The species was identified by the authors according to the Flora Europaea (Tutin *et al.* 2010) and vouchers from each sampling site are available at the University of Arizona herbarium (ARIZ, accession numbers in Supporting Information Table S1).

260 ***Genetic variation within and divergence among native populations***

We previously identified four geographically-structured, genetically-divergent populations in the native range (Fig. 2a) using population genomic analyses of double-digest Restriction site Associated Sequences (ddRADseq; Barker *et al.* 2017). Here we use single nucleotide polymorphism (SNP) information from these ddRADseq reads to quantify genome-wide
 265 sequence divergence among genotypes from these four populations ($N = 155$ individuals; Supporting Information Table S1; NCBI sequence read archive BioProject PRJNA275986). Detailed methods for sequencing and SNP generation are described in Barker *et al.* (2017). Briefly, total genomic DNA was isolated using a CTAB/PVP DNA extraction protocol (Webb & Knapp 1990), and digested with enzymes *PstI* and *MseI* to create fragments for ddRADseq
 270 (Peterson *et al.* 2012). Unique combinations of individual P1 and P2 barcoded adapters were annealed to each sample, and resulting libraries size selected for fragments 350–650 bp. Size-selected libraries were enriched using 14 PCR cycles and sequenced on an Illumina HiSeq 2000 or 2500 to generate 100 bp paired-end reads. Reads were quality-filtered and de-multiplexed using SNOWHITE 2.0.2 (Dlugosch *et al.* 2013). R1 reads were trimmed to a uniform length of 76
 275 bp for final SNP analysis. The `denovo_map.pl` pipeline program in STACKS 1.20 (Catchen *et al.* 2011, 2013) was used to merge identical reads into 'stacks', identify polymorphic sites, create a catalog of loci across individuals, and determine the allelic state at each locus in each individual (Barker *et al.* 2017). Here the 'populations' module in STACKS was used to export polymorphisms for our focal populations, and a locus was required to be genotyped in at least
 280 70% of the samples in each population ($-r\ 0.7$), and have a minimum stack depth ($-m$) of ten. This resulted in 1585 polymorphic ddRADseq SNPs (Dryad doi:##.###/dryad.#####). To quantify genetic variation, we calculated intrapopulation nucleotide diversity (π) as the average number of nucleotide differences among alleles (including invariant sites) for each ddRADseq locus using the 'populations' module in STACKS. To quantify divergence between two
 285 populations, we calculated pairwise interpopulation π (i.e. D_{xy}) between alleles at the same locus from each population. A custom script was used to combine polymorphism data from all loci to obtain π across the total length of all sequence tags.

Experimental crosses

290 Experimental crosses were conducted to compare the performance of matings within and among the four native populations, using parents reared in a common environment (Supporting Information Fig. S1). We reared parents to flowering in a glasshouse at the University of Arizona (as in Dlugosch *et al.* 2015b). Between 9 and 22 parents / site were reared from seeds of different field mothers, or from bulk collections at Asian sites ($N = 332$; Supporting Information

Table S1). Flowering heads (capitula) were covered with fine mesh bags while in bud, and hand pollinated using a single pollen donor when a large fraction of florets were receptive. Strong self-incompatibility in this species was verified by manual self-pollination and by bagging unmanipulated capitula (yielding 0% seed set). Seeds were collected at maturity from a total of 349 successful crosses within and among populations (Dryad doi:##.###/dryad.#####).

Progeny (N = 523, including 1–3 per cross) were reared for growth measurements in the common glasshouse environment. Increased growth is a fundamental metric of heterosis in experimental crosses (Birchler *et al.* 2010; Chen 2013), and it is a key trait whose evolution is associated with increased fitness in invasions of *C. solstitialis* (Eriksen *et al.* 2012; Dlugosch *et al.* 2015b). All size measurements of both ‘source’ genotypes (produced by within-population crosses) and ‘admixed’ genotypes (produced by among population crosses) were made in the same experiment, at both 4 and 5 weeks of age (Supporting Information Fig. S1). Size at both timepoints was measured using a non-destructive size index [(maximum leaf length * maximum leaf width)^{1/2} * leaf number] that has been shown to have a strong linear correlation with total biomass under glasshouse conditions in this species (Dlugosch *et al.* 2015b). Exponential growth rates between these two measurements for each plant were compared using REML Analyses of Variance (ANOVA) with fixed effects of 1) the source population of crossed genotypes; 2) observer (the person measuring the plants); and nested effects of 3) cross direction (the source population of the maternal vs. paternal genotype) and 4) individual parental combination. Least squares means (LSM) and standard errors were extracted from these models for use in analyses below.

