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Global gene flow releases invasive plants from environmental
 constraints on genetic diversity

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96 **Author Contributions**

97 YMB coordinated the PLANTPOPNET network. The founding steering committee (YMB, SPB, EEC, AMC, JE, MBG, A-LL, DAR, RS-G and GW) designed the PLANTPOPNET network and 98 99 wrote the demographic census protocol, while the current steering committee (including DZC, 100 BDE, AF, SM-B and JV) oversee network operation. ALS, YMB and TRH designed the concept, 101 DNA data collection and analytical approach for the current study. ALS conducted all analyses 102 and wrote the code for SNP filtering and analysis. ALS wrote the first manuscript draft with major 103 contributions from YMB, TRH, JV, JAC and AMC. All authors (except SPB and JE) contributed 104 data used in the current study. All authors made contributions to the final manuscript.

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110 Abstract

111 When plants establish outside their native range, their ability to adapt to the new environment is 112 influenced by both demography and dispersal. However, the relative importance of these two 113 factors is poorly understood. To quantify the influence of demography and dispersal on patterns 114 of genetic diversity underlying adaptation, we used data from a globally-distributed demographic 115 research network, comprising 35 native and 18 non-native populations of *Plantago lanceolata*. 116 Species-specific simulation experiments showed that dispersal would dilute demographic 117 influences on genetic diversity at local scales. Populations in the native European range had 118 strong spatial genetic structure associated with geographic distance and precipitation seasonality. 119 In contrast, non-native populations had weaker spatial genetic structure that was not associated 120 with environmental gradients, but with higher within-population genetic diversity. Our findings 121 show that dispersal caused by repeated, long-distance, human-mediated introductions have 122 allowed invasive plant populations to overcome environmental constraints on genetic diversity, 123 even without strong demographic changes. The impact of invasive plants may therefore increase 124 with repeated introductions, highlighting the need to constrain future introductions of species even 125 if they already exist in an area.

126 Significance Statement

We found that long-distance dispersal and repeated introductions by humans have shaped adaptive potential in a globally distributed invasive species. Some plant species therefore do not need strong demographic changes to overcome environmental constraints that exist in the native range; simply mixing genetic stock from multiple populations can provide an adaptive advantage. This work highlights the value of preventing future introduction events for problematic invasive species, even if the species already exists in an area.

134 Main Text

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135 136 Introduction

137 138 Patterns of genetic diversity across a species' range arise from a complex interplay between the 139 diversifying effect of demographic variation across landscapes with different selection pressures, 140 and the homogenising effects of dispersal¹⁻³. On one hand, variability in demographic 141 performance influences genetic diversity through its influence on effective population size⁴. Short-142 lived, highly fecund species generally have higher levels of genetic diversity compared to species 143 that are long-lived or have low fecundity^{5,6}. On the other hand, dispersal modulates these 144 relationships by facilitating gene flow between populations⁷. Gene flow from seed and pollen can 145 increase genetic diversity and reduce genetic differences among populations. While the 146 importance of these forces is widely accepted⁸, there is uncertainty about the relative strength of 147 demography and dispersal in shaping genetic structure across global environmental gradients^{9,10}. 148 149 For invasive species, the situation is even more complex because humans disrupt many of the 150 natural processes that determine genetic diversity (Fig. 1). For example, repeated introductions 151 and long-distance dispersal by humans can release invasive plant species from demographic 152 constraints, such as those imposed by the colonisation-competition tradeoff¹¹. Invasive species

might also overcome climatic constraints on phenotypic traits as a result of rapid adaptation to
 new environments¹² or non-adaptive processes such as repeated introductions which can swamp
 locally adapted phenotypes¹³. Thus, emerging evidence suggests that plants in their non-native
 range can break ecological 'rules' because they are not always constrained by the same
 biological and climatic forces that operate in their native range.

158

Some populations of invasive species lose genetic diversity during invasion through founder
 effects¹⁴, but many have higher genetic diversity outside their native range^{15,16}. The mechanisms

161 underlying this phenomenon include admixture (i.e. new genotypes arising from interbreeding 162 among divergent source populations)¹⁷, hybridisation¹⁸, rapid mutation¹⁹ and exposure of cryptic 163 genetic variation²⁰. Such increases in genetic diversity can enhance colonisation success²¹ and 164 adaptive potential²² in invasive species. Demographic changes can also improve invasive plant 165 performance²³, which is sometimes associated with release from natural enemies²⁴. 166 Unfortunately, demographic and genetic aspects of invasion are often analysed in isolation²⁵, in 167 part because labour-intensive demographic studies are typically done at one or a few sites 168 making them severely limited in spatial replication²⁶. This means we lack understanding about the 169 relative importance of demographic change and global dispersal on biological invasions^{27,28}. 170 171 Here, we present a demographically-informed analysis of neutral and putatively adaptive genetic 172 diversity in Plantago lanceolata L. (Plantaginaceae), a common forb native to Europe and 173 western Asia, which now has a cosmopolitan distribution (Fig. 2). Plantago lanceolata established 174 in its non-native range through long-distance dispersal by humans²⁹, repeated introductions³⁰ and 175 cultivation³¹ – all processes that can increase genetic diversity and invasion success¹⁵. The 176 overarching aim of the study was to analyse the influences of local demography and global 177 dispersal patterns on genetic diversity in P. lanceolata and determine which of these pathways 178 drives adaptive capacity. This knowledge is necessary to understand how future introduction 179 events will influence the spread of invasive plants. This work was made possible by a globally-180 distributed demographic research network (PLANTPOPNET) and is, to our knowledge, the first 181 analysis of genetic diversity at a global scale that integrates field-collected demographic data. 182

183 In addition to demographic data, we sampled DNA from 491 individuals including outgroups, 184 cultivar lines and 53 naturally occurring populations across the native European range (n = 35) 185 and the non-native range (n = 18) in southern Africa, Australasia and North America (Fig. 2). To 186 address our main aim, three hypotheses were tested: 187

- (H1) In absence of dispersal, increases in survival and fecundity will drive increases in genetic diversity. These effects will be diluted by dispersal between populations.
- 190 191 (H2) Patterns of spatial genetic structure among native populations will reflect dispersal 192 limitations across environmental gradients. In the non-native range, gene flow arising from 193 multiple introductions will disrupt spatial genetic structure observed in the native range. 194
 - (H3) Environmental influences on within-population genetic diversity will be explained by demographic variation (density, fecundity and empirical population growth rate). Repeated introductions into the non-native range and long-distance dispersal by humans will weaken this relationship (Fig. 1).

