

Symptoms of population range expansion: lessons from phenotypic and genetic differentiation in hexaploid *Mercurialis annua*

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Background: Range expansion often results in colonisation bottlenecks that should both deplete genetic diversity and increase genetic differentiation towards the margins of a species' geographic distribution.

Aims: We tested whether genetic differentiation increased among populations of the annual plant *Mercurialis annua* after its colonisation of the Iberian Peninsula from Morocco. Previous work showed that this colonisation resulted in a decrease of phenotypic and genetic diversity from the core in North Africa towards the distribution margins of *M. annua* in north-eastern and north-western Spain.

Methods: Seeds were sampled from 20 populations located across the hexaploid range of *M. annua*. Patterns of phenotypic and genetic differentiation among experimentally grown populations were analysed and compared between the Iberian Peninsula and North Africa.

Results: The level of phenotypic and genetic differentiation among populations in the expanded range of the Iberian Peninsula was similar to that in the core range in North Africa.

Conclusions: Our findings imply that the observed effects of range expansion on genetic differentiation may be independent of the effects on genetic diversity. They point to the importance of taking both historic and contemporary processes of migration into account when predicting the results of range expansion.

Keywords: annual mercury; contemporary gene flow; Iberian Peninsula; North Africa; post-glacial colonisation

Introduction

Climatic warming during the Pleistocene allowed many species in the northern hemisphere to expand their ranges northward from core populations in southern refugia (Hewitt 2000). These range expansions, which typically involved only a small sample of the ancestral population, are expected to have had a large impact on patterns of genetic diversity (Austerlitz et al. 1997). For example, as a result of the successive genetic bottlenecks involved in expansion, marginal populations are expected to display reduced within-population genetic diversity, a pattern that has been widely documented (Austerlitz et al. 2000). Indeed, populations in the expanded range often display reduced levels of neutral genetic diversity (Prugnolle et al. 2005; Obbard et al. 2006b), reduced levels of phenotypic variation (Manica et al. 2007; Pujol and Pannell 2008) and reduced levels of quantitative genetic variation (Lavergne and Molofsky 2007; Pujol and Pannell 2008).

Colonisation bottlenecks that reduce population diversity are also expected to increase genetic differentiation among populations (Slatkin 1987; Austerlitz et al. 1997). We thus expect range expansion to generate a pattern of lower diversity associated with higher differentiation in populations of the expanded range. Ultimately, gene flow

into marginal populations after range expansion has taken place will erode differentiation among them and cause within-population genetic diversity to increase (Lavergne and Molofsky 2007). However, dispersal among populations after a range expansion might occur on too small a geographic scale, or might be too recent to homogenise the diversity between core populations and marginal populations. Under such a scenario, gene flow among populations within the expanded range would be expected to reduce differentiation among them even while differences in levels of diversity between the core and the margins persist (Ibrahim et al. 1996).

Here, we analyse the relationship between genetic variation and differentiation observed in the core and in the expanded range of the European plant *Mercurialis annua* L. (Euphorbiaceae) to disentangle historic and contemporary processes of migration (e.g. Kropf et al. 2008). The hexaploid range of *M. annua* probably expanded after the Pleistocene, from a refugium in North Africa along two coastal corridors around the Iberian Peninsula (Obbard et al. 2006c). As a result, northern Iberian populations of hexaploid *M. annua* display much lower genetic diversity than more southern populations, both at neutral loci (Obbard et al. 2006c), as well as at loci affecting several life-history traits (Pujol and Pannell 2008; Pujol et al.

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2009). Although the effects of range expansion on patterns of diversity are well established for hexaploid *M. annua*, the relationship between population differentiation and range expansion has not yet been studied. On the one hand, we might expect population differentiation to be greater in marginal populations of *M. annua* in the Iberian Peninsula compared with core populations in North Africa. Alternatively, if the Strait of Gibraltar acts as an effective barrier to gene flow, and contemporary gene flow occurs among populations only within the expanded range, then we would not expect the reduced genetic variation in the expanded range to be associated with an increase in genetic differentiation. This is because contemporary gene flow will have eroded genetic differentiation among marginal populations in Iberia in the absence of gene flow from the core populations in North Africa that would otherwise have replenished population diversity. We attempted to distinguish these two alternative scenarios by analysing regional patterns of genetic differentiation among populations in relation to patterns of population diversity in *M. annua* published by Obbard et al. (2006c) and Pujol and Pannell (2008). Although our study is narrowly focused on regional patterns of genetic variation of a cosmopolitan European weed, it has broader implications for our understanding of patterns of diversity during and after range expansions where dispersal occurs at different spatial scales.