Mid-parent trait values

Source populations can be genetically divergent from one another in growth rate due to local adaptation or genetic drift, so we tested for evidence of non-additive genetic interactions during admixture by comparing admixed genotypes to mid-parent expectations that assume additivity in the trait (Dlugosch *et al.* 2015b). To calculate these expectations, both parental and admixed genotypes must be products of crosses conducted in a common environment, to minimize transgenerational plasticity effects on the traits of interest. Moreover, it is essential to compare measurements of parental and admixed genotypes at the same life stage within the same experiment, because growth rate is highly sensitive to experimental conditions. To accomplish this for *C. solstitialis*, we generated a distribution of pseudo-mid-parent values by randomly drawing combinations of ‘source’ growth phenotypes from the progeny of within-population crosses (i.e. representatives of parental lineages, produced by crosses in the common glasshouse environment, as described above) that were reared and measured at the same time as admixed genotypes (Supporting Information Fig. S1). Pseudo-parental combinations with observer effects removed were drawn with replacement 1000 times, and their average (mid-parent) growth rates calculated to create a distribution of pseudo-mid-parent values that were compared to growth rates of admixed genotypes using two-tailed t-tests.

Relationship between progeny performance and parental population diversity and divergence

To examine the relationship between growth rates in our experimental crosses and both genetic variation within source populations and genetic divergence between source populations, we used a linear model to explain deviation of growth patterns from mid-parent expectations (Y_{mp}). The

340 model included fixed effects in the form:

$$Y_{mp} = \mu + \pi_m + \pi_p + \pi_{mp} + g_m + g_p + e_{mp}$$

345 where π_m and π_p are maternal and paternal source intrapopulation π respectively, which are predicted to scale negatively with growth deviation due to the benefits of admixture at low within-population variation; π_{mp} is interpopulation π between parental source populations, predicted to influence growth deviation positively where genetic load is increasingly rescued, and negatively where there are epistatic incompatibilities accumulating at high divergence; g_m and g_p are respectively maternal and paternal source population LSM growth rate phenotype ('source' lineages grown in the same experiment as admixed progeny, as described above),
350 predicted to correlate with growth deviation positively where there are transgenerational plasticity/epigenetic effects; and e_{mp} is the residual error. All interaction terms were non-significant ($P > 0.1$) and are not shown. All non-significant terms were removed from the final model.

355 **Literature Survey**

We explored the literature to ask whether reported cases of admixture are being realized under conditions in which we might expect admixture to be favorable to many invaders, and whether there might be limits on these benefits due to negative genetic interactions. Using papers that reported the distribution of molecular genetic variation in native populations and in populations
360 introduced from the native range to new areas (i.e. 'primary' introductions), we extracted metrics of genetic variation and divergence for native populations and recorded whether admixture was reported in the introduced populations. We analyzed an older dataset of this type (Dlugosch et al. 2015a) to identify the effects of study design on reports of admixture (Supplemental Methods). Based on this information, we focused our survey on microsatellite-
365 based studies and controlled for the number of sites surveyed in the native range (see Supplemental Methods and Results). We examined the relationship between reports of admixture and metrics of genetic divergence and within-population genetic variation across native sites using logistic regressions with the number of sites sampled as a covariate (ln transformed). We quantified potential source population divergence using F_{ST} and related
370 metrics (Φ_{ST} , G_{ST}) reported among all native populations in a study. We quantified genetic variation as the mean across all native sampling sites and loci (within a single type of genetic marker) of expected heterozygosity (H_E) and observed heterozygosity (H_O). All statistical tests were performed in JMP 11 (SAS Institute, Cary, USA).

375 **Results**

Experimental Crosses in *C. solstitialis*

Nucleotide diversity (π) varied within and across native *C. solstitialis* populations, and was consistently higher between pairs of populations than within populations (Fig. 2). Intrapopulation π ranged from 0.004 to 0.005 average SNPs/site, with the highest value in
380 western Europe and the lowest in Asia. Interpopulation π increased to 0.005–0.008 average SNPs/site. The largest values of interpopulation π occurred in comparisons of alleles from southern Greece to those from other populations, consistent with our previous observation of a highly differentiated lineage occupying the Apennine-Balkan Peninsulas (Barker *et al.* 2017).

385 Growth rates differed significantly among different admixture combinations of parental source
populations (ANOVA $F_{5,11} = 3.59$, $P = 0.005$), spanning an order of magnitude in exponential
growth rates (Supporting Information Fig. S2). Of the 12 combinations of crosses between
genotypes from different maternal and paternal source populations, we found that seven deviated
390 significantly from pseudo-mid-parent expectations, and all but one of these were in the positive
(heterotic) direction (Fig. 3). Progeny of the maternal source population from western Europe
experienced multiple heterotic interactions with those from other populations. The single
negative interaction occurred in crosses with a maternal genotype originating from eastern
Europe and a paternal genotype originating from the highly divergent population in southern
Greece. In contrast, maternal genotypes originating from southern Greece showed positive
395 genetic interactions with paternal genotypes from other regions, including those from eastern
Europe.