200 A genotypic simulation model, parameterised with empirical demographic data from P. lanceolata, 201 was used to test H1. We then coupled field-collected demographic data (density, empirical 202 population growth rate and fecundity) with single nucleotide polymorphism data (18.166 neutral 203 and 3,024 putatively adaptive SNPs) to test H2 and H3. 204

205 Results and discussion

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Hypothesis 1: Dispersal between populations will dilute demographic effects on genetic diversity 208

209 In two simulated populations unconnected by dispersal, with different rates of juvenile survival (oi = 0.1 and 0.2) and female fecundity (seeds per plant, 8 - 1-100), higher juvenile survival led to 210 211 greater genetic diversity (Fig. 3a). Above the threshold at which populations went extinct ($\beta_{\rm F}$ = 212 15), genetic diversity increased sharply until $\mathcal{S}_{\rm F}$ was approximately 25. Above this point there was 213 little influence of fecundity on genetic diversity (Fig. 3a). Population size at the end of the 214 simulation was larger with higher juvenile survival (Fig. 3b). Thus, variation in female fecundity

appears to have less influence than juvenile survival in determining genetic diversity in *P. lanceolata.* When the two populations were connected by dispersal, differences in heterozygosity
persisted until the number of migrants per generation exceeded 50,000 (Fig. 3c, d). This number
is realistic in natural populations since reproductive individuals typically produce a minimum 20100 seeds and migration refers to propagules dispersed before the recruitment process. Male
fecundity was kept constant in the model as it is very high in *P. lanceolata* (10,000–54,000 pollen
grains per anther³²) and had no influence on genetic diversity.

222

The simulation result supports our prediction (H1) that demography would influence genetic diversity in *P. lanceolata* when dispersal barriers are present and that dispersal would dilute these effects. The simulation also suggests that juvenile survival is an important parameter controlling heterozygosity. When dispersal barriers are removed however, gene flow from pollen and seed will swamp local effects of juvenile survival on heterozygosity. We could therefore expect demographic effects on genetic diversity to become undetectable at the upper range of pollen and seed movement that occurs in *P. lanceolata*.

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231 The increases in genetic diversity with juvenile survival (Fig. 3) might not confer an adaptive 232 advantage since they reflect genetic diversity arising from neutral demographic processes. The 233 relevance of this result however, is that there is enough demographic variability in *P. lanceolata* to 234 shape neutral genetic structure, an assumption underlying the hypotheses in the rest of the study. 235 Thus, we can expect juvenile survival to be the dominant demographic parameter underlying 236 differences in *P. lanceolata* genetic diversity when dispersal is limited at local scales. At 237 continental scales, genetic diversity is probably influenced less by juvenile survival when gene 238 flow is high. This might be especially true in the non-native range where there has been a shorter 239 history of local adaptation³³ and multiple human-mediated introductions (the human activity 240 pathway, Fig. 1).

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Hypothesis 2: Global gene flow from multiple introductions will disrupt spatial genetic structure

244 Admixture analysis of *P. lanceolata* genotypes with fastSTRUCTURE³⁴ revealed strong genetic 245 structure in the native range and a high degree of admixture in the non-native range. The number 246 of genetic clusters at Hardy-Weinberg Equilibrium (K) was between K = 6 (model complexity 247 maximising marginal likelihood) and K = 13 (model components used to explain structure in the 248 data). When K = 6, cultivar lines and outgroups (*P. coronopus* and *P. major*) formed two distinct 249 clusters and the remaining four clusters were present in the native European range with clear 250 spatial structure (Fig. 2). Greece, Italy, the Islands of the North Atlantic and Finland comprised 251 almost 'pure' lines of these four clusters, while other European populations were admixed. 252

253 Genotypes of most non-native populations were admixed and there was relatively little spatial 254 structure at a global scale (Fig. 2). This was supported by a significantly higher Diversity Score in 255 the non-native range (model estimate, SE = 0.34, 0.04), compared to the native range (0.22, 256 0.03) (see SI Appendix, Fig. S6, P = 0.033). Italy and central France were the most similar source 257 material for the dominant genotype in the non-native populations. Some cultivar stock was 258 identified in the Spanish populations, possibly reflecting the Iberian source of material used to 259 breed cultivars. The cultivars were developed in New Zealand, thus the presence of cultivar stock 260 in that population might indicate mixing between the naturalised population and pasture plants 261 (Fig. 2). At the upper range of K, further spatial structure was identified in Europe (e.g. at K = 13262 Norway was differentiated from Finland), while the non-native populations still showed admixture 263 of multiple, mostly Mediterranean sources (see SI Appendix, Fig. S1). The lack of spatial 264 structure at a global scale was supported by Analysis of Molecular Variance (AMOVA) showing 265 that genetic variation between the native and non-native range was only 2.2%, among individuals 266 within populations was 10.7% and among populations within ranges was 11.4%. The remaining 267 genetic variation (75.5%) accounted for individual heterozygosity.

