

Supporting Information

Pujol et al. 10.1073/pnas.0902257106

SI Materials and Methods

Sample Populations. Seeds used to establish the base populations were collected from 20 populations across the hexaploid range of *Mercurialis annua* (Euphorbiaceae) (1–3). Latitudes of populations ranged from 33.7° (Morocco) to 43.15° (northern Spain). Note that diploid populations of *M. annua* occur further north and east, but the diploids expanded from a different refugium in the eastern Mediterranean Basin (2). Coordinates corresponding to population locations are available from the authors upon request. In each population, seed was collected and pooled from ≈20–40 seed-bearing individuals.

Methods for Generation 1: Crosses. In 2005, we grew a bulk of 50 individuals from each of the 20 populations through a single generation of growth and mating in a common glasshouse. Because *M. annua* is wind-pollinated and flowers indeterminately, we could not self and outcross different flowers on the same plants. For each population, therefore, a group of 50 outcrossing plants and three isolated selfing plants were grown and allowed to mate separately in a common glasshouse. Groups of 50 hermaphrodites from each population were isolated from one another by pollen-proof screens. This method of isolation is effective for *M. annua* (1). We also isolated single plants using pollen-proof screens (three plants for each of the 20 populations) to let those self-fertilize on their own. Populations were located in the glasshouse after a random design. At this stage, we removed any males segregating in the experimental populations. Males co-occur in many wild populations of *M. annua*, and their frequency depends critically on the pollen produced by hermaphrodites (4, 5). However, because maleness is determined by a dominant allele (6), by removing males, we effectively established experimental lines that contained only hermaphrodites in subsequent generations. Note that any contamination between lines will have diluted the differences established between them, so that our results can be interpreted as conservative. After mating (*M. annua* is wind-pollinated) and fruit maturation, we harvested seeds for fitness assays. Here and below, all plants were grown in 9 × 9 cm pots on Goundrey's Astro Universal Peat based compost and were watered regularly.

Methods for Generation 2: Inbreeding Depression Experiment. In the summer of 2008, we established 20 inbred and 20 outbred individuals from each population in separate pots in the glasshouse, with each individual being a randomly sampled progeny. The individuals were placed in a random location on the glasshouse bench. When individuals were 6 weeks old, they were harvested and assayed for their male reproductive effort (MRE), female reproductive effort (FRE), aboveground biomass, and height. For each individual, MRE and FRE were, respectively, measured as the dry weight of male flowers and seeds over the plant dry biomass.

Data Analysis. We accounted for temporal variation among individuals in the glasshouse by including the germination day as a covariate in our analysis. All variables were linearized across the whole dataset by using the best linear transformation obtained from a “Box Cox” fitting function; all data were ultimately transformed by exponents ranging from 0.17 to 0.38. On the basis of these values, we estimated inbreeding depression components in terms of MRE, FRE, height, and above-ground plant biomass for each population. We estimated inbreeding depression (δ) as $\delta = 1 - w_{\text{self}}/w_{\text{out}}$ for $w_{\text{out}} > w_{\text{self}}$ and $\delta = w_{\text{out}}/w_{\text{self}} - 1$ for w_{out}

$< w_{\text{self}}$, where w_{out} and w_{self} correspond to the mean linearized phenotypic performance of outbred and inbred progeny, respectively (7). The total fitness of each individual i was estimated as the relative prospective contribution through pollen and seeds of each individual to the progeny, defined as $w_i = s_i + p_i\bar{s}/\bar{p}$, where \bar{s} and \bar{p} are the mean seed production and mean pollen production of all outcrossed individuals in the respective population. This index estimates the inbreeding depression suffered by selfed progeny in an otherwise outcrossing population. All populations' inbreeding depression measures were regressed against latitude, which may be viewed as a proxy for the distance along corridors of range expansion from the core populations in North Africa to the marginal populations in northeastern and northwestern Spain.