Holding maternal source population constant, crosses to increasingly divergent paternal source
populations showed a wide variety of trends in growth performance (Fig. 3), including positive
400 (Asia), negative (eastern Europe), and curvilinear relationships (peaking at intermediate values;
western Europe and southern Greece). Using data from all crosses, the deviations of admixed
progeny from additive expectations were not predicted by parental source population divergence
(interpopulation π), parental source intrapopulation π , or parental lineage phenotypes in a linear
model (model $P = 0.24$). Removing the extreme heterotic datapoint in the cross of Asia \times
405 southern Greece (see Fig. 3a, Supporting Information Fig. S3), however, yielded a highly
significant model, strongly predicting growth deviation ($r^2_{\text{adj}} = 0.92$; $F_{4,10} = 28.6$, $P = 0.005$), in
which the direction of main effects were most consistent with epistatic interactions among loci
(Fig. 1c,d). In particular, deviations in growth were negatively associated with divergence
between parental source populations (Fig. 1c; π_{mp} effect $P = 0.006$), and positively associated
410 with maternal source intrapopulation π , such that high genetic variation in the maternal source
population made heterotic interactions stronger (Fig. 1d; π_m effect $P = 0.0007$).

Progeny performance depended significantly on the source of the maternal vs. the paternal
genotype in the cross (Fig. 4a; ANOVA of progeny performance with nested effect of cross
415 direction: $F_{6,5} = 0.014$, $P = 0.02$). This result could suggest that transgenerational maternal
effects influenced the growth of progeny, in which case a positive relationship would be
predicted between maternal lineage phenotype and progeny deviation from mid-parent
expectations. Yet, maternal lineage growth rate negatively predicted deviation from mid-parent
expectations (Fig. 4b; g_m effect $P < 0.0001$), and its interaction with genetic divergence was also
420 negative ($\pi_{mp} * g_m$ effect $P = 0.0009$). This result is not consistent with maternal effects, but
could be explained by non-additive genetic interactions between the maternally-inherited
cytoplasmic genome and the bi-parentally inherited nuclear genome.

Literature Survey

425 In total, we acquired data from introductions of 34 species (Dryad doi:##.###/dryad.#####),
including reports of admixture in 15 (44%) of these. We found no monotonic relationships
between admixture and either genetic divergence (ln transformed values: $N = 26$, $\chi^2_2 = 0.42$, $P =$
 0.81), or within-population diversity measured as H_E ($N = 29$, $\chi^2_2 = 2.56$, $P = 0.28$; Fig. 1f) or
 H_O ($N = 24$, $\chi^2_2 = 0.03$, $P = 0.98$), which was also the case in the older dataset of 167 plant and

430 non-plant species (Supplemental Results). Inspection of the data revealed a strong curvilinear relationship between admixture and genetic divergence (Fig. 1f).

Discussion

435 Introduced species have opportunities to gain fitness advantages from increases in genetic diversity and novel genetic combinations that are associated with admixture (Frankham 2005; Hufbauer 2008; Rius & Darling 2014; Dlugosch *et al.* 2015a). The fitness benefits of admixture are expected to grow as the divergence between source populations increases and as the genetic variation within source populations decreases, under a variety of mechanisms (Lynch 1991; Dlugosch *et al.* 2015a; Hufbauer 2017). We found significant genetic interactions in over 50%
440 of our controlled crosses among native *C. solstitialis* populations, and all but one were positive (heterotic) in the first generation. Heterotic effects declined in magnitude as divergence among populations increased, however, in a manner consistent with epistatic cytonuclear interactions. Such interactions could limit the benefits of admixture for invaders generally. We found that while admixture has been frequently reported in the literature, it is disproportionately lacking
445 from systems with high genetic divergence among potential source populations. Our findings are consistent with the idea that admixture might often occur under conditions that are likely to be favorable for introduced species, but also that these conditions should be bounded by negative epistatic interactions at high levels of divergence.

450 Epistasis is unique in its potential to generate both positive and negative interactions during admixture. Any positive interactions are expected to diminish and become negative in later generations, as recombination breaks up co-adapted allele combinations (Lynch 1991). Thus benefits from epistasis during admixture are expected to be transient over time. Experiments with first generation crosses should generally demonstrate the maximum benefit of epistasis, and
455 will be conservative with respect to identifying negative interactions that might ultimately constrain interbreeding of divergent populations (see Introduction 3. Epistasis). Importantly, transient *increases* in fitness can be highly beneficial when founding populations are struggling to establish (i.e. the ‘catapult effect’; Drake 2006). Further, negative epistatic interactions from hybrid breakdown in later generations can also be avoided by backcrossing with resident
460 parental populations (Dlugosch *et al.* 2015b), and high fitness of early generation admixed genotypes can be maintained through clonal propagation or polyploidy in some cases (e.g. Facon *et al.* 2008). Indeed, clonal or polyploid spread of hybrids have provided some of our best examples to date of the evolution of invasiveness (Ellstrand & Schierenbeck 2000; Hufbauer 2008).

465 The complexity of these outcomes from epistasis alone argues that we should expect admixture to vary in its contribution to the establishment and invasion of introduced populations, and early generation crosses should be particularly useful for gauging its most powerful effects on fitness. Notably, some of the strongest evidence of heterotic interactions in our crosses of *C. solstitialis*
470 (with some of the highest resulting plant growth rates) occurred when the western European population served as the maternal parent. This lineage appears to be a primary contributor of invasions into the Americas (Eriksen *et al.* 2014; Barker *et al.* 2017). Strong heterotic interactions in this lineage suggest that a possible contributor to its success as an invasion ‘bridgehead’, (an invasion which initiates many subsequent invasions; Lombaert *et al.* 2011),

475 could be its propensity to foster beneficial admixture events (e.g. Turgeon *et al.* 2011).