269 The minimum number of colonising propagules required to produce the observed level of genetic 270 diversity in non-native regions ($Prop_{min}$) depended on sample size (r = 0.99) and ranged from 5.35 271 in New Zealand to 49.95 in North America (Fig. 2). Multiple introductions were therefore required 272 to produce observed levels of genetic diversity in the non-native ranges. Relative to sample size, 273 Prop_{min} ranged from 0.55 to 0.90 indicating that, in each region, more than half the sampled 274 population was required to represent non-native genetic diversity. Propmin was based on the 275 alleles present in the native range, but there were also a number of non-European alleles in each 276 non-native region (12-159, Fig. 2). Thus, we either failed to sample the full extent of the source 277 population (despite extensive sampling across Europe), or new genotypes were produced after 278 colonisation. The latter explanation can arise through transgressive segregation³⁵ and is one 279 mechanism by which invasive species adapt quickly to new environments. However, we also 280 detected private alleles within sites in Europe (see SI Appendix, Table S1) so our sample does 281 not represent the full range of genetic diversity in the species.

282

283 Genetic structure measured by Fst (genetic differentiation between all pairs of populations) was 284 stronger among populations in the native range (mean $F_{ST} = 0.16$) than the non-native range 285 (mean $F_{ST} = 0.09$). To analyse the influence of environmental gradients on F_{ST} , we used three 286 separate generalised dissimilarity models, one for each range type: native range, non-native 287 range and the global population (native and non-native combined). The deviance explained by 288 the native model was 74.3% (bootstrap CI = 68.6, 78.3) and two out of six variables fitted in the 289 model had a significant influence on Fst (Fig. 4, see SI Appendix, Fig. S2). Genetic distance 290 increased with geographic distance (Fig. 4a) and sites with similar levels of precipitation 291 seasonality were more genetically similar (Fig. 4b) after accounting for other variables in the 292 model (see SI Appendix, Fig. S2). No variable significantly affected F_{ST} in the non-native range 293 (deviance explained = 23.1%, bootstrap CI = 9.4, 34.1) or the global population (deviance 294 explained = 10.9%, bootstrap CI = 7.25, 14.33) (see SI Appendix, Fig. S2). Geographic distance 295 was included in each model to account for differences in spatial scale. Thus, if environmental 296 influences on gene flow had persisted in the non-native range, they should have been detectable. 297 Combined with the admixture analysis, these results support our prediction (H2) that multiple 298 introductions from diverse source populations and long-distance dispersal can weaken 299 environment-genetic structure relationships. Plantago lanceolata reproduces clonally as well as 300 sexually and this flexible reproductive mode, combined with high admixture in the non-native 301 range, suggests fast expansion after colonisation. This might allow the species to overcome 302 ecological constraints, without the need for local adaptation³⁶.

303

304 In the native range of *P. lanceolata*, the increase in genetic distance with precipitation seasonality 305 might partially reflect a historic biogeographical pattern (precipitation seasonality was correlated 306 with longitude, r = 0.47). Historical processes occurring along both east-west and north-south 307 axes shape contemporary genetic patterns in European plants. For example, glacial refugia in 308 Iberia, Italy and the Balkans, were reflected in highly divergent lines of Arabidopsis thaliana south 309 of the alpine barrier³⁷. In our dataset, the Italian population was genetically distinct, while two 310 eastern sites in Romania were highly differentiated and genetically related to Greece (Fig. 2). 311 François et al.³⁷ also found evidence for an eastern refuge in *A. thaliana*. Further sampling into 312 the continental Asian range of *P. lanceolata* would help uncover whether the observed patterns 313 arose from movement with agriculture westward across Europe^{38,39} or postglacial colonisers from 314 the Balkans⁴⁰.

- 315
- Hypothesis 3: Global gene flow will weaken demographic effects on genetic diversity within
 populations

We compared a series of linear models, including additive and interactive effects of range
(native/non-native) to address the hypothesis that environmental influences on within population
genetic diversity would differ between the native and non-native ranges (Dataset S1). Our results
offered partial support for Hypothesis 3 because environmental gradients (characterised by mean

temperature, temperature seasonality and mean precipitation) affected population growth rate,

fecundity and neutral and adaptive genetic diversity in native and non-native ranges of *P. lanceolata* (Fig. 5, see SI Appendix, Fig. S3). Our expectation, however, that genetic
responses to the environment could be explained by demographic variation had little support (see
SI Appendix, Fig. S3). Demographic variables responded to environmental gradients, but did not
induce a response on genetic diversity when used as predictor variables. Demographic and
genetic parameters within populations were best explained by environmental gradients and, in
some cases, there were differences in the responses between native and non-native ranges.

331

332 The top-ranked models for population growth rate (Fig. 5a) and fecundity (Fig. 5b) had additive 333 effects of mean temperature, responding similarly in the native and non-native ranges. Globally, 334 warmer sites tended to have lower population growth rates and higher fecundity. Increases in 335 fecundity can occur to offset lower survival in stressful environments⁴¹, a phenomenon which has 336 been recorded in other studies of *Plantago*^{42,43}. There was also an additive effect of temperature 337 seasonality on neutral genetic diversity (Fig. 5c), with highly seasonal sites having greater genetic 338 diversity in the native and non-native ranges. Mean temperature and temperature seasonality 339 were correlated (r = -0.36, p = 0.02, see SI Appendix, Fig. S4). Thus, the observed responses are 340 best thought of as responses to an environmental gradient, with demographic and genetic 341 parameters responding to different aspects of the gradient. High genetic diversity in highly 342 seasonal sites might have been driven by increased fecundity, since we found some evidence of 343 a positive relationship between fecundity and genetic diversity (see SI Appendix, Fig. S3g, 344 Dataset S1).

345

346 Three of the top-ranked models included an interaction between environment and range, showing 347 environmental effects in the native range but not the non-native range. Both neutral (Fig. 5d, 348 bootstrap CI = 0.001, 0.010) and adaptive (Fig. 5f, bootstrap CI = 0.004, 0.021) genetic diversity 349 decreased across a mean precipitation gradient in the native range, but not in the non-native 350 range. Adaptive genetic diversity increased with temperature seasonality, but only in the native 351 range (Fig. 5e, bootstrap CI = -0.021, -0.005). There was also support ($\triangle AICc < 2$) for non-native 352 populations having a weaker response to environmental gradients in terms of fecundity (see SI 353 Appendix, Fig. S3a, b), population growth rate (see SI Appendix, Fig. S3c) and neutral genetic 354 diversity (see SI Appendix, Fig. S3d). Taken together, these results suggest that non-native 355 populations are not constrained by the same environmental forces as their native counterparts.

