Geographical gradients in leaky sex expression and reproductive effort in a dioecious plant are consistent with selection during range expansion

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Keywords: colonisation, self-fertilisation, reproductive assurance, Baker' Law, life-history trade-off, r-selection, ruderal life-history strategy, dispersal

Abstract

Range expansion involves repeated dispersal events and the establishment of new populations through colonisation. Natural selection is therefore expected to favour traits that enhance dispersal and enable uniparental reproduction when mates are scarce. There is substantial evidence that range-edge populations evolve both increased dispersal ability and, in hermaphroditic species, an enhanced capacity for self-fertilisation. By contrast, the evolution of a greater capacity for uniparental production has not yet been demonstrated for dioecious plants. Here, we report results from a large common-garden experiment to assess the life history and sex expression of males and females of the dioecious annual herb *Mercurialis annua* sampled from across its European range. We found that plants from range-edge populations in central and western Europe not only tend to have smaller seeds and greater reproductive effort (RE) than populations from the eastern Mediterranean Basin, from where the species expanded its range, but also that males and females have an increased incidence of leaky sex expression (the production of flowers of the opposite sex). Small seeds and high RE are associated with dispersal, and the colonisation of new populations, and leaky sex expression allows otherwise unisexual individuals to produce progeny by self-fertilisation. Our results suggest that leaky sex expression in this species has been enhanced by selection for reproductive assurance, rather than representing a by-product of developmental instability during floral development or a relic of an incomplete transition to dioecy, as has been proposed for other dioecious species exhibiting leaky sex expression.

Introduction

The geographical distribution of species, especially at mid to high latitudes, is widely the result of range expansions in the past (Hewitt, 2000; Svenning & Skov, 2007). Such range expansions have long been known to leave their traces in the population genetics of the species concerned, not only in response to selection for adaption to new abiotic and biotic environments (Bridle & Vines, 2007; Sexton *et al.*, 2009), but also as a result of the recurrent genetic bottlenecks that occur with successive steps of colonization (Slatkin, 1977; Excoffier *et al.*, 2009; Slatkin & Excoffier, 2012). The genetic bottlenecks increase the effect of random drift on the genome, causing the stepwise depletion of genetic diversity with distance from the pre-expansion 'core' of the species' range (Ibrahim *et al.*, 1996; Hewitt, 2000; Excoffier *et al.*, 2009). This depletion corresponds to both neutral variants, but also to variants with effects on fitness (Excoffier *et al.*, 2009). Accordingly, we expect to see the accumulation of deleterious mutations with recurrent colonization (Peischl *et al.*, 2013, 2015; González-Martínez *et al.*, 2017; Willi *et al.*, 2018), increased homozygosity at the corresponding loci and a consequent reduction in inbreeding depression (Pujol *et al.*, 2009) as well as lower responsiveness to natural selection and fewer selective sweeps towards the range edge (González-Martínez *et al.*, 2017). However, we also expect natural selection to act through the process of colonization itself, favoring phenotypes that confer more successful colonization.

A key idea is that individuals capable of uniparental reproduction, whether by asexual means (Delnevo *et al.*, 2024) or self-fertilisation (Pannell, 2015), should be more likely to succeed in colonizing new habitats than those that need to find a mate to reproduce. Baker (1955) linked observations of an increased selfing capacity in species on oceanic islands to colonization followng long-distance dispersal to islands. This enrichment on islands of species with a capacity to self as a result of selection for reproductive assurance was later labelled Baker's Law by Stebbins (1957), highlighting its broad relevance across both plants (Baker, 1955, 1967) and animals (Longhurst, 1955; Stebbins, 1957). Since then, the concept has been invoked to explain the prevalence of uniparental reproduction not only in island colonists but also in metapopulations and as a result of range expansions (reviewed in Cheptou, 2012; Pannell, 2015; Pannell *et al.*, 2015). For instance, a comparative study of more than 1,500 species found a significant over-representation of self-compatible species on islands in all three considered

68 families *Asteraceae*, *Brassicaceae* and *Solanaceae* (Grossenbacher *et al.*, 2017). In
69 metapopulation systems, higher extinction rates and greater patchiness are associated with
70 increased frequencies of self-fertilising individuals in species with mixed mating, including
71 *Silene vulgaris* (Taylor *et al.*, 1999) and *Mercurialis annua* (Obbard *et al.*, 2006b). In the context
72 of range occupation and expansion, autonomously apomictic species, e.g., in the *Asteraceae* and
73 *Ranunculus*, tend to occupy broader distributions than their sexually reproducing relatives
74 (Hörandl *et al.*, 2008; Cosendai *et al.*, 2013; Hörandl, 2023). In *Senecio squalidus*, an increased
75 selfing ability at the introduced range has been linked to reductions in self-incompatibility and S-
76 allele diversity, perhaps favoured by selection for reproductive assurance (Brennan *et al.*, 2013).
77
78 Selection on a colonisation ability may also apply to phenotypic variation in dioecious species,
79 as originally proposed by Baker (1967). The occurrence of dioecious species on islands might
80 appear surprising, given that usually dioecy implicates biparental rather than uniparental
81 reproduction. However, Baker drew attention to the fact that many dioecious plants exhibit
82 inconstant sex expression, with males and females producing some flowers with the opposite
83 sexual function, potentially allowing them to self-fertilise. This phenomenon, referred to as
84 inconstancy or 'leakiness' in sex expression (e.g., Baker, 1967; Lloyd & Bawa, 1984;
85 Korpelainen, 1998), is common in dioecious species (Ehlers & Bataillon, 2007; Cossard &
86 Pannell, 2019); in what follows, we refer to it simply as 'leakiness'. Although Ehlers and
87 Bataillon (2007) and Crossman and Charlesworth (2014) modelled the evolution of leakiness in
88 terms of a response to selection for reproductive assurance, this possibility has been underplayed
89 in the literature and has not been demonstrated empirically. For instance, it has been suggested
90 that its occurrence may simply reflect developmental noise (Lloyd & Bawa, 1984), or that is may
91 be a plastic response to enviromental factors (Charnov & Bull, 1977; Freeman *et al.*, 1980; Case
92 & Barrett, 2001, 2004; Delph, 2003) such as temperature, drought, herbivory (Delph & Wolf,
93 2005; Golenberg & West, 2013; Villamil *et al.*, 2022). Lloyd (1980) interpreted manifestations
94 of leakiness in dioecious species as evidence of still incomplete transitions from combined to
95 separate sexes. Cossard et al. (2021) demonstrated that variation in leakiness in the dioecious
96 annual plant *Mercurialis annua* has a genetic basis and is responsive to selection. However, to
97 our knowledge evidence that leakiness can be promoted specifically by selection for

reproductive assurance is still lacking from wild populations, not least those that have expanded their range through repeated colonization.

Selection during range expansions should also act on dispersal traits (reviewed in Hargreaves & Eckert, 2014; Chuang & Peterson, 2016; Weiss-Lehman & Shaw, 2022). Increased dispersal rates and dispersal distances are expected to be favored by selection, through the process of colonization itself (Travis & Dytham, 1999) and as a result of assortative mating of highly dispersive individuals at the expansion front, a phenomenon labelled the olympic village effect (Shine *et al.*, 2011). Notable examples include the invasive cane toad *Rhinella marina* in Australia, which has evolved longer legs enabling faster dispersal at the invasion front (Phillips *et al.*, 2006, 2007). Similarly, in plants, the ragwort *Senecio inaequidens* exhibits increased pappus size, enhancing wind-dispersal distance toward its range margin (Monty & Mahy, 2010) and the dune plant *Abronia umbellata* shows increased wing index toward the range limit, presumably because this trait facilitates greater dispersal distances (Darling *et al.*, 2008). Cody and Overton (1996) argued that greater dispersal ability in mainland plants than on old islands that had been colonized from the mainland reflected advantages of dispersal among available habitat patches on the mainland whereas island populations would be comprised of the descendants of plants that did not disperse. Cheptou et al. (2008) recorded a similar observation of reduced dispersal in older populations in urban metapopulations of the plant *Crepis sancta*. This phenomenon is part of the well-known 'island syndrome', which includes the maintenance of flight in animals on continents and its loss on islands (Foster, 1964; Adler & Levins, 1994; Jezierski *et al.*, 2024). The evolution of dispersal and related traits as a result of selection during colonization has been labelled the 'metapopulation effect' (Olivieri & Gouyon, 1997), but it should apply similarly to colonization during range expansions.

The evolution of traits conferring greater dispersal should come at a cost in other traits (Tilman, 1990; Burton *et al.*, 2010; Urquhart-Cronish *et al.*, 2024). Specifically, we expect greater dispersal and colonization ability to trade-off against traits conferring competitive ability. For instance, in the parlance of the classic *r*/*K* continuum, colonizers should often be *r*-strategists, prioritizing high reproductive output and dispersal over long-term survival and competitive ability, while species in stable, crowded environments should tend to be *K*-strategists

129 (MacArthur & Wilson, 1967; Pianka, 1970; Reznick *et al.*, 2002; Travis *et al.*, 2023). Similar
130 ideas form the basis of the competition-ruderal axis in Grime's (1974) C-S-R triangle, where
131 ruderal refers to species with traits that allow rapid colonisation at the expense of their
132 competitiveness within crowded habitats. This notion aligns in general with the fast-slow life-
133 history continuum (Stearns, 1983, 1989), where fast, ruderal strategies with rapid development,
134 early reproduction, short lifespans, are traded off against traits that promote long-term
135 persistence and competitive ability, such as greater plant height and biomass. Shifts toward
136 greater colonization ability in plants are particularly evident for seed traits, with ruderal species
137 tending to produce more, lighter and smaller seeds that disperse easily but having fewer
138 resources to cope with competition (Venable, 1992; Turnbull *et al.*, 1999; Leishman, 2001;
139 Maron *et al.*, 2021). For example, the lodgepole pine *Pinus contorta* ssp. *latifolia* showed
140 reduced seed mass and wing loading toward its invasion front (Cwynar & MacDonald, 1987),
141 and multiple invasive *Rumex* species were found to produce more (though not smaller) seeds in
142 introduced ranges (Bufford & Hulme, 2021). On the basis of these ideas, we should expect to
143 find plants at the edge of a range expansion to produce more and smaller seeds and to allocate
144 less to size and vegetative competitiveness. This would also correspond to a strategy of increased
145 reproductive effort (Harper & Ogden, 1970; Perry *et al.*, 2012) in plants towards the range edge.
146
147 Here, we test the hypothesis that selection should have favoured increased leakiness in sex
148 expression, smaller seed sizes, smaller biomass and greater reproductive effort in range-
149 expanded populations of the European annual dioecious plant, *Mercurialis annua* L.
150 (Euphorbiaceae). Populations of dioecious *M. annua* in western Europe have lower within-
151 population genetic diversity (Obbard *et al.*, 2006b) and display signatures of increased genetic
152 load with fewer selective sweeps than those in the east (González-Martínez *et al.*, 2017),
153 observations that are all consistent with a range expansion into western Europe from the eastern
154 Mediterranean Basin. *M. annua* has sex chromosomes (Russell & Pannell, 2015; Veltsos *et al.*,
155 2018; Gerchen *et al.*, 2022) and strong sexual dimorphism (Harris & Pannell, 2008; Hesse &
156 Pannell, 2011; Sánchez Vilas & Pannell, 2011; Tonnabel *et al.*, 2019), but it also shows
157 substantial leakiness in both sexes (Cossard & Pannell, 2019; Cossard *et al.*, 2021). Previous
158 research showed that the removal of males from experimental populations of the species elicited
159 a strong response to selection on the sex allocation of females, substantially increasing their

leakiness (Cossard *et al.*, 2021). We expected that selection for reproductive assurance during the range expansion of *M. annua* into Europe should be evident in a pattern of enhanced leakiness with distance from the inferred geographic origin in the eastern Mediterranean Basin. We also expected plants in the expanded range to produce more, smaller seeds and to allocate less to vegetative size.