Both the identity of the maternal parent population of origin and genetic diversity within the maternal population were important to the performance of *C. solstitialis* crosses, which strongly suggests that the divergence of maternal (cytoplasmic) DNA from the paternal nuclear genome
480 is the underlying cause of the interactions that we observed. There is increasing evidence that cyto-nuclear interactions within species are common and can have important phenotypic effects in both animals and plants (Ballard & Melvin 2010; Bock *et al.* 2014). The additional effect of genetic diversity within the maternal population would seem to suggest that maternal
485 heterozygosity in some way reflected the potential for positive interactions between maternally inherited cytoplasmic genes and nuclear genes from a divergent paternal population. We are not aware of an established mechanism that could account for this pattern, though it seems plausible that a recent evolutionary history with a greater diversity of nuclear backgrounds might predispose the maternal cytotype to have favorable interactions with novel paternal genotypes. Intriguingly, crosses between maternal genotypes from the invaded range in California (USA)
490 and paternal genotypes from the invasion's origin in Spain showed reduced seed set in a previous study (Montesinos *et al.* 2012). Compared to other populations across the species range, those from western Europe and California have some of the lowest levels of genetic divergence between them, but the highest phenotypic divergence (Barker *et al.* 2017), suggesting that adaptation might be driving the accumulation of negative epistatic interactions in this case.

495 Negative epistatic interactions are expected to have increasing fitness costs as populations diverge, ultimately resulting in reproductive isolation and speciation (Orr & Turelli 2001). Given multiple introductions of divergent material, pre- or post-zygotic isolation among particularly divergent source populations could prevent the formation and establishment of admixed
500 genotypes (Rius & Darling 2014). The only significant negative interaction among our crosses occurred when eastern European maternal genotypes were crossed with paternal genotypes originating from an adjacent area in southern Greece. The population in southern Greece is particularly divergent from other populations, and might belong to a distinct subspecies (Barker *et al.* 2017). The geographic boundary separating southern Greece and eastern European
505 populations could be a region in which early speciation dynamics might fruitfully be studied. These particular interactions raise questions about whether particular lineages are serving as the maternal parents in admixture events between differentiated populations in the native range and in the invasions of *C. solstitialis*.

510 Epistasis has rarely been discussed as a mechanism of major importance to admixture's role in invasions, and there have been few previous tests for epistatic interactions in introduced species. A small number of empirical studies have found evidence of epistasis underlying both negative and positive interactions. Keller and colleagues (Keller *et al.* 2000) crossed native populations of three widespread agricultural weeds and found evidence of negative epistatic interactions in
515 either the first generation (F1) cross or the backcross (F2) in each. Johansen-Morris and Latta (Johansen-Morris & Latta 2006) crossed two invading genotypes of *Avena barbata* in their introduced range and found evidence for epistasis underlying both hybrid vigor in early generation hybrids (F2) and reduced fitness in later (F6) generations. Notably, later generations were highly variable and some individual lines showed potential for outperforming parental

520 genotypes, revealing rare opportunities for beneficial admixture even when there is an overall pattern of deleterious hybrid breakdown.

Other authors have pointed out that admixed progeny might also suffer from the loss of local adaptation to the introduced range, due to introgression of a divergent source that is not as well adapted to local conditions (Verhoeven *et al.* 2011). This has been demonstrated in *Mimulus guttatus*, where first generation crosses among and between native and invading populations are generally heterotic (though with reduced benefits in the F₂; van Kleunen *et al.* 2015; Li *et al.* 2018), but crosses between ranges are maladapted to the invaded environment (Pantoja *et al.* 2018). In general, negative epistasis could contribute to such outbreeding depression, as a result of divergence due to both local adaptation and genetic drift. In the absence of negative epistatic interactions, it becomes much more likely that natural selection will favor admixed progeny that retain locally adapted alleles, and introgression at some level will occur. Invaders might also be uniquely poised to avoid problems with loss of local adaptation from the native range during natural admixture events, given that they occupy a novel environment (Rius & Darling 2014).

535 Our survey of the literature revealed that while admixture is commonly reported in phylogeographic studies of invaders, it is disproportionately observed at intermediate values of divergence. At low values of divergence, admixture might not be particularly favorable, given that the fitness benefits of introgression increase with divergence under all proposed underlying mechanisms (Fig. 1a,b). Perhaps more importantly, admixture is also likely to be underestimated at low levels of divergence due to low power to identify multiple source populations (Dlugosch *et al.* 2015a), and indeed we found evidence for a variety of study design effects that suggest power is an important issue (see Supplemental Results). In contrast, the decline in observations of admixture at the highest levels of divergence, where the ability to detect sources of admixture should be strong, suggests that introgression between increasingly divergent sources is less likely.