356

Population growth rate and neutral and adaptive genetic diversity were all higher in the non-native range (Fig. 5, Dataset S1), suggesting that invasive populations have a greater capacity for colonisation and adaptation. Higher population growth rates in non-native populations were probably driven by increases in survival rather than fecundity, since fecundity was lower in the non-native range (Fig. 5b, Dataset S1). Thus, our simulation experiments and our field data indicated stronger effects of survival than of fecundity on genetic diversity and population growth, respectively.

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365 Increases in genetic diversity can arise when environmental heterogeneity drives population 366 turnover through increases in sexual reproduction, population growth and survival^{6,44}. In our study 367 however, population growth was affected by mean temperature, not variability in temperature; 368 cooler sites generally had higher rates of population growth across the first two demographic 369 censuses. This is consistent with previous work showing that high mean temperature was 370 associated with mortality in P. lanceolata⁴². Thus, we did not find a clear demographic 371 explanation for the effect of temperature seasonality on genetic diversity. Temperature stability 372 might have promoted clonality in *P. lanceolata*, leading to lower genetic diversity⁴⁵. However, 373 rates of sexual and clonal reproduction within species are often inversely related⁴⁶ and genetic 374 diversity was unaffected by rates of sexual reproduction in our study. The influence of global 375 variation in clonality on genetic diversity needs further investigation, particularly because clonality 376 combined with sexual reproduction can increase invasion success³⁶.

378 Our prediction that environmental effects on genetic diversity could be explained by demographic 379 variation had only little support, even in the native range. Except for a weak increase in neutral 380 genetic diversity with density (see SI Appendix, Fig. S3f) and fecundity (see SI Appendix, Fig. 381 S3g), there was little direct influence of demographic variables on genetic diversity. There are at 382 least two explanations for this general lack of a demographic relationship. First, genetic structure 383 can arise even under frequent dispersal⁴⁴. Thus, although we found strong spatial genetic 384 structure in the native range, it is possible that dispersal was high enough to mask any influence 385 of demography on genetic diversity (the natural dispersal pathway, Fig. 1). Second, the fine scale 386 of demographic sampling within sites (a few m²) might not reflect effective population size⁴⁷. This 387 fits with our understanding of abiotic filters operating at all scales, while biotic filters, such as 388 inter- and intra-specific interactions affecting demographic performance, generally operate at 389 localised scales^{10,13}. Plantago lanceolata is also highly genetically variable, within and outside its 390 native range. Thus, the low power within sites might have limited our ability to draw conclusions 391 about demographic influences on genetic diversity. Sampling more individuals per site in future 392 might reveal stronger effects of fecundity, survival and population growth on genetic diversity. 393

394 In summary, genetic diversity in *P. lanceolata* appears to be shaped predominantly by 395 temperature and precipitation gradients related to gene flow and admixture, rather than 396 demographic variation. Our data support the prediction, that high dispersal would dilute 397 demographic effects on genetic diversity (H1). Globally, our analyses suggest that genetic 398 diversity in the non-native range is shaped by admixture from multiple source populations and 399 ongoing introductions, leading to high neutral and adaptive genetic diversity (H2). Our data 400 suggest that invasive populations can establish in a broad range of environments, without the 401 need for associated demographic change. Thus, there was little support for the prediction that 402 demographic variation could explain environmental effects on genetic diversity (H3). Our unique 403 global demographic data set provides new evidence that invasive species can overcome ecological 'rules' in their non-native range¹¹⁻¹³. Reducing long-distance dispersal and further 404 405 introductions of invasive plants is important, even in areas where they already exist, as this will 406 limit future increases in genetic diversity and the formation of new genotypes that confer an 407 adaptive advantage in new environments.

409 Methods

410 411 Study overview

412

408

413 *Plantago lanceolata* is a short-lived (mean, max = $2.8, 8 \text{ yr}^{48}$), perennial forb, native to Europe. It 414 reproduces sexually and vegetatively, with gynodioecy, self-incompatibility and protogyny to 415 enhance outcrossing⁴⁹. Flowers are wind pollinated and seeds mature in summer. The species 416 occurs in a wide range of habitats including semi-natural grasslands, roadsides, disturbed sites, 417 abandoned fields and agricultural land⁵⁰. Seeds are dispersed locally by wind but seed dispersal 418 distances are estimated to be within centimetres or metres of the mother plant⁵¹. Widespread 419 propagule movement by humans²⁹ and repeated introductions as seed contaminants³⁰ has led to 420 the global distribution of P. lanceolata. It has been present in Australia since before 1850 421 (www.ala.org.au), in North America since before 1832³⁰ and for an unknown time in South 422 Africa⁵². It is cultivated as a commercial pasture plant in New Zealand because it grows well in 423 the mild winter and limits soil nitrification³¹. The species is classed as invasive in its non-native 424 range⁵² because it reproduces prolifically and spreads over large areas⁵³. We follow this definition 425 of 'invasive' to refer to P. lanceolata and other plant species with this characteristic. We use the 426 term 'non-native' to refer to the geographic range outside of Europe where the species exists.

427

428 We used field-collected demographic and DNA data from populations of P. lanceolata to analyse 429 spatial variation in demographic rates and genetic diversity. The demographic data were used to 430 parameterise the simulation part of the study (H1) and to analyse the demographic influence on

431 genetic diversity across global environmental gradients (H3). For the genetic data set, we

432 sampled 454 individuals from 53 naturally-occurring populations in 21 countries across the native European range (35 populations: Denmark, Estonia, Finland, France, Germany, Greece,
Hungary, Ireland, Italy, Norway, Romania, Spain, Sweden, Switzerland, United Kingdom) and the
non-native range (18 populations: Australia, Canada, Japan, New Zealand, South Africa, USA)
(Fig. 2). The latitudinal range of sampling, in absolute terms, was 27.5–61.4°. Forty-four
populations (83%) were established sites in the PLANTPOPNET network (www.plantpopnet.com)
undergoing an annual demographic census, while the remaining nine were sampled for DNA only
(see SI Appendix, Table S1).