Leakiness can be measured both by its incidence (the proportion of individuals producing opposite-sex flowers) and its degree (the number of opposite-sex flowers produced per individual) (Cossard & Pannell, 2019). We expected reproductive assurance to favour increased incidence of leakiness in both sexes, but increased degree only in males, because a mildly leaky female could self-fertilise many ovules and establish a population, whereas the success of a colonising male would depend on it producing many seeds, requiring a substantial degree of leakiness. An greater degree of leakiness in males than females is also expected as an outcome of selection towards female-biased sex allocation due to local mate competition (Hamilton, 1967; Roux *et al.*, 2024).

Sex expression in *M. annua* is mediated by the plant hormones auxin (masculinising) and cytokinin (feminizing) (Louis & Durand, 1978; Dauphin-Guerin *et al.*, 1980; Hamdi *et al.*, 1987; Li *et al.*, 2019). Previous research found that exogenous application of these hormones was able bring about sex change in individuals sampled from western but not eastern European populations (unpublished observations). Accordingly, we complemented our study by testing the hypothesis that plants from populations further from the species' core would be increasingly responsive to exogenous hormone application (i.e., becoming leakier in terms of incidence, as well as of degree).

To test our hypotheses, we collected plants as seeds from 26 populations spanning the geographic range of diploid *M. annua* and grew them under common garden conditions. We then assessed among-population variation in leakinesss, hormone-induced leakiness, seed size, plant size and reproductive effort. Because the range expansion of *M. annua* led to the occupancy of habitats under different climates (typically cooler, more mesic climates in the north and west compared to the eastern Mediterranean), we also included in our analysis potential trait

associations with climate – though we had no *a priori* expectations for the direction of any effect of climate on leakiness.

Methods

Sampling of populations across the geographic range

To examine variation in leakiness and reproductive effort across the geographic range of diploid dioecious *M. annua*, we collected seeds from 26 populations in autumn 2023 and spring 2024 (Fig. 1, Tab. S1). For each population, seeds were bulked from at least 20 females, except for the Albanian population, where only five individuals could be sampled due to its small size.

Experimental set up, plant growth, and hormone treatments

In autumn 2024, we sowed seeds from 26 populations in trays with a peat-based substrate. After one month, when most plants had begun flowering, we transplanted between 80 and 120 individuals per population into 0.68 L pots filled with Ricoter 140 soil. Exceptions were Populations 1 and 8, where only 43 and 55 plants germinated, respectively, and all of these were used. For logistical reasons, we grew males and females in separate glasshouses; our design thus effectively involves two independent experiments, one for each sex. We were not interested in a direct sex comparison and measured a different set of variables on each sex. In each glasshouse, plants were randomly distributed across seven tables in a random block design.

After a month of growth, we applied hormone treatments to half of the plants in 24 of the populations (we excluded the two populations with lower plant numbers, i.e., these were not treated). Specifically, in addition to automatic watering, half of the males and half the females, respectively, received 55 ml of cytokinin and 55 ml of auxin twice a week; control plants received 55 ml of water. Following Louis and Durand (1978), we used 2 µM BAP for the cytokinin treatment and a mixture of 1×10^{-3} g/L IAA and 1×10^{-2} g/L GA_3 for the auxin treatment. Randomization ensured a balanced distribution of plants from each population and treatment across tables.

Measurements of reproductive effort and leakiness

After three weeks of growth following the onset of the hormone treatments, we harvested plants table by table in each glasshouse, thus retaining table and harvest time together as the blocking unit. For most tables, all plants were harvested within two days. For each plant, we recorded the number of flowers of the opposite sex (i.e., fruits on males and male flowers on females), plant height and above-ground biomass. Additionally, for a subset of five plants of each sex, population and hormone treatment (i.e., 5 x 2 x 25 x 2 for a total of 500 plants), we counted the number of female fruits on females and male flowers on males. These data were used to calculate an index of reproductive effort, defined as the number of male flowers on a male or the number of female fruits on a female divided by its respective biomass. The reproductive effort of plants without hormone treatment was used to calculate the population average male or female reproductive effort, which we used to compute the degree of leakiness.

We computed two metrics of leakiness: (i) the incidence of leakiness (whether a plant was leaky or not, i.e., in producing at least one flower of the opposite sex at the time of observation) and (ii) the degree of leakiness (how leaky a plant was in quantitative terms). We followed Cossard and Pannell (2019) to compute the degree of leakiness for females as $d_f=\lambda_f/M$, where λ_f is the given female's male flower production (i.e., its male RE), and M is the male reproductive effort of an average male from the same population as the female. Due to phenotyping constraints, M for each population was calculated as the average of five untreated males selected for detailed phenotyping. The degree of leakiness for males was computed as $d_m=\lambda_m/F$, where λ_m and F are defined analogously to λ_f and M, respectively.

<u>Measurements of seed size and seed mass</u>

We weighed and photographed approximately 50 randomly chosen seeds from the bulk field-collected seeds of 23 of the 26 populations. In the remaining three populations, all seeds had to be used for the common garden experiment, precluding estimates of seed size. Seed images were analyzed using ImageJ (Schneider *et al.*, 2012), employing the "Analyze Particles" function to estimate the projected shadow area of each seed . We estimated the average seed mass for each population by dividing the total mass of ~50 seeds by the number of seeds.

<u>Models predicting the recorded traits</u>

We constructed generalized linear mixed models (GLMMs) and linear models to assess the dependence of seven plant traits on distance from the range core: plant biomass, plant height, incidence of leakiness, degree of leakiness, seed weight, seed size, and reproductive effort. We defined the species range core as a midpoint between the two easternmost collection sites (GPS: 32.781618, 35.013214; Fig. 1). We computed each site's distance to the core (Tab. S1) using two metrics: (1) geodesic distance (Vincenty, 1975) and (2) shortest walking distance minimizing water crossings (via Google Maps). The two distance measures were highly correlated, with outliers being the populations in central and southern Italy (Tab. S1, Fig. S2). Our results were accordingly very similar for the two measures, so we present only results for geodesic distances in what follows.

To analyse trait variation recorded in each of the (male and female) common gardens, we used GLMMs with a consistent fixed and random effects structure, differing only in response variables, distribution families, offset terms, and zero-inflation components when applicable. Random effects included collection site and the glasshouse table-sampling time block effect. Fixed effects were distance from the core, hormone treatment (binary), and the interaction between distance and hormone treatment. For the two seed traits, which were measured from field-collected seeds, our model excluded the hormone treatment.

We modelled the incidence of leakiness as a binary variable. The degree of leakiness was modeled using a zero-inflated negative binomial GLMM, with counts of leaky flowers (males) or fruits (females) as the response, offset by the log-transformed product of plant biomass and population-average reproductive allocation. The zero-inflation component included distance as a fixed effect, based on results from the binomial model. Reproductive effort was modeled with a negative binomial GLMM, using counts of fruits or male flowers, offset by log biomass. Plant height and biomass were modeled with Gaussian GLMMs; biomass was square-root transformed. Of the seed traits estimated from field samples, seed weight was modeled with a linear model using population-average seed weight, and seed area was modeled with a Gaussian GLMM, including collection site as a random effect. Both models included distance from the core as fixed effects.

283 Note that our model for female biomass as a function of geodesic distance failed to converge. To
284 address this issue, we removed greenhouse table from the random effects, retaining collecting
285 site as the only random effect. This approach was taken because variance among tables in female
286 biomass was effectively zero (5.79×10^{-32}) but substantially higher among collecting site levels
287 (162). We also compared the AIC of two models, each including only one random effect, either
288 table or collecting site. The model with collecting site had a substantially lower AIC (10300)
289 than the model with table ID (11200), further supporting our choice to remove table from the
290 final model.
291
292 <u>The significance of distance from the core when accounting for climate</u>
293 Given that plant traits might have been influenced not only by the history of range expansion but
294 also by environmental factors and local adaptation especially to climate after colonization, we
295 asked whether the significant patterns we observed across the species range (in terms of the
296 incidence of leakiness and reproductive effort; see Results) remain significant when
297 climatological factors are included in the models. To do this, we first analyzed the climatological
298 data for the collection sites and then rebuilt models for these focal traits, incorporating climate
299 factors as fixed effects while excluding all non-significant terms from the previous models.
300
301 To characterize the overall local climates of our sampling sites, we extracted climatological data
302 from 1981 to 2010 using the CHELSA global climate dataset (version 2.1; Karger *et al.*, 2017).
303 After cleaning the dataset and removing missing values, we retained 65 climatological variables
304 (Tab. S8), across 26 collection sites. We then used principal component analysis (PCA) on the
305 scaled values of these variables to reduce the data dimensions to two principal components
306 (PCs), which together explained approximately 70% of the total climatic variance (PC1: 54.7%;
307 PC2: 14.2%). We tested for pairwise correlations among the two distance metrics and the two
308 climate PCs using Pearson correlation tests.
309
310 We next built generalized linear mixed models (GLMMs) for the incidence of leakiness and
311 reproductive effort using geodesic distance from the species core and the two climate PCs as
312 fixed effects. These models were selected based on previous analyses that showed significant
313 associations between these traits and distance. All hormone-related terms, previously found to be

non-significant, were excluded from these models. Random effects, data distribution families, and offset terms were retained, consistent with the previous models.

To evaluate the relative contributions of distance from the species core and local climate on trait variation, and to address multicollinearity arising from the inclusion of two distance metrics and two climate PCs in the same model, we applied variation partitioning we conducted commonality analysis (Ray-Mukherjee *et al.*, 2014), i.e., variation partitioning (Goldstein *et al.*, 2002), for all the models of these four traits, using geodesic distance and two climate PCs as fixed effects. We compared the marginal R^2 (Nakagawa *et al.*, 2017), the proportion of variance in the response variable being explained by the fixed factors, for full models with models with subsets of fixed effects, quantifying variance explained by each factor.

Note that analogous analyses using land distance instead of geodesic distance are presented in Supporting Information. Additionally, GLMMs were constructed using climate principal components (PCs) in place of distance from the core, and variation partitioning was performed using both distance metrics and climate PCs. Results are provided in Supporting Information.

All analyses were performed in R (R Core Team, 2025). All GLMMs and LMs were fitted using the 'glmmTMB' function from the 'glmmTMB' package in R (Brooks *et al.*, 2017). We checked the diagnostic fit of all chosen models for significant deviations, overdispersion, and zero-inflation (when applied) using the 'DHARMa' package (Florian, 2016) in R, with 5,000 simulation points. Correlation tests were performed using the 'cor' function from the 'stats' package (Bolar, 2019). Variation partitioning followed the method in Legendre (2008), modified for the higher number of fixed factor in our models. Marginal R^2 of the models were extracted using the 'performance' package in R (Lüdecke *et al.*, 2021). The scripts and data are provided on https://github.com/maithunguyen/Leakiness-sex-expression-and-reproductive-effort-Mercurialis-annua-range-expansion.