A disproportionate lack of admixture at high levels of divergence could be due either to poor establishment of admixture events, as we have argued should be expected under epistasis, or to a lack of opportunity for admixture events to occur between highly divergent sources in the first place. How the sources of introduced species are distributed across the native range, particularly as a function of the genetic divergence of those sources, has not been investigated to our knowledge. If introductions tend not to be sampled from across geographic barriers that are also barriers to gene flow, this could result in (a lack of vectors) a lack of admixture at high genetic divergence among native population, though multiple introductions from different parts of the native range appear to be frequent within species introductions (Dlugosch & Parker 2008a; Uller & Leimu 2011). This possibility could be studied given knowledge of specific source populations for both failed and successful multiple introductions, as well as the geographic distribution of genetic variation within the native range. Such datasets might be available (or possible to accumulate) for groups with particularly well-documented introduction attempts, such as birds (e.g. Maitner *et al.* 2012).

560 Finally, we note that the fitness effects of admixture are also expected to be affected by genetic diversity and genetic load within the admixing populations. In our experimental crosses, we

found that genetic diversity in the maternal lineage was positively associated with heterosis, opposite of our predictions, and there was no effect of diversity in the paternal lineage on performance. We also found that admixture was not identified more often in the literature for species with lower levels of heterozygosity. Several factors may obscure our ability to interpret whether introduced species are often in a situation to benefit from an increase in genetic diversity. Heterozygosity and haplotype diversity are less sensitive measures of genetic variation than metrics that better capture rare alleles, such as allelic richness. On the other hand, heterozygosity should reflect the history of standing variation in a population (Lohr & Haag 2015), and rare alleles, by virtue of being rare, will not strongly influence most of the mechanisms that underlie the effects of admixture (with the notable exception of Evolutionary Rescue).

We also emphasize that we have measured diversity in potential source populations rather than in founding populations that would have experienced admixture, but this information is unattainable for already admixed populations. Previous surveys have indicated that genetic bottlenecks are not typically severe during founding events, such that founder population genetic diversity largely resembles native population diversity, though this is not true for all invaders and expansion fronts (Dlugosch & Parker 2008a; Excoffier *et al.* 2009; Uller & Leimu 2011; Dlugosch *et al.* 2015a). For example, several studies have found evidence consistent with inbreeding depression in natural invading populations and its rescue by admixture (e.g. Bailey & McCauley 2006; Nolte *et al.* 2009; Mullarkey *et al.* 2013; Keller *et al.* 2014; Rius & Darling 2014; van Kleunen *et al.* 2015). It may be that founding populations with particularly low genetic variation and high genetic load, which would be predicted to benefit most from admixture, are relatively rare across species introductions as a whole, although admixture could provide substantial benefits to founding populations when severe genetic bottlenecks do occur, and these benefits may be realized over evolutionary time, which is not readily observed in experimental crosses (Szűcs *et al.* 2014, 2017a; b; Wagner *et al.* 2017).

Conclusions

Early discussions of admixture in species introductions recognized its potential to provide genetic and evolutionary rescue, and favorable genetic interactions (Novak & Mack 1993; Ellstrand & Schierenbeck 2000). These benefits were hailed as the resolution to what has been called the ‘genetic paradox’ of invasions, wherein invaders are somehow successful despite experiencing founding events that are expected to have unfavorable effects on genetic diversity and fitness (Allendorf & Lundquist 2003; Kolbe *et al.* 2004; Hufbauer 2008). Particularly given that multiple introductions seemed to be common in successful invasions (Dlugosch *et al.* 2015a), admixture offered the potential to resolve the genetic paradox for many if not most invaders. We now know that introduced species do not often suffer from large reductions in genetic diversity during founding events (Dlugosch & Parker 2008a; Uller & Leimu 2011; Dlugosch *et al.* 2015a). Admixture will still be beneficial to introduced species under a variety of mechanisms (Ellstrand & Schierenbeck 2000; Frankham 2005; Drake 2006; Facon *et al.* 2008), but there should be limits to these benefits, particularly at high divergence among source populations due to negative epistasis. This pattern is a general prediction of epistatic interactions among loci that have been diverging among populations (Carroll *et al.* 2003; Phillips 2008), though epistasis is rarely mentioned in the context of invasions (Rius & Darling 2014). Here we

610 observed increased performance of experimentally admixed progeny which diminished and
became negative as divergence between parental populations increased, consistent with epistasis.
Additional experimental investigations across many taxa will be essential to understanding the
mechanisms shaping the fitness effects of admixture in natural systems. Also needed are studies
of systems with known introduction attempts, wherein it is possible to identify opportunities for
615 admixture, whether those opportunities have been realized, and whether the prevalence of
admixture is associated with the degree of fitness benefits. In general, our analyses argue that it
may be possible to resolve conflicting evidence for the benefits of admixture during invasion by
examining genetic divergence between and diversity within source populations. If the different
outcomes of admixture are generally predictable, it will be possible to clarify whether we should
620 realistically expect that admixture is a general mechanism for the success of introduced species.