441 We characterised the environment at each site using four variables from BioClim⁵⁴ at 30" 442 resolution: annual mean temperature, annual mean precipitation, temperature seasonality 443 (standard deviation of annual mean temperature) and precipitation seasonality (coefficient of 444 variation in annual mean precipitation). We selected these variables because they were important 445 for morphological variation in P. lanceolata in preliminary analyses and multi-collinearity was not 446 high (variance inflation factor < 3, maximum *r* between pairs of environmental variables = 0.43 447 (mean temperature and seasonality in precipitation) and between range (native/non-native) and 448 environment (mean temperature) = 0.59)⁵⁵.

449

450 Field demographic census & DNA sampling 451

452 PLANTPOPNET is an ongoing research project that began in 2014 and annual censuses of P. 453 lanceolata populations are planned for the long-term. Our analysis used data collected between 454 2014 and 2017, but not all sites began data collection at the same time (i.e. year 0 varied among 455 sites, see SI Appendix, Table S1). In most populations (61%), year 0 was 2015 and 73% of 456 populations were sampled twice during this study period (number of annual censuses per 457 population = 1-3, see SI Appendix, Table S1). At each census site in year 0, a series of adjacent 458 50 x 50 cm quadrats was established along transects until the quadrats covered 100 individual 459 plants. Researchers established transects where P. lanceolata was present in sufficient numbers 460 for demographic studies, so density estimates might reflect upper estimates across local 461 populations. Quadrats were permanently marked to enable repeat censuses from year 1 462 onwards. Each plant was individually tagged and all rosettes on each plant were measured 463 according to a standard protocol⁵⁶ which included leaf length, number of flowering stems, 464 inflorescence length and stage of seed development.

465

466 At each site, fresh leaf tissue from seven to nine individuals was collected and placed 467 immediately in silica gel (see SI Appendix, Table S1). Sampled individuals were close to 468 (approximately 5-20 m), but outside of, census plots and were separated from each other by 469 approximately 5-10 m. Thus, we avoided damage to permanently marked individuals in the 470 census population, ensured that samples were closely related to the census population and 471 minimised the chance of sampling clones. We included two samples each from one population of 472 P. coronopus (Spain) and four populations of P. major (Australia x 2, Ireland x 1, Romania x 1) as 473 outgroups. To investigate if naturally occurring populations were influenced by genetic stock from 474 commercial pasture lines, we included nine individuals from each of three cultivar lines derived 475 from *P. lanceolata*: AgriTonic, Ceres Tonic and Tonic Plantain. The whole data set thus included 476 491 individuals. The data are publicly available in Dataset S2

- 477 (https://doi.org/10.5281/zenodo.3579579).
- 478
- 479 Genotyping

480

Samples were genotyped at Diversity Arrays Technology P/L (Canberra, Australia) using double
 restriction enzyme complexity reduction and high-throughput sequencing (DArTseq). Total

483 genomic DNA was extracted with a NucleoSpin 96 Plant II Core Kit (MACHEREY-NAGEL) and

484 purified using a Zymo kit (Zymo Research). The enzymes Pstl and Msel were chosen following

tests of different enzyme combinations for *P. lanceolata*. DNA samples were processed in

486 digestion / ligation reactions following Kilian et al.⁵⁷ but substituting the single Pstl adaptor for two

487 adaptors corresponding to restriction enzyme-specific overhangs. The Pstl adaptor was modified 488 to include Illumina sequencing primers and variable length barcodes following Elshire et al.⁵⁸. 489 Mixed fragments (PstI-MseI) were amplified in 30 rounds of PCR using the following reaction 490 conditions: 94 °C for 1 min, then 30 cycles of 94 °C for 20 sec, 58 °C for 30 sec, 72 °C for 45 sec, 491 followed by 72 °C for 7 min. After PCR, equimolar amounts of amplification products from each 492 sample were bulked and applied to c-Bot (Illumina) bridge PCR followed by single-read 493 sequencing on an Illumina Hiseg2500 for 77 cycles. Raw sequences were processed using 494 DArTseg analytical pipelines (DArTdb) to split samples by barcode and remove poor quality 495 sequences. Genotypes for co-dominant, single nucleotide polymorphisms (SNPs) were called de 496 novo (i.e. without a reference genome) from 69 bp sequences using DArTseq proprietary 497 software (DArTsoft). Replicate samples were processed to assess call rate (mean = 79%). 498 reproducibility (mean = 99 %) and polymorphic information content (mean = 22%).

- 499
- 500 SNP filtering 501

502 Starting with 37,692 SNPs that passed DArTseq quality control, we filtered the data for minimum 503 minor allele frequency (1%), call rate (50%) and reproducibility (98%) using custom R scripts⁵⁹ 504 (Dataset S2), Loci in Hardy-Weinberg (HW) and linkage disequilibrium hold important biological 505 information about population structure but extreme disequilibrium can indicate genotyping errors 506 which bias estimates of population structure⁶⁰. Within sites, there was limited power to reliably 507 test for patterns of HW and linkage disequilibrium (7–9 individuals per site). It was not possible to 508 combine samples from multiple populations because we detected strong genetic structure, even 509 within countries, which would have produced biologically meaningful patterns of disequilibrium 510 arising from the Wahlund effect⁶¹. Thus, to identify SNPs with consistent patterns of HW 511 disequilibrium, we tested each locus in every population separately using Fisher's exact tests⁶² 512 and used un-adjusted P values given the low power within sites. Loci which deviated from HW 513 equilibrium in > 5 populations were removed⁶³. We used the correlation between genotype 514 frequencies⁶⁴ to test for linkage disequilibrium between each pair of loci in each population. 515 Following the same rationale as for HW disequilibrium, we removed a locus if it was in a 516 correlated pair (r > 0.75) in > 5 populations. To reduce the chance of disequilibrium from physical 517 linkage, we also filtered SNPs that occurred in the same 69 bp sequence as another SNP, 518 keeping the one with the highest call rate. The data comprised 21,190 SNPs after applying these 519 filters. 520