Material and methods

Study species and populations

We sampled populations of the plant *Mercurialis annua* across its hexaploid range in North Africa and across the Iberian Peninsula. Note that these populations correspond to the synonymous species *M. ambigua* L. Fil., which also includes tetraploid populations from further south in Morocco (Durand 1963). However, we prefer the broader nomenclature, that is, *M. annua* sensu lato, because this taxon is monophyletic whereas *M. ambigua* is not (Krähenbuhl et al. 2002; Obbard et al. 2006a). None of our samples were from diploid populations, which occur further north and east and are thought to have expanded from a different refugium in the eastern Mediterranean Basin (Obbard et al. 2006b). The correspondence between ploidy levels and their geographic distribution was discussed by Thomas (1958) and Durand (1963) and confirmed for the populations sampled here by Obbard et al. (2006a) using flow cytometry.

Seeds for our study were collected from 20 androdioecious or monoecious populations in coastal regions in North Africa and the Iberian Peninsula (see Figure S1 and Table S1 of the supplementary material which is available via the supplementary content tab on the article's webpage at <http://dx.doi.org/10.1080/17550874.2010.516027>). For each population, seeds were randomly selected from the pooled progeny of approximately 20 to 40 individuals. Populations ranged in latitude from 33.7° N (Morocco) to 43.15° N (northern Spain).

Genetic data

For each of the 20 populations sampled, 42 plants were established from seeds in a glasshouse at the University of Oxford, between November 2000 and August 2003 (total $n = 840$). These populations were part of the larger dataset analysed by Obbard et al. (2006b), who determined multi-locus genotypes at six isozyme loci; we extracted the genotypic data for the 20 populations studied here from this earlier study. Our analyses (see below) effectively assume that individuals sampled were representative of the populations from which they were drawn and that they were independent. Given that *M. annua* can self-fertilise at low population densities (Eppley and Pannell 2007), the assumption of independence may have been violated for some populations. Nevertheless, because our sample closely represents a process of choosing individuals randomly from the next generation of potential adults, we believe that our protocol represents an adequate representation of the genetic variation found in populations. Importantly, there is no indication that density-dependent self-fertilisation rates should differ among regions sampled, because populations vary similarly in their density among regions (M.E. Dorken and J.R. Pannell, unpubl. data). A summary table of genetic parameters for isozyme diversity in the 20 populations can be found in the supplementary online material (Table S2).

Phenotypic measures

We grew a new bulk of individuals from each of the 20 populations presented above. Fifty seeds per population were established as seedlings ($n = 1000$) through a single generation of growth and mating in a common glasshouse at the University of Oxford. Here, and below, all plants were grown in 9 × 9 cm pots on Goundrey's Astro Universal peat-based compost and watered regularly. The 50 hermaphrodites from each population were allowed to mate in separate groups. Populations were randomised in space in a common glasshouse and were isolated in their groups. *M. annua* is wind-pollinated, so that plants could mate freely within their groups. However, we prevented gene flow among populations through the use of pollen-proof screens, as described by Pujol and Pannell (2008). We also removed any males segregating in the experimental populations. Males co-occur with hermaphrodites in many wild populations of *M. annua* and their frequency depends critically on the pollen produced by hermaphrodites. However, because maleness is determined by a dominant allele (Pannell 1997), by removing males we effectively established experimental lines that contained only hermaphrodites in subsequent generations.

Following mating and fruit maturation, we harvested seeds from all plants. The following summer, we established 20 individuals from each population ($n = 400$), all produced by different mother plants, in separate pots in the glasshouse. Each mother plant was randomly chosen from the previous generation. The individuals were allocated a

random location in a grid on the glasshouse bench from the time of their germination to the time of harvest. When individuals were 42 days old, they were harvested and assayed for their male reproductive effort (MRE), female reproductive effort (FRE) and above-ground biomass. MRE and FRE, respectively, were estimated by the sum of the dry weight of all male flowers and seeds from a plant divided by the above-ground vegetative biomass of the plant. While *M. annua* is variable for a number of characters, we measured only reproductive effort because these characters were the focus of a larger study of responses to selection in the same populations (Pujol and Pannell 2008).

Statistical analyses

We estimated the phenotypic differentiation among populations within regions (i.e. North Africa, eastern Iberia and western Iberia) by calculating the percentage of phenotypic variation that was explained by differences between populations within each region (coefficient of determination r^2 in a general linear model determined by using PROC GLM in the SAS software; SAS-institute 2000). Values for MRE, FRE and above-ground biomass were linearised across the whole data set by using the best linear transformation obtained from the 'Box Cox' fitting function of the R software (R Development Core Team 2007). We then tested whether phenotypic differentiation within regions followed a model of isolation by distance. We used a Mantel test to test for a correlation between the geographical distance separating populations and individuals' pair-wise phenotypic similarity estimated by the Moran Index, which we calculated using the programs autocorr (Hardy et al. 2000) and Genalex (Peakall and Smouse 2006). We then tested whether phenotypic differentiation differed significantly between regions. We conducted a non-parametric Wilcoxon two-sided test for tied data comparisons by using the procedure Npar1way in the SAS software (SAS-institute 2000) to test whether the magnitude of pair-wise similarity between individuals from different populations was different between pairs of regions.