Simulation Model. We simulated the effects of a range expansion on patterns of inbreeding depression by allowing a population to expand its range from a single source population along a 1D corridor by stepping-stone colonization and migration. We assumed a habitat string of 100 sites, each with a carrying capacity of 1,000 individuals. We characterized the genotype of each individual in terms of two haploid genomes, each with $L = 100$ unlinked loci. At each locus, the haplotype could be loaded with a deleterious mutation, or it could be mutation free. The fitness of each individual depended on its multilocus genotype. Specifically, multiplicative fitness that was assumed for individual j was given by

$$w = \prod_{i=1}^L (1 - \lambda_i s_i), \quad [1]$$

where s is the selective coefficient, $\lambda_i = h$ if the locus is heterozygous, $\lambda_i = 1$ if the locus is homozygous for the deleterious allele, $\lambda_i = 0$ if the locus is homozygous for the nond deleterious allele, and h is the dominance coefficient. Our model assumed that the selection and dominance coefficients were the same across all loci.

Before range expansion, we first allowed the source population to reach an equilibrium between mutation to deleterious alleles at multiple loci and selection. Equilibrium was ascertained by running two simulations in parallel, one in which all individuals were started free of deleterious mutations, and the other in which all individuals began as heterozygotes. Once equilibrium in the core population had been reached, we allowed it to expand its range along a single array of 99 further habitat patches by stepping-stone colonization and migration. For this process, we allowed M seeds, randomly chosen, to move from any filled population i into populations $i - 1$ and $i + 1$ (with edge populations receiving potential migrants only from the single adjacent site). After migration, mating and reproduction was as follows: (i) an individual was randomly chosen from the M (in the case of newly colonized sites) or $n = 1,000$ individuals (in the case of filled habitat), with a probability of choosing a particular individual being proportional to its relative fitness; (ii) a haploid gamete was generated for this individual by allowing free recombination between all loci and assuming, for each locus independently, a probability of μ that the chosen allele suffered a deleterious mutation; (iii) a second individual was chosen as in i , by sampling from the population with replacement, and a second haplotype was formed, as in ii ; and (iv) steps i through iii were repeated until the population contained N individuals.

With this scheme, recently colonized populations grew to their carrying capacity in a single generation, as in classic metapopulation models (8, 9). Note that we assumed only forward mutation to a deleterious state, so that a loaded locus was not affected further by subsequent mutations. We let $U = \mu L$ be the genomic mutation rate.

We ran this stepping-stone model until all of the 100 demes had reached their carrying capacity. From that point in time, we recorded inbreeding depression and the mean frequency of deleterious mutations per locus in each deme at 10-generation intervals. Specifically, we recorded inbreeding depression as follows:

$$\delta = 1 - \frac{\bar{w}_{A,B}}{\bar{w}_{A,C}}, \quad [2]$$

where subscripts A, B, and C identify the haplotype composition of the individual “assayed” for fitness. Thus, genotypes composed of haplotypes A and B were formed by sampling haplotypes from the same individual (a selfing event), whereas genotypes composed of haplotypes A and C were formed by sampling two haplotypes, each from a different individual within the same deme. We also recorded the mean frequency of deleterious mutations with each deme.

We repeated above procedure 20 times for each parameter set. All results are reported as averages across 20 independent replications.

1. Pujol B, Pannell JR (2008) Reduced responses to selection following species range expansion. *Science* 321:96.
2. Obbard DJ, Harris SA, Pannell JR (2006) Sexual systems and population genetic structure in an annual plant: Testing the metapopulation model. *Am Nat* 167:354–366.
3. Durand B (1963) Le complexe *Mercurialis annua* L. sl. Une étude biosystématique. *Ann Sci Nat Bot* 12:579–736.
4. Dorken ME, Pannell JR (2008) Density-dependent regulation of the sex ratio in an annual plant. *Am Nat* 171:824–830.
5. Eppley SM, Pannell JR (2007) Density-dependent self-fertilization and male versus hermaphrodite siring success in an androdioecious plant. *Evolution* 61:2349–2359.
6. Pannell J (1997) Mixed genetic and environmental sex determination in an androdioecious population of *Mercurialis annua*. *Heredity* 78:50–56.
7. Husband BC, Schemske DW (1996) Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50:54–70.
8. Slatkin M (1977) Gene flow and genetic drift in a species subject to frequent local extinction. *Theor Popul Biol* 12:253–262.
9. Pannell JR, Charlesworth B (2000) Effects of metapopulation processes on measures of genetic diversity. *Philos Trans R Soc Lond Ser B Biol Sci* 355:1851–1864.