Results

Overall, we found the expected association of geographic distance from the species core with reproductive traits, but not with vegetative traits. Toward the range margin populations, plants

345 exhibited significantly higher incidence in leakiness, increased reproductive effort, and lower
346 seed weight (Fig. 2, S1; Tab. S2, S4). We found no significant associations between the degree
347 of leakiness and distance. Land and geodesic distances generally showed associations in the
348 same direction, but associations with land distance consistently showed weaker significance
349 across all traits except for seed weight and male leakiness ability (Tab. S2, S4). In the following
350 sections, we primarily present analyses using the geodesic distance metric unless stated
351 otherwise.
352
353 Hormone treatment showed effects on vegetative but not reproductive traits
354 We found no effect of the hormone treatment on any of the reproductive traits studied, including
355 leakiness ability, leakiness degree, or reproductive effort in either sex (Tab. S2). However,
356 hormone treatment had a significant positive effect on two measured vegetative traits: biomass
357 ($Z = 9.49$, $P < 0.001$ for females; $Z = 3.57$, $P < 0.001$ for males) and height ($Z = 7.57$, $P < 0.001$
358 for females; $Z = 4.80$, $P < 0.001$ for males), as well as significant interactions with distance from
359 the core in predicting plant size (Tab. S2). Since the effect of hormones on plant size was not the
360 focus of our study, we do not analyze this further.
361
362 Geographic variation in leakiness
363 The incidence of leakiness increased toward the range margins in both sexes when using
364 geodesic distance (females: $Z = 4.29$, $P < 0.001$; males: $Z = 2.38$, $P < 0.05$; Fig. 2A, Tab. S2).
365 This pattern was further supported by the zero-inflation components of models investigating the
366 degree of leakiness, showing that the proportion of excess zeros (i.e., non-leaky individuals)
367 declined significantly with increasing distance from the species core (females: $Z = –4.76$, $P <$
368 0.001; males: $Z = –3.29$, $P < 0.01$; Tab. S2). Similar patterns were found for land distance (Tab.
369 S4).
370
371 Geographic variation in reproductive effort
372 Reproductive effort increased toward the range margins in both sexes using geodesic distance
373 (females: Z = 2.53, $P < 0.05$; males: $Z = 1.96$, $P = 0.05$; Fig. 2B, Tab. S2). Using land distance,
374 the same positive trend was observed, though statistical significance was weaker (females: $Z =$
375 1.94, $P = 0.052$; males: not significant; Tab. S4).

376

377 Geographic variation in plant and seed size

378 We found no significant association between either geodesic or land distance and vegetative
379 traits (biomass or height) in either sex (Tab. S2, S4). Seed mass decreased significantly with land
380 distance ($Z = -2.20$, $P < 0.05$; Fig. 2C, Tab. S4) but showed no significant relationship with
381 geodesic distance (Tab. S2). Projected seed area showed no significant relationship with either
382 distance measure (Tab. S2, S4).

383

384 Geographic variation in traits after including local climate data

385 PC1 of local climate and land distance were moderately to highly correlated (Fig. 3), reducing
386 the predictive power of each factor in models that included both geodesic distance and climate
387 PC1, and complicating interpretation when only a subset of the correlated terms was included.
388 When both principal components (PCs) of local climate were added to the models, the
389 associations between the incidence of leakiness and geodesic distance remained positive but fell
390 short of statistical significance (males: $Z = 1.91$, $P = 0.057$; females: $Z = 1.85$, $P = 0.064$; Tab.
391 S3). Leakiness showed no significant association with either of the climate PCs ($P > 0.2$ for
392 both; Tab. S3). Geodesic distance showed no significant association with reproductive effort in
393 either sex, and land distance showed no association with leakiness ability or seed weight when
394 climate PCs were included (Tab. S3). PC1 of climate was significantly negatively associated
395 with the incidence of leakiness in females ($Z = -2.91$, $P < 0.01$, Tab. S5) when modeled with
396 land distance, but this association disappeared when geodesic distance replaced land distance
397 (Tab. S3).

398

399 Our analysis of variation partitioning revealed that distance metrics (geodesic distance alone,
400 land distance alone, or both combined) were the dominant factors explaining variance in the
401 incidence of leakiness in males, female reproductive effort, and seed mass (Fig. 4, S3, S4).
402 Distance from the species core accounted for more than half of the marginal variance, i.e., the
403 variance explained by all fixed predictors combined, in models including both distance and
404 climate PCs. By contrast, most of the variance in the incidence of leakiness in females and male
405 reproductive effort was explained by the combined effects of distance and climate. Across all

406 five focal traits, distance metrics consistently explained a larger proportion of marginal variance
407 than climate PCs (Fig. 4, S3, S4).
408
409 **Discussion**
410 Among-population variation in leakiness in sex expression
411 We found a significant increase in the incidence of leakiness in sex expression in both males and
412 females of *M. annua* with distance along the species' inferred range expansion. This pattern is
413 consistent with the idea that increased leakiness should evolve in response to selection for
414 reproductive assurance during colonisation in a range expansion. Baker (1955) first drew
415 attention to the phenomenon of selection for a capacity for uniparental reproduction in the
416 context of the colonisation of oceanic islands. Later, Baker's law was invoked to predict and
417 explain the evolution of a selfing ability in metapopulations (Pannell & Barrett, 1998), and its
418 relevance for range expansions was also highlighted (Cheptou, 2012; Pannell, 2015; Pannell *et*
419 *al.*, 2015). Our results here now provide further correlative evidence for an expanded view of
420 Baker's Law beyond the colonisation of oceanic islands and metapopulations.
421
422 We also expected to find a difference in the patters of the degree of leakiness (i.e., how many
423 male flowers or fruits a leaky female or male should procued) between males and females, but
424 our results revealed no such difference. It is likely that mild leakiness is largely sufficient for
425 successful colonisation by females, so our result for them is as expected. In contrast, we
426 expected that colonisation success for males would be enhanced by their ability to produce many
427 seeds, i.e., by a high degree of leakiness. However, here, too, there was no pattern across the
428 species range. It is possible that the advantages of a high degree of leakiness in males during
429 colonisation are countered by the disadvantages associated with producing many seeds in
430 established populations. Because males disperse a great deal of pollen to the wind, any ovules
431 they produce are likely to be be self-fertilised, even in established populations, and self-fertilised
432 seeds might suffer inbreeding depression (Charlesworth & Charlesworth, 1987; Pannell &
433 Jordan, 2022).
434
435 Neither males nor females in our experimenet responded to their respective hormone treatments
436 in their incidence or degree of leakiness, despite the fact that the hormone treatment did have a

437 mild but significant effect on plant growth. Given this lack of any treatment effect on leakiness,
438 we were thus unable to test our hypothesis that plants towards the edge of the range expansion
439 should be more sensitive to hormone application in their sex expression, i.e., more ready to
440 express leakiness.
441
442 Among-population variation in life-history traits
443 We found that male and female reproductive effort (RE) were both higher toward the western
444 range margins of *M. annua*, when measured in terms of the geodesic distance, with female RE
445 showing a stronger trend than male RE. Our results for seed size were similar, though the
446 significant trend of seeds with lower biomass towards the range margin was found when distance
447 from the expansion orgin was measured in terms of land distance. Our results for life history are
448 thus rather more ambiguous than for the incidence of leakiness, but it is nonetheless worth
449 noting a tendency for life history variation in the direction predicted by life-history theory under
450 a colonisation-competition trade-off. To this extent, our results contribute to a substantial body
451 of empirical work supporting a major expectation from life-history theory (see Introduction),
452 notably strengthening correlative evidence for selection of traits associated with a ruderal life
453 history during colonisation in range expansions (Cwynar & MacDonald, 1987; Phillips *et al.*,
454 2006; Darling *et al.*, 2008; Monty & Mahy, 2010; Bufford & Hulme, 2021). Note that we did not
455 find a corresponding decrease in plant size or height, as expected for a scenario of reduced
456 competition during range expansion. Given that *M. annua* populations grow quickly after
457 colonisation (Dorken *et al.*, 2017) and plants in established dense population must be highly
458 competitive, this result is in retrospect perhaps not surprising.
459
460 Associations of leakiness in sex expresssion and life history with climate
461 We found that both leakiness and life-history traits were strongly associated with climate, as
462 summarised by the first principle component of all the climatic variables included in our
463 analyses. This association was entirely expected, given that the range expansion involved
464 movement across a steep gradient from a Mediterranean to a temperate climate. The association
465 between distance along the range expansion and climate should diminish any confidence we
466 might have that in one or the other factor as a sole causitive factor underlying the distribution of
467 variation in leakiness and life-history variables. Nevertheless, it is difficult to see why a

468 temperate climate should have selected for increased leakiness, and we had no *a priori* reason
469 for hypothesising that it should. While *M. annua* has a strict winter annual life history under a
470 Mediterranean climate, it is able to persist over the entire year in temperate western Europe, with
471 overlapping generations (personal observations). If this means that plants in temperate
472 populations face stronger competition, then one might expect such populations to have larger
473 seeds and possess vegetative characters conferring a greater competitive ability. If anything, our
474 results point to the opposite pattern, which should strenthen rather than weaken the basis of our
475 inference of the importance of selection during the range expansion itself.
476
477 Concluding remarks
478 Our study provides another example of the likely effect of selection on disperal and life-history
479 traits during colonisation. While the populations we observe in nature are usually well-
480 established, it is well to remember that the traits we find expressed in them may reflect not only
481 selection under the local conditions but also infrequent episodes of colonisation in their history.
482 Our results also provide further support for the intuitive idea, originally advanced by Baker , that
483 colonisation should act, in particular, as a selective agent favouring an ability for self-
484 fertilisation. This advantages of unisexual reproduction also apply in the context of geographic
485 parthenogenesis as an explanation for an enrichment of a tendency towards asexual reproduction
486 in otherwise sexual lineages towards the high-latitude edges of their geographic distributions
487 (Bierzychudek, 1985; Jacquemyn *et al.*, 2005; Hörandl *et al.*, 2008; Cosendai *et al.*, 2013;
488 Hörandl, 2023).
489
490 While Baker (1967) and others (Ehlers & Bataillon, 2007; Crossman & Charlesworth, 2014;
491 Cossard & Pannell, 2019; Cossard *et al.*, 2021) have recognised that the tendency in many
492 dioecious species towards inconstant or leaky sex expression should facilitate their colonisation
493 potential, to our knowledge ours is the first study to provide strong, albeit correlational, evidence
494 that selection during repeated colonisation may enhance this tendency. An important implication
495 is that leaky sex expression found in natural populations of dioecious species may be actively
496 maintained by natural selection during occasional periods where mates are limited, either in low-
497 density populations or during colonisation. In other words, leaky sex expression in dioecious
498 plants may not always be just a relict of an unfinished transition to fully separate from

hermaphroditism or monoecy (Lloyd, 1980) or an expression of developmental noise (Lloyd & Bawa, 1984). Indeed, dioecy in the genus *Mercurialis* is ancestral, not derived (Krähenbühl *et al.*, 2002; Obbard *et al.*, 2006a), and the evolution of leaky sex expression in *M. annua* can almost certainly be regarded as an evolved adaptation to its colonising habit that coincided a transition to an annual life history (Pannell *et al.*, 2008). This adaptation appears to have been enhanced not only by ongoing colonisations in dynamic metapopulations (Obbard *et al.*, 2006b; Eppley & Pannell, 2007; Dorken *et al.*, 2017) but also, as now shown here, by range expansion.