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Data Accessibility

- 885 • Original sequence data are available at NCBI sequence read archive BioProject PRJNA275986.
- ddRADseq polymorphism data are available at Dryad doi:##.###/dryad.#####
- Data from experimental crosses are available at Dryad doi:##.###/dryad.#####
- Data from the literature survey are available at Dryad doi:##.###/dryad.#####

890 Author Contributions

KMD conceived the study. SRA, JEB, FAC, HDG, and KMD performed the literature review. BSB performed the genomic analyses. JEC performed the experimental crosses. BSB and KMD analyzed the data and wrote the manuscript, which was edited by all authors.

895 Figure Legends

Fig 1. Identifying the conditions under which admixture might be most favorable during invasions. Shown are predicted relationships for the performance of admixed populations as a function of (a) the genetic divergence between their source populations and (b) the genetic variation within their initial founding populations, under different genetic and evolutionary mechanisms. In a linear model of progeny performance in experimental crosses the invasive plant *C. solstitialis* (excluding heterotic outlier [Asia × southern Greece]), genetic divergence (interpopulation π) had a significant negative effect (c, $P < 0.01$) and genetic variation (intrapopulation π) in maternal populations had a significant positive effect (d, $P < 0.001$) on the deviation of progeny from mid-parent genotype expectations. Data points in (c,d) show progeny deviation from mid-parent as partial residuals after taking into account all other effects in the

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model. In the literature, genetic admixture in introduced plant populations is reported more often at intermediate genetic divergence (F_{ST} and related metrics for microsatellite markers) among potential source populations in the native range (e), and shows no relationship with average genetic variation (H_E) within native populations (f).

Fig 2. Sampling sites and sequence variation in the native range of *C. solstitialis*. (a) Sampling sites for this study (large dots) span genetically divergent populations in western Europe (WE, blue), eastern Europe (EE, purple), southern Greece (SG, red), and Asia (AS, green) as previously identified from population genomic analyses (previous sampling indicated by large and small dots; Barker *et al.* 2017). (b) Average intrapopulation nucleotide diversity (π) across the total length of all ddRADseq reads within each sampling site (dots) and for all individuals pooled within a region (bars). (c) Average interpopulation π in pairwise comparisons between individuals from different populations.

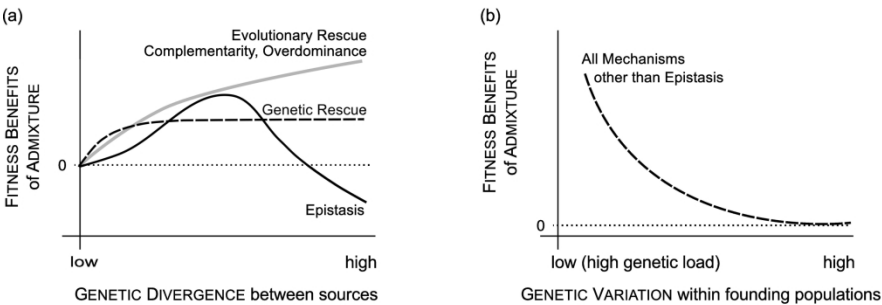
Fig 3. For each maternal region, panels (a–d) show growth rates of *C. solstitialis* progeny from experimental crosses to fathers from other regions and the mid-parent expectations for each cross. Growth rates are shown versus average pairwise nucleotide diversity (π) between parental populations. Color codes for each cross are as in Fig 2. Within-region crosses are indicated by solid colors, and between-region crosses by paternal region color outlined by maternal region color. Mid-parent expectations are shown as means (black bars) \pm s.e.m. (gray bars). Growth rates are least squares means \pm s.e.m., and significant deviations from mid-parent distributions are indicated as: *** $P < 0.0001$; ** $P < 0.001$; * $P < 0.05$.

Fig 4. (a) Growth rates of *C. solstitialis* progeny from experimental crosses with all possible combinations of mother and father population-of-origin. Color codes for each cross indicate maternal and paternal population as in Fig 2, with paternal region color outlined by maternal region color. Growth rates are least squares means \pm s.e.m., and significant differences between reciprocal crosses of the same parental populations are indicated * $P < 0.05$. (b) In a linear model of progeny performance (excluding heterotic outlier [Asia \times southern Greece]), growth rate of genotypes from the maternal region had a significant negative relationship ($P < 0.0001$) with the deviation of progeny from mid-parent expectations. Data in (b) show progeny deviation from mid-parent as partial residuals after taking into account all other effects in the model.

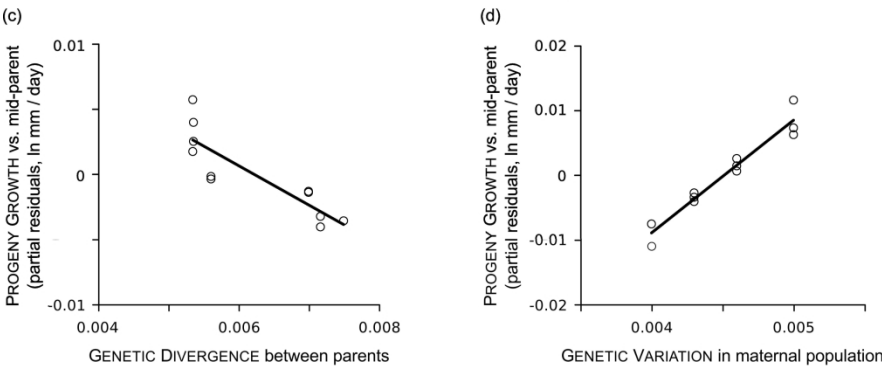
Supporting Information

Supporting information is provided as a single PDF document.