521 Detecting loci under putative selection 522

523 Neutrality was an assumption underlying the population structure models we used, thus, we 524 investigated if SNPs were putatively under selection using one population-level method 525 (BayeScan) and two individual-level methods (PCAdapt and LFMM). BayeScan uses an MCMC 526 algorithm to examine outlier loci against background values of population differentiation (F_{ST}) 527 among pre-defined populations⁶⁵. PCAdapt and LFMM both define background population 528 structure as K principal components derived from individual genotypes^{66,67}. In PCAdapt, each 529 SNP is regressed against each principal component. LFMM uses the principal components as 530 latent factors in a Gaussian mixed model, where the genotype matrix is modelled as a function of 531 an environmental matrix⁶⁷. While BayeScan is suitable for our population-level sampling design, 532 PCAdapt and LFMM are more reliable for species with complex, hierarchical population structure 533 (e.g. multiple divergence events) and are less sensitive to admixed individuals and outliers in the 534 data^{68,69}. Thus, we considered outliers identified in any of the three methods to be putatively 535 under selection.

536

537 For BayeScan, we set the prior odds at 200 (appropriate for the number of markers in our data⁷⁰), 538 ran the model using default parameters (100,000 iterations with a thinning interval of 10, a burn-in 539 of 50,000 and 20 pilot runs of 5,000 iterations), and checked the distribution of the log likelihood

across iterations to ensure model convergence (see SI Appendix, Fig. S5). For both individual-

541 level methods, we examined scree plots to determine K and used the first 10 components which 542 captured the majority of population structure in the data (see SI Appendix, Fig. S5). We defined 543 the LFMM environmental matrix using the four 30" BioClim variables described above and three 544 additional variables: elevation (metres above sea level, measured at the site) and two variables 545 extracted from CliMond⁷¹ at 5' resolution: annual mean moisture index and seasonality in 546 moisture (CV of annual mean moisture). To control for false discovery rate, we calculated q-547 values from p-values and classed SNPs as outliers where q < 0.05 for BayeScan and PCAdapt 548 and q < 0.1 for LFMM (to account for the small number of loci identified with this method, see SI 549 Appendix, Fig. S5). The three analyses identified a total of 3,026 outlier SNPs and, as commonly reported in other studies⁶⁹, there was little overlap among methods (see SI Appendix, Fig. S5). 550 551 After filtering the putatively adaptive loci, our final data set comprised 18,164 neutral SNPs.

552

554

553 Simulated genetic diversity (Hypothesis 1)

We conducted two simulation experiments in MetaPopGen 0.0.4⁷² to determine if realistic levels of variation in *P. lanceolata* survival and fecundity would influence genetic diversity and whether dispersal would override demographic influences on genetic diversity. Gametes in the model are produced via Mendelian segregation and mating is random⁷². We modelled two distinct populations to examine different rates of juvenile survival and female fecundity. In Experiment 1, the two populations were unconnected by dispersal, while in Experiment 2 they were connected by varying levels of dispersal.

563 Male fecundity $\delta_{\rm M}$ in *P. lanceolata* is high (10,000–54,000 pollen grains per anther³²) and had no 564 influence on genetic diversity. Thus, we set δ_M at 10,000 and focussed on variation in female 565 fecundity (seeds per plant) \mathcal{B}_{F} , adult σ_{a} and juvenile σ_{i} survival rate, and between-population 566 dispersal δ (number of migrants per generation). In both experiments each of the two populations 567 *i*, had two age classes x (juvenile x_i , adult x_a), three genotypes p representing all combinations of 568 two alleles (00, 01 and 11) and a starting size N_{xp} of 25,000 individuals. The model was not 569 spatially explicit, but we wanted each population to represent a 1 ha site with a density of 15 570 individuals / m² (based on census data from year 0). Generation time in *P. lanceolata* is 571 approximately 3 years (range 1–3 years^{73,74}). Thus, we ran the model for 100 time steps to 572 represent population dynamics over 100-300 years, accounting approximately for the time P. 573 lanceolata has been present in its non-native range. Population sizes reached a steady state 574 within 10 time steps. We estimated juvenile carrying capacity as $K = (A_F^* (N^* p))^* q$, where q is 575 the estimated field germination rate (0.039). We kept K time- and population-constant. 576 MetaPopGen can only simulate one locus at a time, so we repeated the experiments 300 times to 577 simulate sampling 300 independent loci (following⁷²).

578

579 In Experiment 1, we tested the influence of $\mathcal{B}_{\rm F}$ on genetic diversity (1–100, based on census data 580 from year 0) and σ (σ_{ii1} = 0.1; σ_{ai1} = 0.84; σ_{ii2} = 0.2; σ_{ai2} = 0.71) with no dispersal between populations (δ =0). Survival rates were based on a total population estimate of 5% alive after five 581 years (exp(log(0.05)/5)) (ref. 73) and adjusted for commonly reported low survival in juveniles⁴². In 582 Experiment 2, we tested the influence of δ (migration rate: 0–0.04 = number of migrants: 0– 583 584 60,000) on the difference in genetic diversity between populations. Each population had the same 585 survival rates as Experiment 1 and $\beta_{\rm F}$ was kept constant at 20. The migration rates produce large 586 numbers of migrants because each plant produces 20 'newborns' and migration occurs before 587 recruitment in the model⁷². Thus, δ is influenced by K and will always be higher than recruitment. We summarised expected heterozygosity at the end of each simulation and calculated the mean 588 589 and 95% confidence interval across the 300 loci. The experiments can be reproduced with the 590 code in Dataset S2.