Acknowledgements

We thank Shengman Lyu, Antoine Guissan, Valentin Verdon and Kai-Hsiu Chen for their comments on our analysis; Leonardo Cantarro, Sebastian Cato, Theophanis Constantinidis, Stefano Doglio, Galina Gavrilenco, Thijs Heijman, Gabriele Lagrasta, Ramy Maalouf, Arjan Ndoni, Thomas Schorr, Gertrude Seiser, Kathy Vivian, Jean-Paul Wollf, Walter Wimmer for their help with seed sampling; Jessica Bainbridge, Kai-Hsiu Chen, Camilo Ferron Martinez, Ivan Giordano, Xia Jiang, Ehouarn Le Faou, Marjolaine Rousselle, Philipp Schenkel, Suhaas Sehgal, as well as numerous undergraduate assistants, for their help in setting up experiments and measuring plant phenotypes; and the Swiss National Science Foundation (Grant 31003A_163384) and the University of Lausanne for funding.

Author contributions

Conceived the project and designed the experiment: JRP and MTN; conducted the experiment and analyzed the data: MTN; revised and wrote the final version of the manuscript: MTN and JRP. Both authors approved the final version of the manuscript.

Conflict of interest

We have no conflict of interest to declare.

Data availability statement

The scripts and data are provided on https://github.com/maithunguyen/Leakiness-sex-expression-and-reproductive-effort-Mercurialis-annua-range-expansion.

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Figures and Tables

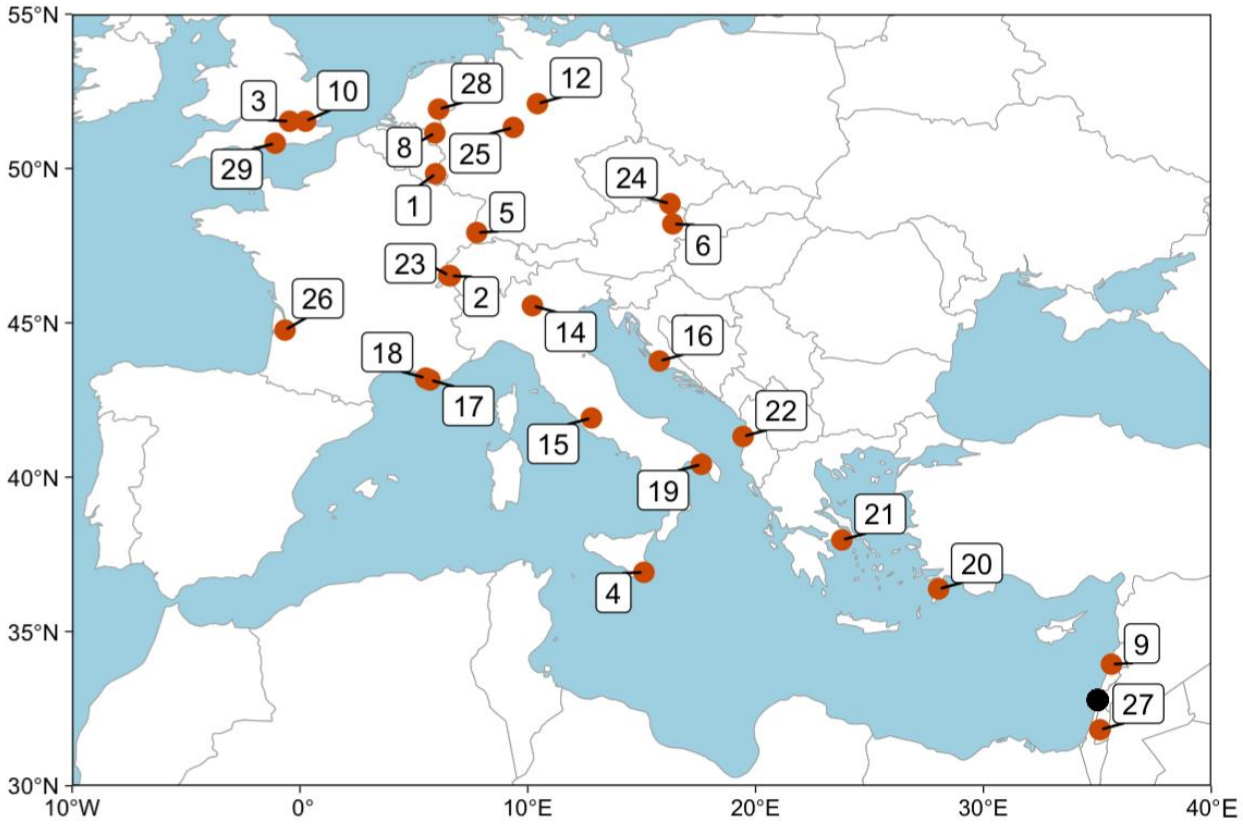

Figure 1. The map of sampling sites (red points) with their labels (detailed in Tab. S1). The black point is midway between populations 9 and 27 and was used for the origin of the species' range core in our analyses as a function of distance.

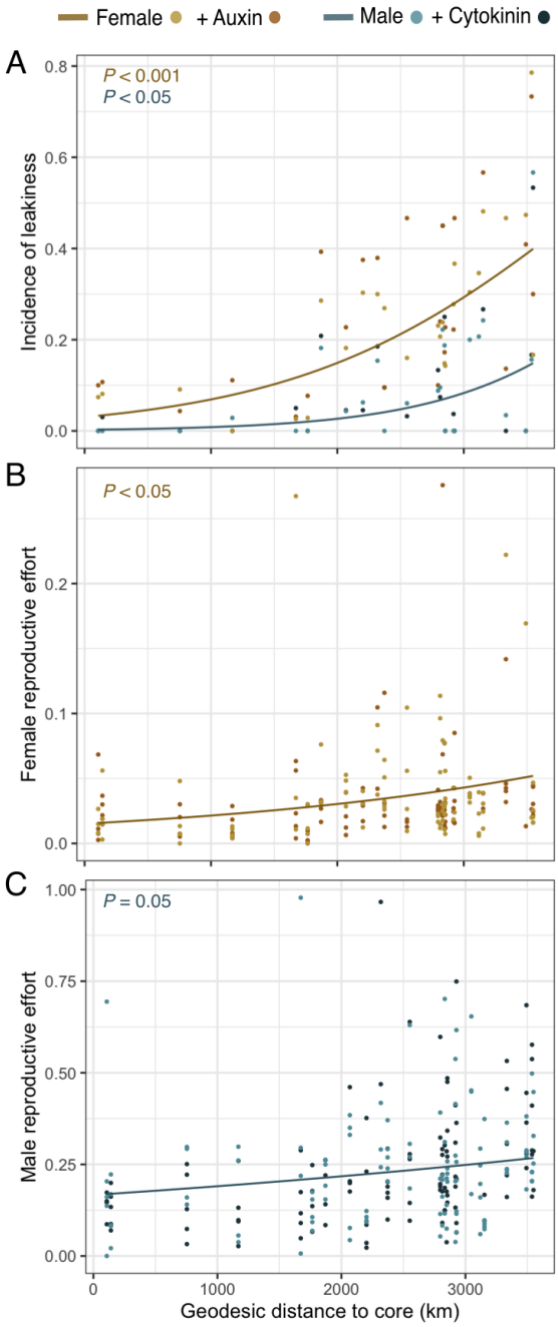

Figure 2. Observed data and linear regression predictions showing the association between population distance from the species' range core and (A) incidence of leakiness, and reproductive effort (number of flowers per unit biomass) of (B) females and (C) males. Each point represents trait values measured in the common-garden experiment. Point colors indicate sex and hormone-treatment status. Lines show GLMM-predicted responses (Tab. S2) as functions of distance, with colors denoting sex and significance levels indicated in the upper left of each panel.

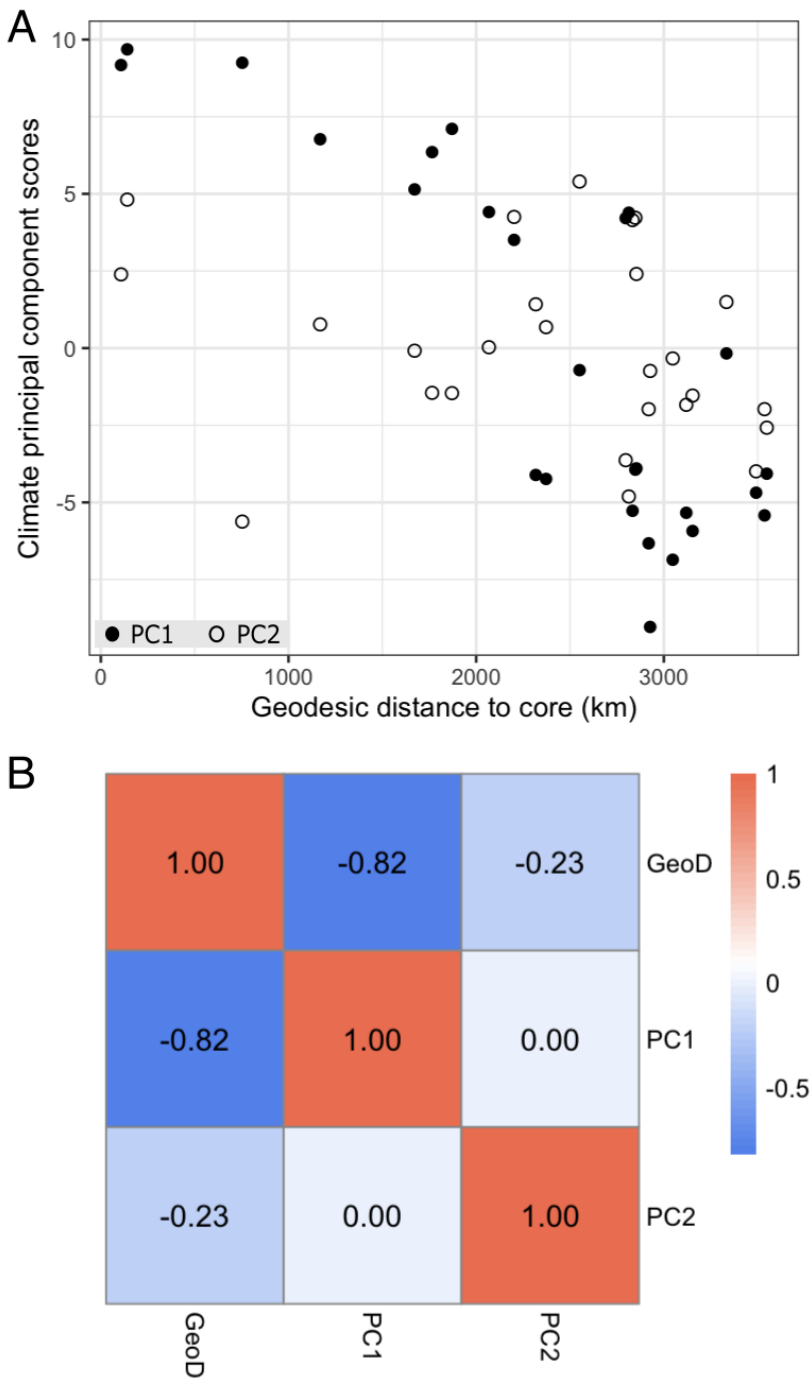

Figure 3. Correlations between principal component (PC) scores and population geodesic distance from the species' range core. (A) PC1 and PC2 scores plotted against geodesic distance to the range core. (B) Heatmap showing color-coded Pearson correlation coefficients (values annotated) for pairwise correlations between geodesic distance and climate PC scores.