PREDICTIONS: Under what conditions do we expect admixture to have the greatest benefits to fitness?



EXPERIMENT: Under what conditions is heterosis greatest during experimental admixture of *C. solstitialis*?



LITERATURE SURVEY: Under what conditions has admixture been most prevalent?

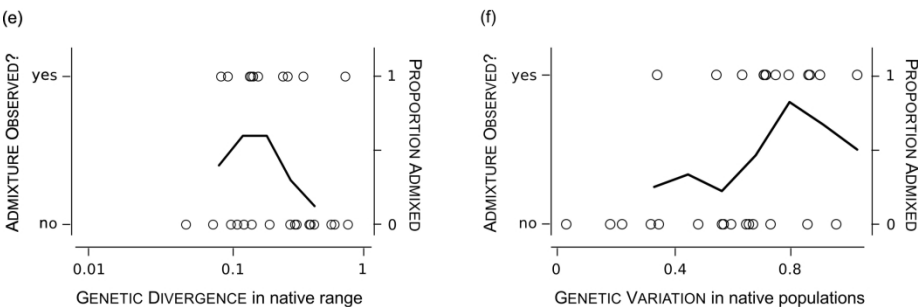


Figure 1

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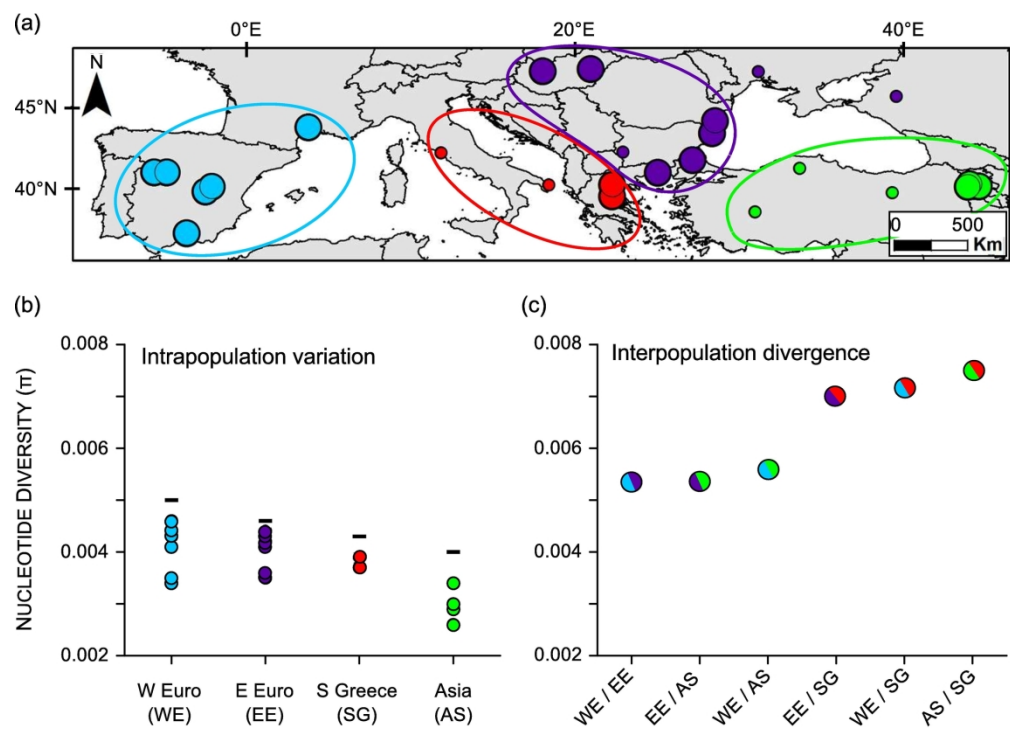