592 Population genetic structure (Hypothesis 2)

593

594 All population structure analyses used our panel of neutral SNPs; a choice dictated by the model 595 assumptions being based on Hardy-Weinberg and linkage equilibrium. We first conducted an 596 Analysis of Molecular Variance in poppr 2.8.0⁷⁵ to determine how neutral genetic diversity was 597 partitioned across levels: within individuals, among individuals within populations, among 598 populations within ranges, and between the native and non-native range. To assess genomic 599 relationships and the degree of admixture in the global data set, we used fastSTRUCTURE³⁴. 600 This model determines the number of genetic clusters in the data that would maximise Hardy-601 Weinberg and linkage equilibrium (K). We investigated K=1 to K=20 and assigned each individual 602 to a cluster based on the model complexity that maximised marginal likelihood and the model 603 components used to explain structure in data³⁴. To quantify the level of admixture for each 604 individual (i) across the most likely K, we calculated a Diversity Score⁷⁶ as:

605

DS =	$-\Sigma_{i=1}^{K}C_{i}$	$\cdot \ln(C_i)$
	$-H_{rr}$	nax

606

607 where C_i is the cumulative admixture and H_{max} is a scaling factor $(H_{\text{max}} = K \cdot ((1/K) \cdot \ln(1/K)))$, 608 making DS relative to complete evenness for each individual. We used a linear mixed model to 609 evaluate whether there was a difference in DS between the native and non-native range, with site 610 fitted as a random effect. 611

612 To determine whether multiple introductions of *P. lanceolata* had occurred in non-native regions 613 (Australia, Japan, New Zealand, North America and South Africa) we estimated the minimum 614 number of propagules required to produce the observed level of genetic diversity in non-native 615 regions $(Prop_{min})^{77}$. We defined the source population as all of Europe because non-native 616 individuals were usually composed of admixed genotypes from multiple European populations. 617 For each non-native region, we calculated the number of alleles not present in Europe and 618 removed these from the reference panel of non-native alleles. Individuals from the native range 619 were then randomly cumulatively sampled without replacement. Propmin was the number of 620 individuals sampled at the point when all alleles in the non-native panel were represented 621 (Dataset S2). We repeated the process 1000 times to obtain a mean and standard error. We also 622 calculated the number of unique alleles in each of the 53 sites as a measure of uniqueness. 623

624 To assess the influence of environmental gradients on spatial genetic structure, we used 625 generalised dissimilarity models^{78,79}. We fitted one model for the native range, a second for the 626 non-native range and a third for the global data set (native and non-native). We calculated 627 genetic differentiation as F_{ST} between all pairs of populations in GENEPOP 4.6⁸⁰. Environmental 628 distances between all pairs of populations i and j were calculated from the four BioClim variables 629 $x (x_i - x_i)^{79}$. For each of the three data sets, we fitted geographic distance and all environmental 630 distances as predictor variables in a single model. The importance of each variable, given all 631 other variables, was assessed by comparing the fitted model to 500 models with a permuted 632 environmental matrix⁷⁹. Thus, the effect of each environmental variable can be interpreted 633 independently and differences in spatial scale are accounted for by the geographic distance 634 variable. P values were Bonferroni-adjusted across all terms within each model. We used 635 deviance explained to assess goodness-of-fit of the three models. Given samples size differences 636 between the three data sets, we used a bootstrap estimate from 10,000 replicates of the deviance 637 explained to assess the accuracy of the model fit. We assumed the deviance explained to be 638 accurate if bootstrap 95% confidence interval (CI) did not include zero.

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Demographic & dispersal effects on genetic diversity (Hypothesis 3) 641

642 We used linear regression to determine if environmental influences on within-population genetic 643 diversity could be explained by demographic variation and whether this effect would be weakened by mass dispersal into the non-native range (Hypothesis 3). The observation-level for all analyses
was the population and the number of observations was 44 (i.e. all populations with genetic and
demographic data, see SI Appendix, Table S1).

648 Genetic diversity was calculated as allelic richness in hierfstat⁸¹, separately for the neutral 649 (18,166 SNPs) and adaptive (3,024 SNPs) datasets. Allelic richness was highly correlated with 650 expected heterozygosity (H_{e}) (r = 0.98) and, because it was standardised for sample size, it 651 eliminated a weak correlation we observed between He and sample size. We characterised the 652 environment using the four BioClim variables. For demography, we used three variables that can 653 influence genetic diversity (Table 1): population density (rosettes/m²), fecundity and empirical 654 population growth rate. For fecundity, we used reproductive effort, estimated as the rosette-level 655 inflorescence length x number of flowering stems per m². Empirical population growth rate was 656 calculated as $r = \log(N_{t+1}/N_t)$, indicating the strength and direction of change in rosettes/m² in the 657 first two years of the study (for 38 of the 44 populations with two years of data, see SI Appendix, 658 Table S1). Thus, r reflects the combined influence of fecundity and survival (the variables 659 explored in simulation Experiment 1). We used rosette-level data for all metrics to reduce 660 potential observer bias in assessing clonality, but plant- and rosette-level metrics were highly 661 correlated (r = 0.94). Fecundity was log-transformed to address a strongly skewed distribution 662 and all predictors were standardised prior to analysis (x - mean(x)/SD(x)).

663

664 We tested environmental and demographic effects separately, to determine which variables best 665 described variation in genetic diversity. The analysis comprised two stages. First, we analysed 666 the effect of each environmental variable on genetic diversity. Here, we also modelled the 667 environmental effect on demography (i.e. using the three demographic variables as response 668 terms) to establish a baseline for environmental influences on demographic rates. Second, we 669 examined whether each demographic variable influenced genetic diversity. In both stages we 670 analysed environmental and demographic interactions with range (native/non-native). Because 671 data limitations (n = 44) it was not possible to fit complex models with multiple interaction terms 672 so we modelled each predictor separately.