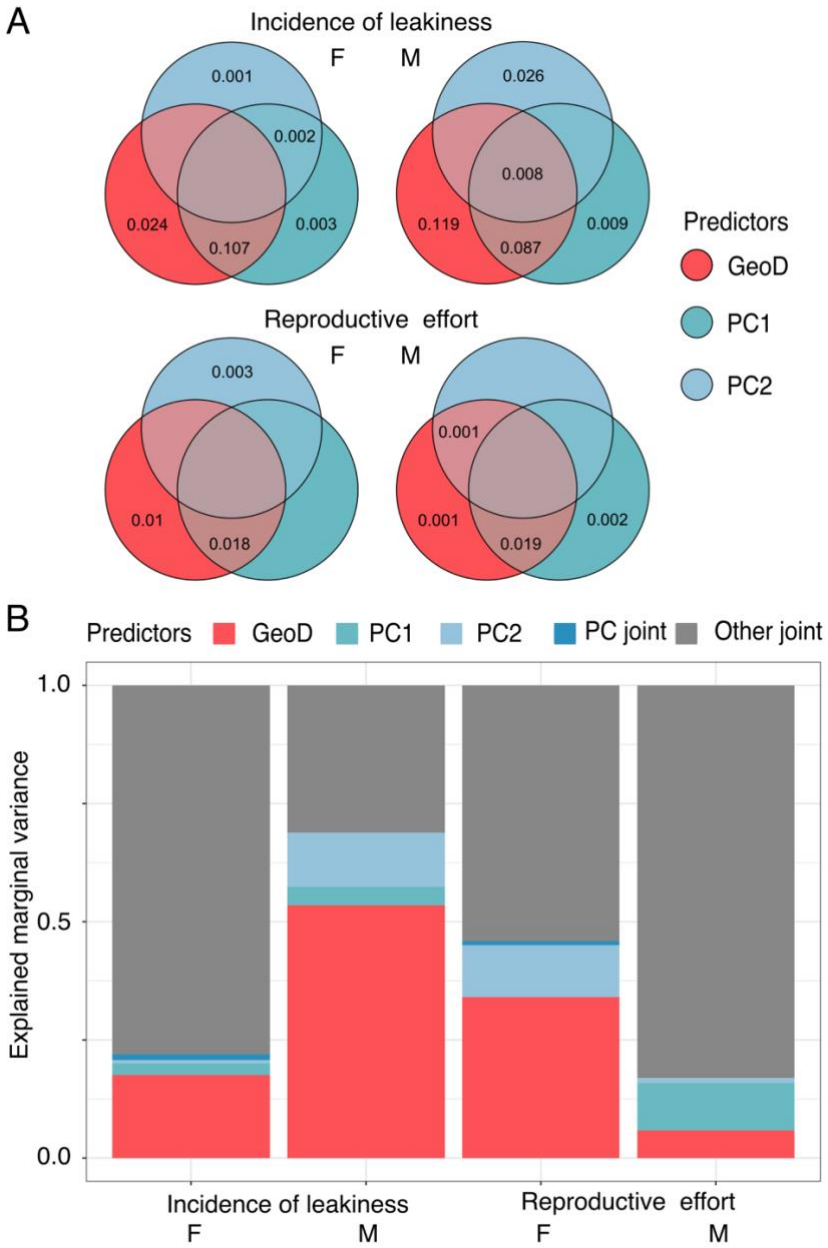

Figure 4. Variation partitioning of GLMMs for each trait and sex that showed significant associations with geodesic distance. (A) Venn diagrams showing the proportion of total variance in each response trait explained by geodesic distance (red), two climate principal components (blue shades), and by their joint effects. Empty fractions represent non-positive contributions to explained variance. (B) Stacked bar plots showing marginal variance, i.e., the proportion of variance in each response trait explained by fixed effects, partitioned into components attributed to geodesic distance (red), climate (blue shades; including PC1, PC2, and their joint effect), and the shared contribution of distance and climate (grey).

Supporting Information

Table S1. The ID and coordinates of the 26 sampling sites and the range core, the distances of each site to the core.

ID	N (degree)	E (degree)	Geodesic distance (km)	Land distance (km)
1	49.83	5.95	3047	4101
2	46.52	6.61	2848	4062
3	51.53	-0.46	3536	4626
4	36.92	15.10	1871	4861
5	47.93	7.76	2833	3917
6	48.20	16.37	2317	3205
8	51.15	5.92	3119	4137
9	33.94	35.62	141	130
10	51.53	0.24	3492	4615
12	52.10	10.42	2919	3882
14	45.56	10.20	2551	3626
15	41.92	12.80	2202	4013
16	43.76	15.77	2068	3069
17	43.15	5.71	2797	4176
18	43.21	5.52	2813	4186
19	40.42	17.62	1765	4421
20	36.38	28.03	754	1643
21	37.97	23.79	1169	2880
22	41.32	19.45	1672	2705
23	46.53	6.53	2854	4081
24	48.86	16.24	2372	3274
25	51.33	9.37	2927	3904
26	44.76	-0.66	3334	4671
27	31.82	35.11	108	142
28	51.92	6.08	3153	4167
29	50.82	-1.09	3548	4676
Core	32.78	35.01	0	0

Table S2. Estimates for fixed effects of GLMMs and LMs using geodesic distance. Estimates are presented with 95% confidence intervals. Significance codes : 0 *** 0.001 ** 0.01 * 0.05. In bold are estimations of fixed factors with the significance levels of less than or equal 0.05.

	Trait ~ Fixed terms + (1| Population ID) + (1| Table ID)				Trait ~ Fixed terms
	Incidence of leakiness		Reproductive effort		
	Male	Female	Male	Female	Seed weight
Intercept	-5.99 *** (-8.55, -3.42)	-3.46 *** (-4.50, -2.43)	-1.79 *** (-2.16, -1.42)	-4.19 *** (-4.79, -3.58)	1.82×10^{-3} *** (1.49×10^{-3}, 2.16×10^{-3})
Geodesic distance	**1.19×10^{-3} *** **(0.21×10^{-3}, 2.18×10^{-3})**	**8.61×10^{-4} ***** **(4.67×10^{-4}, 12.5×10^{-4})**	**1.33×10^{-4}** **(0.00, 2.67×10^{-4})**	**3.48×10^{-4} *** **(0.78×10^{-4}, 6.17×10^{-4})**	-8.90×10^{-8} (-22.2×10^{-8}, 4.38×10^{-8})
Hormone treatment	0.75 (-2.67, 4.16)	0.67 (-0.55, 1.89)	-0.354 (-0.771, 0.063)	2.46 (-1.52, 1.53)	-
Geodesic distance x Hormone treatment	-0.19×10^{-3} (-1.55×10^{-3}, 1.16×10^{-3})	-1.79×10^{-4} (-6.78×10^{-4}, 3.19×10^{-4})	1.45×10^{-4} (-0.20×10^{-4}, 3.10×10^{-4})	-6.90×10^{-5} (-83.4×10^{-5}, 69.6×10^{-5})	-

	Trait ~ Fixed terms + (1| Population ID) + (1| Table ID)				Trait ~ Fixed terms + (1| Population ID)
	Male's biomass	Female's biomass	Male's height	Female's height	Seed size
Intercept	36.4 *** (26.9, 45.9)	39.7 *** (26.3, 53.1)	23.2 *** (18.5, 29.9)	23.6 *** (17.2, 29.9)	3.70×10^{-3} *** (3.44×10^{-3}, 3.96×10^{-3})
Geodesic distance	0.80×10^{-3} (-2.87×10^{-3}, 4.47×10^{-3})	2.78×10^{-3} (-2.43×10^{-3}, 8.00×10^{-3})	0.11×10^{-3} (-1.71×10^{-3}, 1.93×10^{-3})	0.68×10^{-3} (-1.77×10^{-3}, 3.13×10^{-3})	3.40×10^{-8} (-6.83×10^{-8}, 13.6×10^{-8})
Hormone treatment	**4.30 ** (1.73, 6.87)**	**10.8 *** (7.54, 14.1)**	**2.31 ** (0.83, 3.79)**	**4.50 *** (2.68, 6.32)**	-
Geodesic distance x Hormone treatment	-0.93×10^{-3} (-1.95×10^{-3}, 0.08×10^{-3})	**-1.85×10^{-3} **** **(-3.15×10^{-3}, -0.546×10^{-3})**	-3.68×10^{-4} (-9.53×10^{-4}, 2.17×10^{-4})	**-0.77×10^{-3} *** **(-1.50×10^{-3}, $- 0.05 \times 10^{-3}$)**	-

	Trait ~ Fixed terms + (1| Population ID) + (1| Table ID), zero inflation ~ Distance			
	Male's degree of leakiness		Female's degree of leakiness	
	Conditional model	Zero-inflation model	Conditional model	Zero-inflation model
Intercept	-6.85 *** (-10.7, 3.03)	2.94 ** (1.05, 4.84)	-6.55 *** (-7.83, -5.28)	1.50 ** (0.55, 2.45)

Geodesic distance	6.16×10^{-4}	**-1.22×10^{-3} ****	3.33×10^{-4}	**-9.55×10^{-4} *****
	(-7.08×10^{-4}, 19.4×10^{-4})	**(-1.94×10^{-3}, -0.49×10^{-3})**	(-1.11×10^{-4}, 7.78×10^{-4})	**(-13.5×10^{-4}, -5.61×10^{-4})**
Hormone treatment	1.36 (-1.50, 4.22)	-	0.29 (-0.97, 1.55)	-
Geodesic distance x Hormone treatment	-5.27×10^{-4}	-	-1.43×10^{-4}	-
	(-14.9×10^{-4} , 4.39×10^{-4})		(-5.90×10^{-4}, 3.05×10^{-4})	

Table S3. Estimates for fixed effects of GLMMs and LM using geodesic distance and PCs of climate as fixed factors. Estimates are presented with 95% confidence intervals. Significance codes : 0 *** 0.001 ** 0.01 * 0.05. In bold are estimations of fixed factors with the significance levels of less than or equal 0.05.