Figure 2

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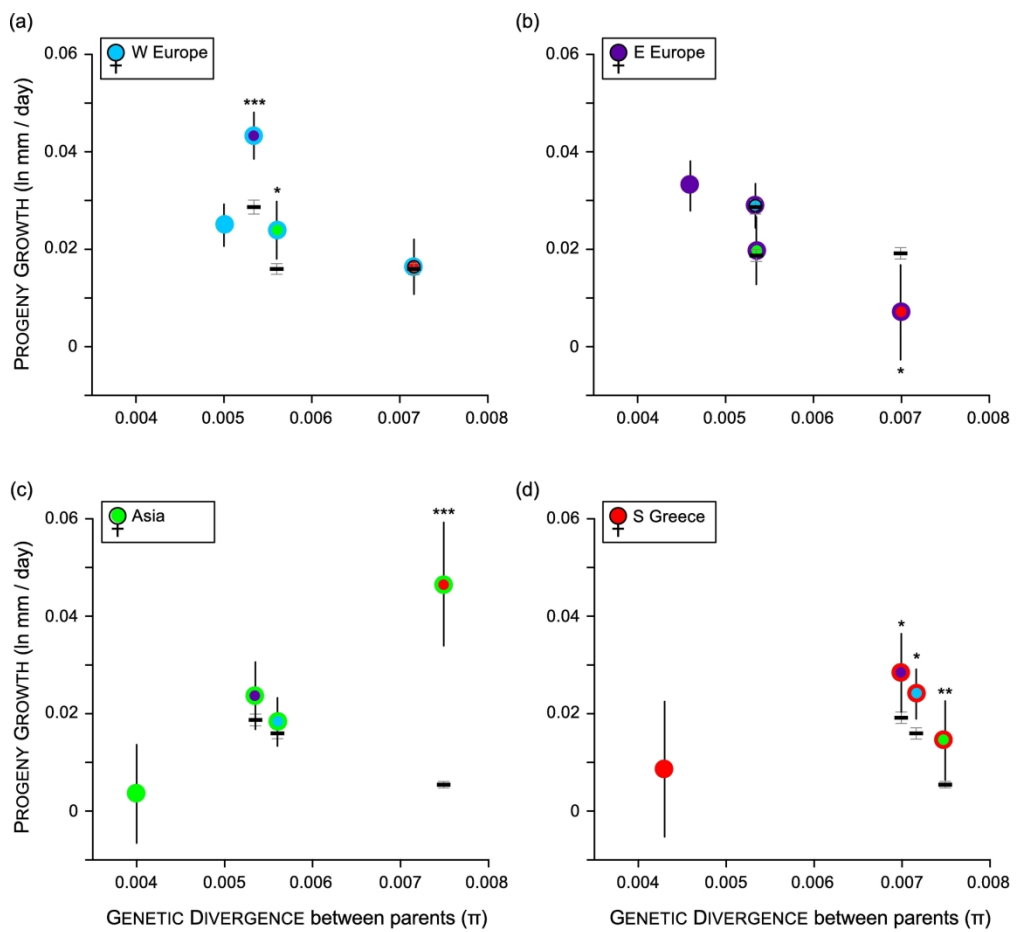


Figure 3

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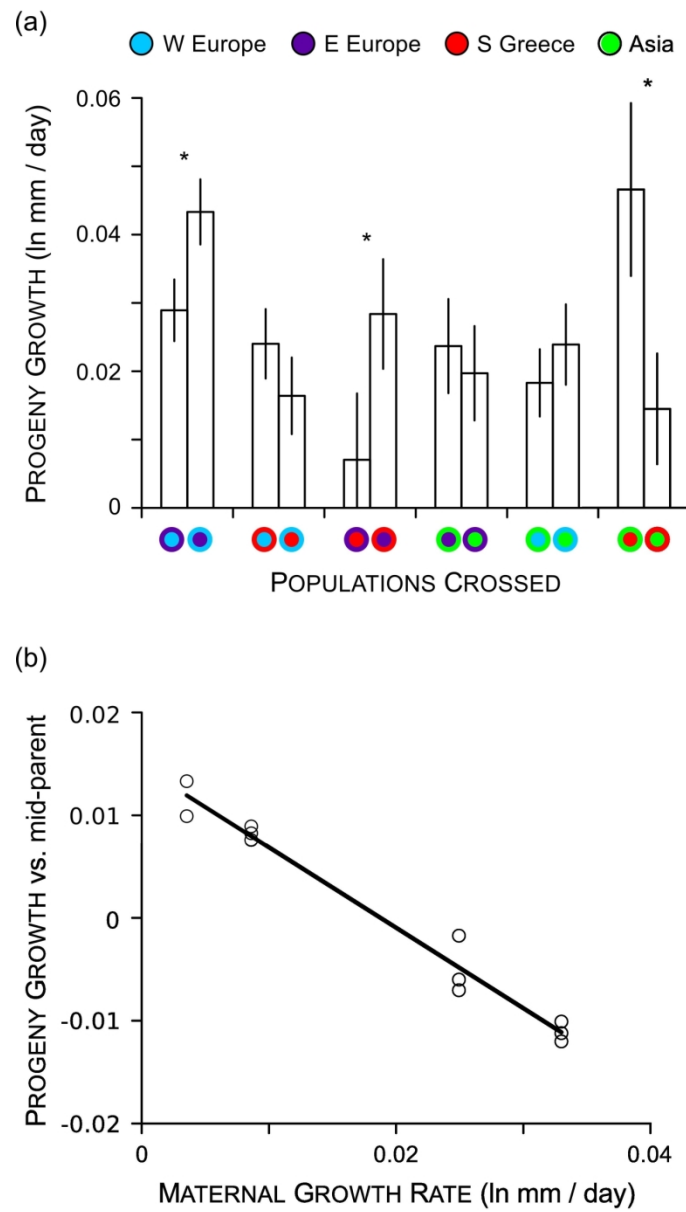


Figure 4

114x203mm (300 x 300 DPI)