673

685

674 To determine the importance of each environmental or demographic predictor, we used AICc to 675 compare model fit across five alternative model forms: a null model (no predictor variation), a 676 predictor only model, a range only model, an additive model (predictor + range) and an interactive 677 model (predictor x range). We considered a model to have support from the data if it improved the 678 fit over the null model by $\Delta AICc > 2$ (ref. ⁸²). Among models that out-fitted the null, those within 679 Δ AICc \leq 2 of each other were considered to have equal support from the data. In these cases, we 680 presented the top-ranked model in the main document and supported models in the Supporting 681 Information. To interpret interaction models in light of sample size differences between the native 682 (30) and non-native (14) ranges (e.g. a strong response in the native range and no response in 683 the non-native range), we obtained a bootstrap 95% confidence interval (CI) from 10,000 684 bootstrap replicates of the interaction coefficient using the adjusted bootstrap percentile method.

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887 Figures and Tables

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889 Figure 1. Conceptual diagram showing how demographic performance and dispersal collectively 890 shape genetic diversity in plant populations (+ indicates a positive relationship expected). Genetic 891 diversity is influenced through natural pathways (solid line), such as local environmental 892 conditions which affect demographic performance and effective population size⁴. Environmental 893 conditions also affect genetic diversity though dispersal (e.g. by facilitating dispersal vectors or 894 creating dispersal barriers). Dispersal can increase genetic diversity directly by providing a source 895 of new genetic material (outcrossing) or indirectly through immigration and consequent effects on 896 demography. High propagule pressure arising from high fecundity can influence source-sink 897 dynamics^{7,83}, increasing rates of dispersal (hence the double arrow between demography and 898 dispersal). Human activity can affect genetic diversity (dashed lines) by altering environmental 899 conditions (e.g. climate change) and by changing dispersal rates and dispersal pathways (e.g. 900 admixture). When this occurs, demographic performance can also be affected (e.g. through 901 enemy release associated with dispersal across biogeographic boundaries) which can cause 902 invasive plants to overcome biotic constraints on life-history¹¹ and environment-trait 903 relationships¹³. Although genetic architecture can influence demography and dispersal, the 904 overall quantity of neutral genetic diversity across the genome is more likely to be the outcome of 905 demographic and dispersal processes, hence the one-sided arrows between these panels. 906 907

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910 Figure 2. Global genetic structure in Plantago lanceolata. (a) Coloured bars represent the 911 proportion of individual genotypes in each population assigned to one of six genetic clusters 912 identified with fastSTRUCTURE. For clarity, multiple sites were aggregated where overlapping bars had similar assignment probabilities (e.g. southern Ireland, Switzerland). Dark grey points 913 914 are *P. lanceolata* records from GBIF/BIENGBIF^{84,85}. For each non-native region, the minimum 915 number of propagules (mean ± standard error), overall (Propmin) and relative to sample size 916 (Prop_{min} / N), indicates that multiple introductions would be required to produce observed levels of 917 genetic diversity. The number of non-European alleles indicates that more genetic diversity was 918 present in non-native regions than could be explained by the native sample. (b) Probability of 919 assignment for 491 individuals to six genetic clusters, with individuals grouped by population 920 within region. Three commercial cultivar lines and two outgroups (P. coronopus and P. major) 921 were included. Country codes for each population are shown on the x-axis. 922 923



925 Figure 3. The simulated effect of demography and dispersal on genetic diversity (expected 926 heterozygosity, ± 95% confidence interval) in two populations of *Plantago lanceolata*. (a) When 927 there was no dispersal between populations, the population with high juvenile survival ($o_j = 0.2$) 928 had greater genetic diversity than the population with low juvenile survival ($o_i = 0.1$). At very low 929 levels of female fecundity $\mathcal{S}_{\rm F}$, populations went extinct (†) but $\mathcal{S}_{\rm F}$ had little influence on genetic 930 diversity at approximately > 25 seeds per plant. (b) Variation in o_i influenced population size at 931 the end of the simulation. (c) The difference in heterozygosity between the two populations was 932 influenced by dispersal between them (where fecundity was kept constant at 20 seeds / plant). (d) 933 Genetic differences persisted until high levels of dispersal (> 50,000 migrants per generation) 934 indicated by the 95% confidence interval crossing zero. 935



- Figure 4. Genetic distance (F_{ST}) between pairs of *Plantago lanceolata* populations in the native European range was explained by two variables: (a) geographic distance and (b) distance in precipitation seasonality (coefficient of variation of annual mean precipitation) between sites. A generalised dissimilarity model indicated these variables had a significant (adjusted P < 0.001) effect on FST, given all other variables in the model (geographic distance, mean temperature, mean precipitation, temperature seasonality and precipitation seasonality). Deviance explained by the model was 74.3% and the model splines are shown in SI Appendix, Fig. S2.



947 Figure 5. Environmental influences on demography and genetic diversity within populations in the 948 native European (n = 30) and non-native (n = 14) range of Plantago lanceolata (model estimates 949 and 95% confidence intervals shown over raw data). First-ranked models are shown for 950 environmental influences on (a) population growth rate, (b) reproductive effort, (c-d) neutral 951 genetic diversity and (e-f) adaptive genetic diversity. In all models except (e), the additive and 952 interactive models both had support from the data (△AICc < 2, see SI Appendix, Fig. S3 and 953 Dataset S1). For (e), the interaction between temperature seasonality (standard deviation of 954 annual mean temperature at each site) and range (native/non-native) was the only model 955 supported by the data (AICc weight = 0.95). 956



Table 1. Demographic variables used to analyse population processes that are important to
 genetic diversity. The relevance of demographic variables to genetic diversity is outlined in Fig. 1
 and described in detail by Ellegren and Gaultier⁴

Demographic	Used as a proxy for	Relevance to	Formula
variable measured		genetic diversity	
Density	Population size	Effective population	Number of rosettes /
		size	m ² (<i>N</i>)
Reproductive effort	Fecundity	Fitness	(inflorescence length x
per unit area			no. flowering stems) /
			m ²
Empirical population	Combined effects of	Fitness	$\log(N_{t+1}/N_t)$
growth rate	survival & fecundity		