Trait ~ Fixed terms + (1| Population ID) + (1| Table ID)				
	Incidence of leakiness		Reproductive effort	
	Male	Female	Male	Female
Intercept	-6.36 *** (-10.1, -2.63)	-2.60 *** (-4.06, -1.13)	-1.71 *** (-2.23, -1.13)	-4.27 ** (-5.83, -2.71)
Geodesic distance	1.41×10^{-3} (-0.039×10^{-3}, 2.86×10^{-3})	5.47×10^{-4} (-0.325×10^{-4}, 11.3×10^{-4})	9.88×10^{-5} (-10.5×10^{-5}, 30.3×10^{-5})	3.48×10^{-4} (-3.00×10^{-4}, 9.95×10^{-4})
PC1	0.046 (-0.150, 0.243)	-4.61×10^{-2} (-13.1×10^{-2}, 3.91×10^{-2})	-1.98×10^{-2} (-5.25×10^{-2}, 1.29×10^{-2})	0.25×10^{-2} (-8.85×10^{-2}, 9.35×10^{-2})
PC2	0.108 (-0.124, 0.340)	1.77×10^{-2} (-8.25×10^{-2}, 11.8×10^{-2})	-6.95×10^{-3} (-45.3×10^{-3}, 31.4×10^{-3})	0.037 (-0.036, 0.110)

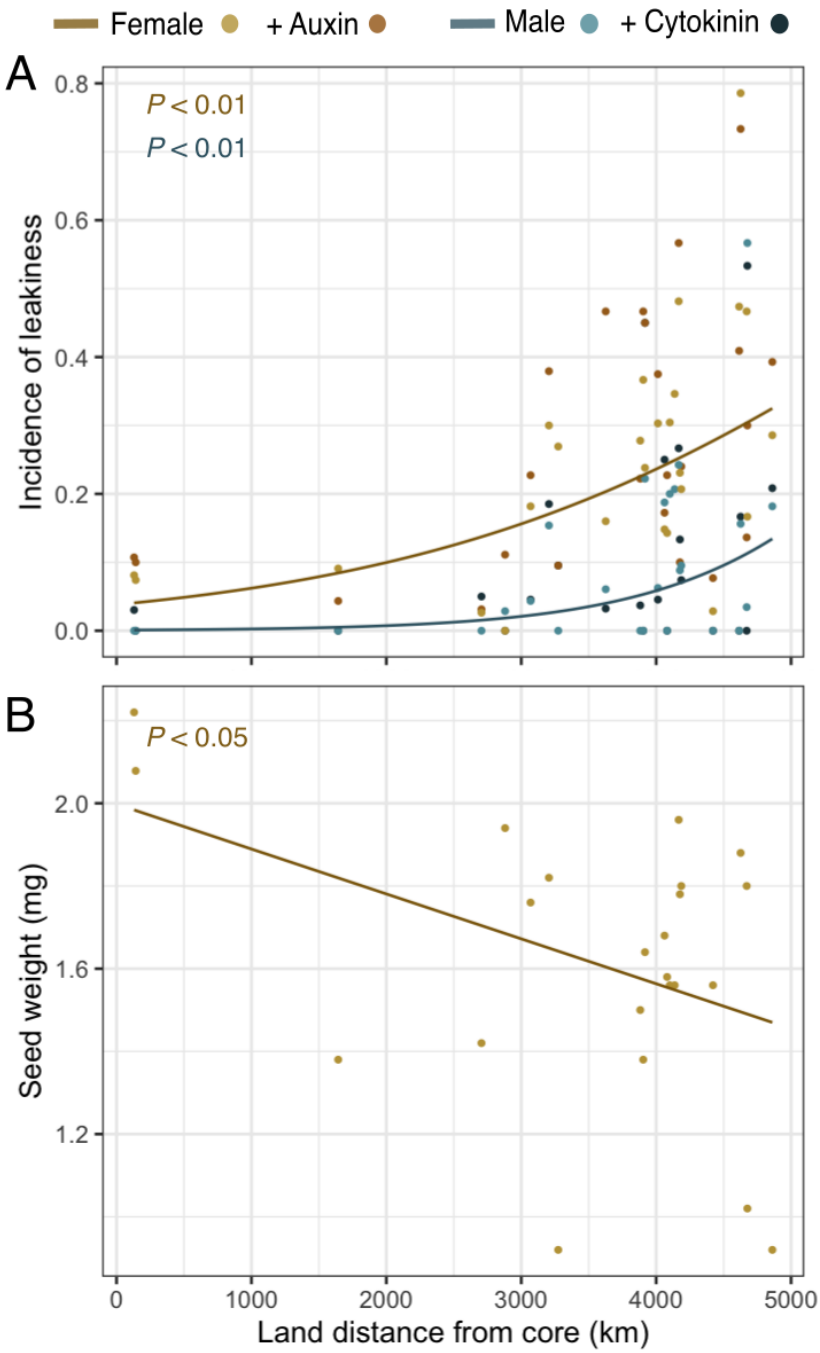

Figure S1. Observed data and linear regression predictions showing the association between population distance from the species' range core and (A) incidence of leakiness, and (B) seed weight. Each point represents trait values measured in the common-garden experiment or from seeds collected from the field. Point colors indicate sex and hormone-treatment status. Lines show (G)LMM-predicted responses (Tab. S4) as functions of distance, with colors denoting sex and significance levels indicated in the upper left of each panel.

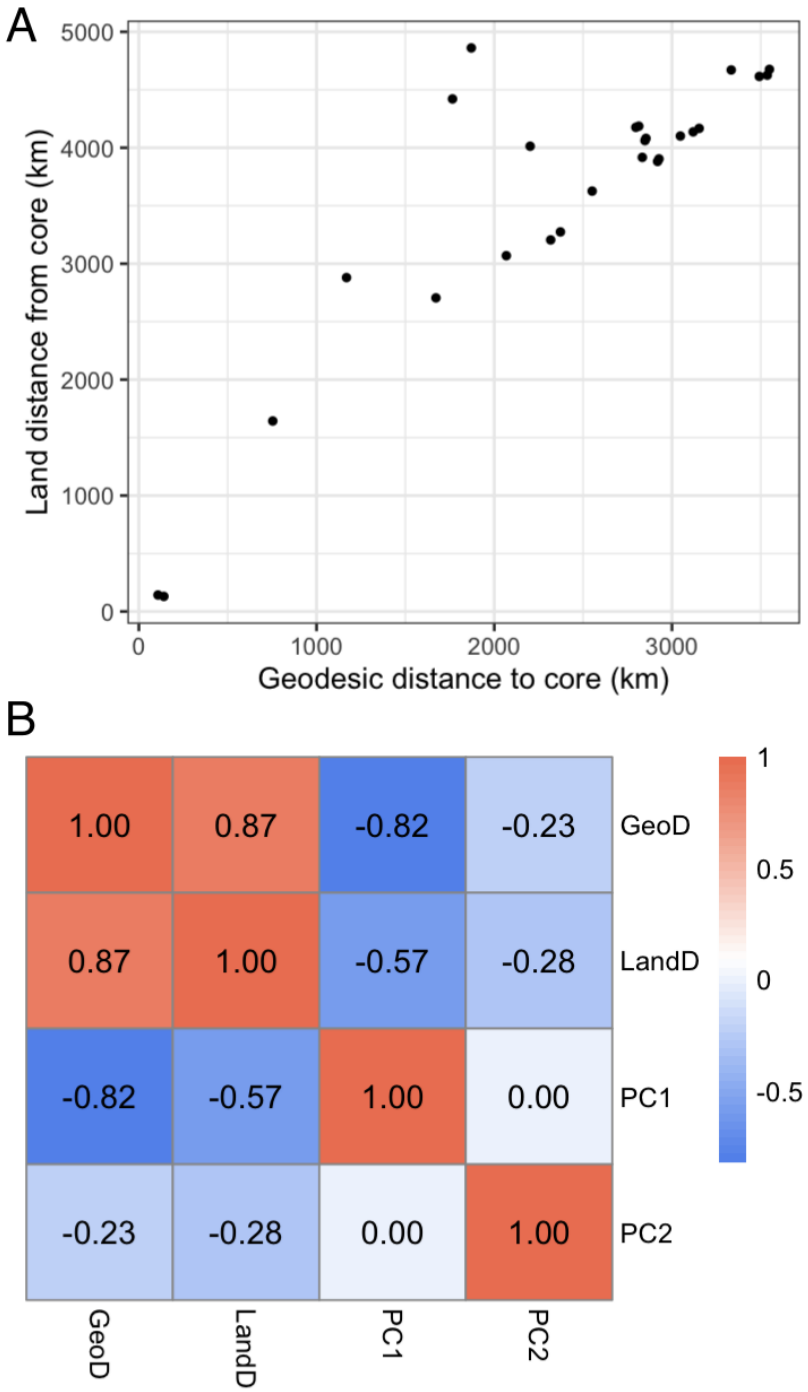

Figure S2. Correlations between principal component (PC) scores and distance metrics. (A) Land distance plotted against geodesic distance. (B) Heatmap showing color-coded Pearson correlation coefficients (values annotated) for pairwise correlations between geodesic distance and climate PC scores. The heatmap showed color coded with value annotated Pearson correlation coefficients of pairwise correlation tests among the distances and climate PCs.

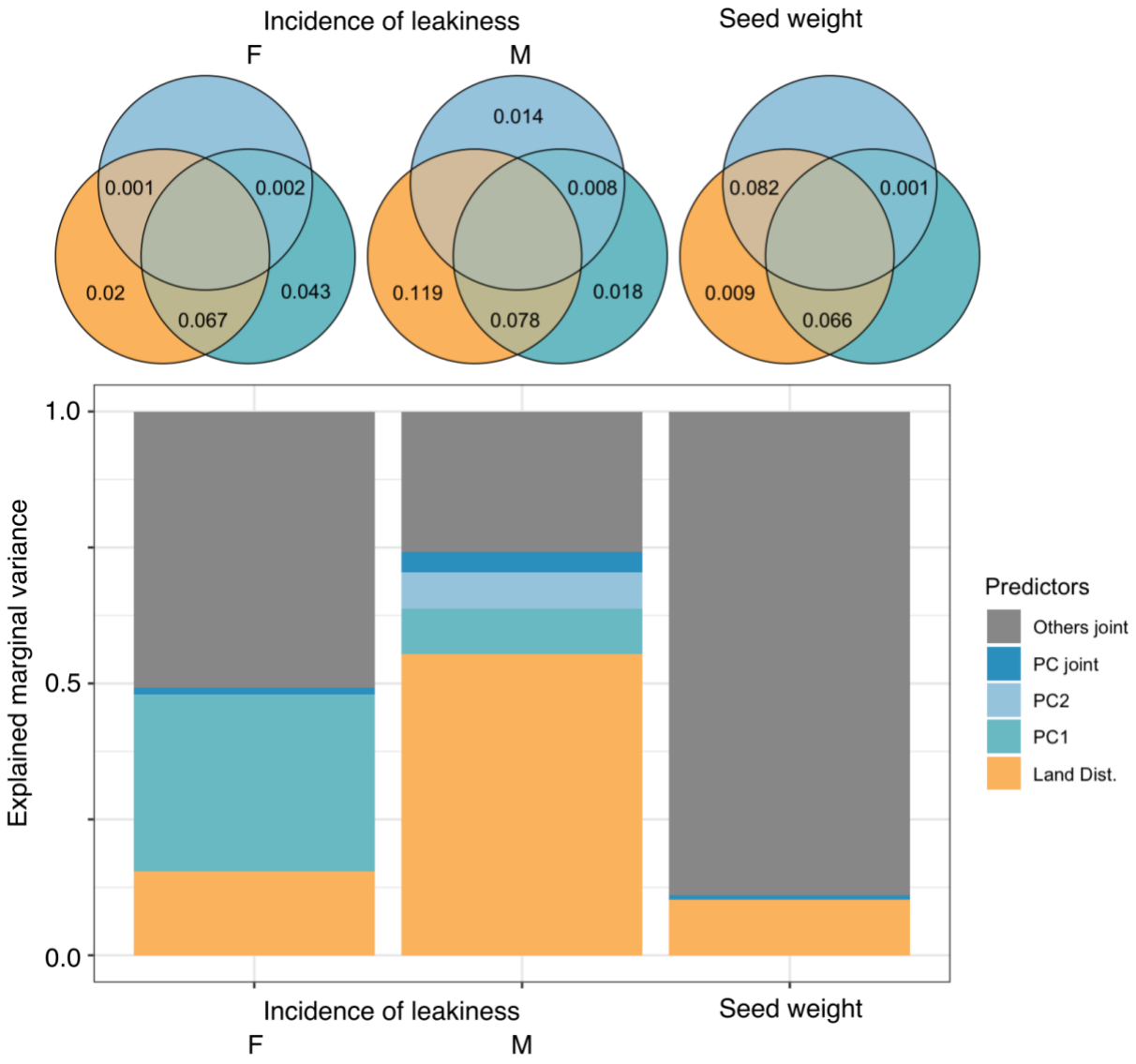

Figure S3. Variation partitioning of (G)LMMs for each trait and sex that showed significant associations with land distance. Venn diagrams showing the proportion of total variance in each response trait explained by land distance (orange), two climate principal components (blue shades), and by their joint effects. Empty fractions represent non-positive contributions to explained variance. Stacked bar plots showing marginal variance, i.e., the proportion of variance in each response trait explained by fixed effects, partitioned into components attributed to geodesic distance (orange), climate (blue shades; including PC1, PC2, and their joint effect), and the shared contribution of distance and climate (grey).

Table S4. Estimates for fixed effects of GLMMs and LMs using land distance. Estimates are presented with 95% confidence intervals. Significance codes : 0 *** 0.001 ** 0.01 * 0.05. In bold are estimations of fixed factors with the significance levels of less than or equal 0.05.

	Trait ~ Fixed terms + (1| Population ID) + (1| Table ID)				Trait ~ Fixed terms
	Incidence of leakiness		Reproductive effort		
	Male	Female	Male	Female	Seed weight
Intercept	-7.03 *** (-10.3, -3.77)	-3.23 *** (-4.45, -2.00)	-1.62 *** (-2.09, -1.15)	-3.99 *** (-4.66, -3.31)	2.00×10^{-3} *** (1.64×10^{-3}, 2.36×10^{-3})
Land distance	**1.06×10^{-3} ****	**5.14×10^{-4} ****	3.94×10^{-5}	1.73×10^{-4}	**-1.09×10^{-7} * (-2.05×10^{-7}, -0.120×10^{-7})**
	(0.26×10^{-3}, 1.86×10^{-3})	**(2.01×10^{-4}, 8.26×10^{-4})**	(-7.94×10^{-5}, 15.8×10^{-5})	(-0.01×10^{-4}, 3.48×10^{-4})	
Hormone treatment	1.70 (-1.13, 4.53)	0.44 (-0.70, 1.57)	-0.393 (-0.868, 0.083)	0.072 (-0.542, 0.686)	-
Land distance x Hormone treatment	-3.64×10^{-4}	-0.62×10^{-4}	1.07×10^{-4}	-6.65×10^{-5}	-
	(-10.4×10^{-4}, 3.10×10^{-4})	(-3.47×10^{-4}, 2.23×10^{-4})	(-0.20×10^{-4}, 2.34×10^{-4})	(-23.3×10^{-5}, 10.0×10^{-5})	

	Trait ~ Fixed terms + (1| Population ID) + (1| Table ID)				Trait ~ Fixed terms + (1| Population ID)
	Male's biomass	Female's biomass	Male's height	Female's height	Seed size
Intercept	30.9 *** (20.3, 41.6)	32.5 *** (17.4, 47.6)	20.1 *** (14.7, 25.4)	21.0 *** (13.8, 28.1)	3.86×10^{-3} *** (3.57×10^{-3}, 4.16×10^{-3})
Land distance	2.09×10^{-3}	3.72×10^{-3}	9.36×10^{-4}	1.18×10^{-3}	-2.25×10^{-8} (-10.2×10^{-8}, 5.67×10^{-8})
	(-0.726×10^{-3}, 4.90×10^{-3})	(-0.258×10^{-3} , 7.70×10^{-3})	(-4.69×10^{-4}, 23.4×10^{-4})	(-0.707×10^{-3}, 3.06×10^{-3})	
Hormone treatment	**6.90 *** (3.97, 9.83)**	**13.7 *** (10.0, 17.4)**	**3.94 *** (2.24, 5.64)**	**5.32 *** (3.25, 7.39)**	-
Land distance x Hormone treatment	**-1.33×10^{-3} *****	**-2.05×10^{-3} *****	**-7.06×10^{-4} ****	**-7.38×10^{-4} ****	-
	(-2.12×10^{-3}, -0.553×10^{-3})	**(-3.04×10^{-3}, -1.06×10^{-3})**	**(-11.6×10^{-4}, 2.52×10^{-4})**	**(-12.9×10^{-4}, -1.85×10^{-4})**	

	Trait ~ Fixed terms + (1| Population ID) + (1| Table ID), zero inflation ~ Distance			
	Male's degree of leakiness		Female's degree of leakiness	
	Conditional model	Zero-inflation model	Conditional model	Zero-inflation model
Intercept	-5.03 (-11.0, 0.98)	5.18 *** (2.56, 7.80)	-5.96 *** (-7.56, -4.37)	1.36 * (0.192, 2.53)

Land distance	-0.325×10^{-4}	**-1.35×10^{-3} *****	0.428×10^{-4}	**-6.69×10^{-4} *****
	(-14.9×10^{-4}, 14.2×10^{-4})	**(-2.06×10^{-3}, -0.65×10^{-3})**	(-3.46×10^{-4}, 4.32×10^{-4})	**(-9.67×10^{-4}, -3.72×10^{-4})**
Hormone treatment	2.06 (-2.54, 6.65)	-	-0.399 (-1.92, 1.12)	-
Land distance x Hormone treatment	-5.42×10^{-4}	-	0.808×10^{-4}	-
	(-16.2×10^{-4}, 5.39×10^{-4})		(-2.96×10^{-4}, 4.58×10^{-4})	

Table S5. Estimates for fixed effects of GLMMs and LM using distance and PCs of climate as fixed factors. Estimates are presented with 95% confidence intervals. Significance codes : 0 *** 0.001 ** 0.01 * 0.05. In bold are estimations of fixed factors with the significance levels of less than or equal 0.05.

	Trait ~ Fixed terms + (1| Population ID) + (1| Table ID)		Trait ~ Fixed terms
	Incidence of leakiness		
	Male	Female	Seed weight
Intercept	-5.59 ** (-9.81, -1.36)	-2.21 ** (-3.65, -0.766)	1.91×10^{-3} *** (1.44×10^{-3}, 2.37×10^{-3})
Land distance	7.27×10^{-4} (-3.41×10^{-4}, 17.9×10^{-4})	2.57×10^{-4} (-1.13×10^{-4}, 6.28×10^{-4})	-8.07×10^{-8} (-20.9×10^{-8}, 4.77×10^{-8})
PC1	-5.93×10^{-2} (-19.2×10^{-2}, 7.35×10^{-2})	**-8.82×10^{-2} ** (-14.8×10^{-2}, -2.87×10^{-2})**	3.29×10^{-6} (-22.2×10^{-6}, 28.8×10^{-6})
PC2	7.48×10^{-2} (-16.3×10^{-2}, 31.2×10^{-2})	5.12×10^{-3} (-94.8×10^{-3}, 105×10^{-3})	2.80×10^{-5} (-2.20×10^{-5}, 7.80×10^{-5})

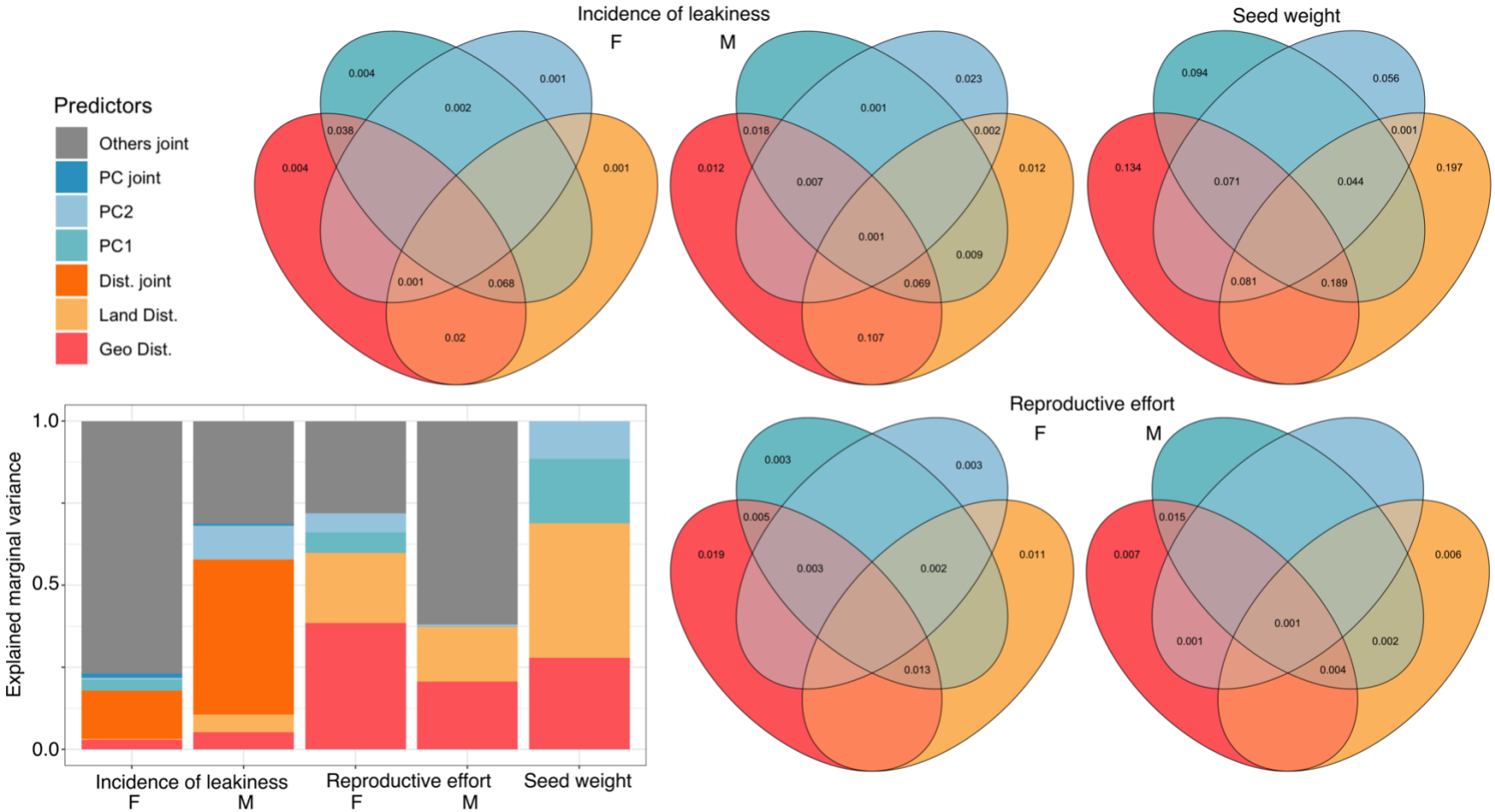

Figure S4. Variation partitioning of (G)LMMs for each trait and sex that showed significant associations with land or geodesic distance. The stacked bar plot shows the marginal variance—that is, the proportion of variance in the response trait explained by all fixed predictors—partitioned into components attributed to distance in red shades (including variance explained by geodesic distance

852 alone, land distance alone, and by the combination of these two), to climate in blue shades (including variance explained by PC1
853 alone, PC2 alone, and the combination of these two), and to the combination of distance and climate together in grey. Venn diagrams
854 illustrate the proportion of total variance in each response trait explained by each predictor alone or jointly by combinations of
855 predictors. Empty fractions represent non-positive contributions to explained variance.

Table S6. Estimates for fixed effects of GLMMs and LMs using PC1 of climate. Estimates are presented with 95% confidence intervals. Significance codes : 0 *** 0.001 ** 0.01 * 0.05. In bold are estimations of fixed factors with the significance levels of less than or equal 0.05.

	Trait ~ Fixed terms + (1| Population ID) + (1| Table ID)				Trait ~ Fixed terms
	Incidence of leakiness		Reproductive effort		
	Male	Female	Male	Female	Seed weight
Intercept	-3.04 *** (-3.75, -2.33)	-1.38 *** (-1.75, -1.01)	-1.47 *** (-1.67, -1.28)	-3.37 *** (-3.65, -3.08)	1.62×10^{-3} *** (1.48×10^{-3}, 1.75×10^{-3})
PC1	**-0.120 * (-0.239 , -0.001)**	**-0.119 *** (-0.175, -0.063)**	**-0.029 * (-0.052, -0.007)**	**-0.041 * (-0.076, -0.006)**	1.20×10^{-5} (-0.968×10^{-5}, 3.37×10^{-5})
Hormone treatment	0.171 (-0.262, 0.604)	0.216 (-0.064, 0.496)	0.003 (-0.154, 0.161)	-0.146 (-0.362, 0.069)	-
PC1 x Hormone treatment	-0.014 (-0.098, 0.070)	0.005 (-0.045, 0.057)	-0.009 (-0.037, 0.020)	-0.006 (-0.043, 0.031)	-

	Trait ~ Fixed terms + (1| Population ID) + (1| Table ID)				Trait ~ Fixed terms + (1| Population ID)
	Male's biomass	Female's biomass	Male's height	Female's height	Seed size
Intercept	38.3 *** (34.8, 41.8)	45.8 *** (40.6, 51.0)	23.4 *** (21.7, 25.2)	25.2 *** (22.8, 27.5)	3.78×10^{-3} *** (3.69×10^{-3}, 3.88×10^{-3})
PC1	0.134 (-0.461, 0.729)	-0.109 (-0.97, 0.749)	0.071 (-0.223, 0.366)	-0.026 (-0.424, 0.372)	-1.12×10^{-5} (-2.73×10^{-5}, 0.48×10^{-5})
Hormone treatment	**2.10 *** (1.07, 3.12)**	**6.48 *** (5.21, 7.76)**	**1.47 *** (0.878, 2.07)**	**2.73 *** (2.02, 3.44)**	-
PC1 x Hormone treatment	-0.045 (-0.126, 0.217)	0.026 (-0.191, 0.243)	-0.041 (-0.140, 0.058)	-0.009 (-0.129, 0.112)	-

	Trait ~ Fixed terms + (1| Population ID) + (1| Table ID), zero inflation ~ PC1			
	Male's degree of leakiness		Female's degree of leakiness	
	Conditional model	Zero-inflation model	Conditional model	Zero-inflation model
Intercept	-5.58 *** (-6.60, -4.57)	-1.15 (-3.14, 0.846)	-5.76 *** (-6.22, -5.30)	-0.995 (-2.01, 0.023)
PC1	-0.054 (-0.229 , 0.121)	0.210 (-0.004, 0.424)	-0.036 (-0.106, 0.035)	**0.194 *** (0.093, 0.296)**
Hormone treatment	-0.182 (-0.794, 0.432)	-	-0.046 (-0.398, 0.306)	-
PC1 x Hormone treatment	-0.062 (-0.186 , 0.061)	-	0.030 (-0.035, 0.095)	-

Table S7. Estimates for fixed effects of GLMMs and LMs using PC2. Estimates are presented with 95% confidence intervals. Significance codes : 0 *** 0.001 ** 0.01 * 0.05. In bold are estimations of fixed factors with the significance levels of less than or equal 0.05.

	Trait ~ Fixed terms + (1| Population ID) + (1| Table ID)				Trait ~ Fixed terms
	Incidence of leakiness		Reproductive effort		
	Male	Female	Male	Female	Seed weight
Intercept	-3.08 *** (-3.85, -2.30)	-1.38 *** (-1.82, -0.932)	-1.47 *** (-1.69, -1.26)	-3.37 *** (-3.67, -3.08)	1.63×10^{-3} *** (1.50×10^{-3}, 1.75×10^{-3})
PC2	-0.016 (-0.261, 0.229)	-0.068 (-0.204, 0.068)	-0.002 (-0.055, 0.050)	0.004 (-0.069, 0.078)	3.97×10^{-5} (-0.804×10^{-5}, 8.73×10^{-5})
Hormone treatment	0.192 (-0.215, 0.598)	0.196 (-0.080, 0.471)	-0.011 (-0.168, 0.147)	-0.170 (-0.386, 0.046)	-
PC2 x Hormone treatment	0.043 (-0.092, 0.177)	0.085 (-0.006, 0.176)	-0.024 (-0.078, 0.029)	0.026 (-0.044, 0.096)	-
	Trait ~ Fixed terms + (1| Population ID) + (1| Table ID)				Trait ~ Fixed terms + (1| Population ID)
	Male's biomass	Female's biomass	Male's height	Female's height	Seed size
Intercept	38.3 *** (34.7, 41.8)	45.8 *** (40.7, 51.0)	23.4 *** (21.8, 25.1)	25.2 *** (22.9, 27.5)	3.80×10^{-3} *** (3.71×10^{-3}, 3.88×10^{-3})
PC2	0.307 (-0.855, 1.47)	0.238 (-1.42, 1.90)	0.465 (-0.086, 0.102)	0.363 (-0.389, 1.11)	**4.75×10^{-5} ** (1.51×10^{-5}, 7.99×10^{-5})**
Hormone treatment	**2.13 *** (1.11, 3.15)**	**6.37 *** (5.12, 7.62)**	**1.45 *** (0.858, 2.04)**	**2.67 *** (1.97, 3.37)**	-
PC2 x Hormone treatment	0.155 (-0.169, 0.479)	**1.18 *** (0.778, 1.60)**	0.014 (-0.174, 0.202)	**0.470 *** (-0.241, 0.699)**	-
	Trait ~ Fixed terms + (1| Population ID) + (1| Table ID), zero inflation ~ PC2				
	Male's degree of leakiness		Female's degree of leakiness		
	Conditional model (Non-converged)	Zero-inflation model (Non-converged)	Conditional model	Zero-inflation model	
Intercept	-6.10	-19.35	-6.06 *** (-6.54, -5.59)	-2.58 ** (-4.39, -0.759)	
PC2	-0.097	-0.666	0.019 (-0.130, 0.167)	**0.415 * (0.089, 0.741)**	
Hormone treatment	-0.084	-	-0.105 (-0.429, 0.219)	-	
PC2 x Hormone treatment	-0.145	-	0.077 (-0.032, 0.187)	-	

861 **Table S8.** Climatological variables used for the analysis of local climates at 26 sites.

Variable names - CHELSA database	Description
bio1	Mean annual temperature calculated as the average of mean monthly temperatures over the year
bio2	Mean diurnal temperature range computed as the average of monthly
bio3	Isothermality; compares day–night variability to annual temperature range
bio4	Temperature seasonality given by the standard deviation of mean monthly temperatures
bio5	Highest monthly mean of daily maximum temperatures across the year; indicates peak thermal conditions
bio6	Lowest monthly mean of daily minimum temperatures across the year; characterizes winter cold intensity
bio7	Annual temperature range; measures amplitude between warmest and coldest months
bio8	Average monthly mean temperature over the wettest 3-month period of the year
bio9	Average monthly mean temperature over the driest 3-month period of the year
bio10	Average monthly mean temperature over the warmest 3-month period of the year
bio11	Average monthly mean temperature over the coldest 3-month period of the year
bio12	Sum of monthly precipitation totals across the year
bio13	Maximum monthly precipitation total
bio14	Minimum monthly precipitation total
bio15	Coefficient of variation of monthly precipitation totals
bio16	Average monthly precipitation during the wettest 3-month period of the year
bio17	Average monthly precipitation during the driest 3-month period of the year
bio18	Average monthly precipitation during the warmest 3-month period of the year
bio19	Average monthly precipitation during the coldest 3-month period of the year
clt_max	Highest total cloud cover percentage
clt_mean	Average total cloud cover percentage
clt_min	Lowest total cloud cover percentage
clt_range	The difference between highest and lowest total cloud cover percentage
cmi_max	Highest monthly climate moisture index

cmi_mean	Average monthly climate moisture index
cmi_min	Lowest monthly climate moisture index
cmi_range	The difference between highest and lowest monthly climate moisture index
gdd0	Sum of daily mean temperatures above 0 °C accumulated over the year
gdd5	Sum of daily mean temperatures above 5 °C accumulated over the year
gdd10	Sum of daily mean temperatures above 10 °C accumulated over the year
gsl	Number of days between the first and last occurrence of growing season conditions
gsp	Total precipitation accumulated during the growing season period
gst	Average daily mean temperature over all growing season days
hurs_max	Highest near-surface relative humidity
hurs_mean	Average near-surface relative humidity
hurs_min	Lowest near-surface relative humidity
hurs_range	The difference between highest and lowest near-surface relative humidity
kg0	Köppen–Geiger climate classification
kg1	Köppen–Geiger climate classification without As/Aw differenciation
kg2	Köppen–Geiger climate classification after Peel et al. 2007
kg3	Climate classification after Wissmann 1939
kg4	Climate classification after Thornthwaite 1931
kg5	Climate classification after Troll-Pfaffen
ngd0	Total number of days in a year with mean daily temperature above 0 °C
ngd5	Total number of days in a year with mean daily temperature above 5 °C
ngd10	Total number of days in a year with mean daily temperature above 10 °C
npp	Production of carbon' means the production of biomass expressed as the mass of carbon which it contains
pet_penman_max	Highest monthly potential evapotranspiration
pet_penman_mean	Average monthly potential evapotranspiration
pet_penman_min	Lowest monthly potential evapotranspiration
pet_penman_range	The difference between highest and lowest monthly potential evapotranspiration
rsds_max	Highest surface downwelling shortwave flux in air
rsds_mean	Average surface downwelling shortwave flux in air
rsds_min	Lowest surface downwelling shortwave flux in air
rsds_range	The difference between highest and lowest surface downwelling shortwave flux in air

scd	Number of days per year with snow cover present at the surface
sfcWind_max	Highest near-surface wind speed
sfcWind_mean	Average near-surface wind speed
sfcWind_min	Lowest near-surface wind speed
sfcWind_range	The difference between highest and lowest near-surface wind speed
swb	Total water equivalent of snowpack accumulated over the year
vpd_max	Highest vapor pressure deficit
vpd_mean	Average vapor pressure deficit
vpd_min	Lowest vapor pressure deficit
vpd_range	The difference between highest and lowest vapor pressure deficit