To analyse regional patterns of genetic differentiation, we used F'_{ST} values calculated between pairs of these populations using six isozyme markers (Obbard et al. 2006b). F'_{ST} is an allelic phenotypic-based differentiation

statistic suited to the analysis of polyploid genotypes that is informative for various levels of ploidy; for a thorough description of the method see Obbard et al. (2006c). Briefly, although F'_{ST} and F_{ST} are not necessarily directly comparable, they are analogous and appear to have similar statistical properties (Obbard et al. 2006c). F'_{ST} is based on an assessment of phenotypic differences between isozyme gel profiles. It can be applied to polyploids with disomic inheritance (such as allopolyploids) in which allele presence/absence can be scored but where allele copy-number (and thus genotype) cannot (Obbard et al. 2006c). Population F'_{ST} estimates of genetic differentiation based on six isozyme loci proved to be sufficiently powerful to detect genetic differentiation among the 20 populations in our sample, with similar numbers of individuals sampled (Obbard et al. 2006b, 2006c). These data were used to compare genetic differentiation within regions; we used a non-parametric Wilcoxon two-sided test for tied comparisons computed through the procedure Npar1way of the SAS software (SAS-institute 2000) to test whether genetic differentiation was similar in different regions. We corrected the significance of the P values in the three between-region comparisons through the use of a serial Bonferroni correction (Rice 1989).

Results

Patterns of genetic differentiation

F'_{ST} values (Table 1) were similar between North Africa and eastern Iberia and between North Africa and western Iberia but differed significantly between eastern and western Iberia ($P = 0.037$, although note that this difference did not remain significant after a Bonferroni correction; see Figure 1). Our results also showed that genetic differentiation was not correlated with distance in any of the three regions studied (Mantel test of correlation between pairwise F'_{ST} and geographical distance: $P = 0.973$ in North Africa; $P = 0.501$ in eastern Iberia; and $P = 0.685$ in western Iberia).

Patterns of phenotypic differentiation

Both MRE and FRE differed significantly among populations within regions (i.e. North Africa, western Iberia and eastern Iberia), whereas biomass differed significantly

Table 1. Percentage of variation (r^2) in male and female reproductive effort (MRE and FRE, respectively) and biomass, and the levels of genetic differentiation, measured as the average pair-wise population F'_{ST} (Obbard et al. 2006c), in different regions of the geographic range of *Mercurialis annua*.

Region	MRE		FRE		Biomass		Isozymes
	r^2 (%)	P -value	r^2 (%)	P -value	r^2 (%)	P -value	F'_{ST}
North Africa	15.8	0.001	11.4	0.012	6	0.220	2.01
Eastern Iberia	24.4	<0.001	31.3	<0.001	0.6	0.963	2.09
Western Iberia	13.1	0.005	33.2	<0.001	9.1	0.049	1.82

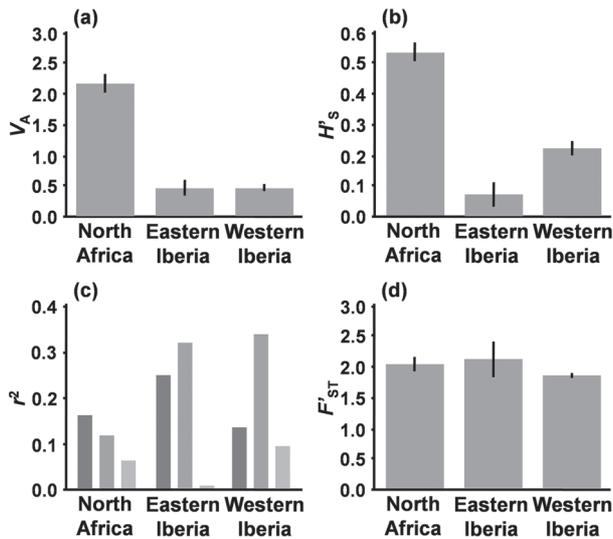


Figure 1. Quantitative genetic variation in *Mercurialis annua* estimated by: (a) population additive genetic variance V_A (data from Pujol and Pannell 2008); (b) within-population genetic diversity estimated as H'_S , the average number of alleles by which pairs of individuals differ (data from Obbard et al. 2006c); (c) phenotypic differentiation estimated by r^2 , the percentage of global variation in the respective phenotypic trait that differs among populations; and (d) genetic differentiation estimated by F'_{ST} , the degree of differentiation between isoloci within a polyploid genome, for North Africa, eastern Iberia and western Iberia. Black bars in (a), (b) and (d) represent standard errors. Dark, medium and light grey bars in (c) represent male and female reproductive effort (MRE and FRE, respectively) and biomass.

among populations only in western Iberia (Table 1). Over all regions and phenotypic traits, the percentage of variance explained by differences among populations ranged from 9.1% to 33.2% (Table 1). These estimates of phenotypic differentiation among populations in the expanded range (i.e. western Iberia and eastern Iberia) were quantitatively similar to, or higher than, those between core populations (i.e. North Africa).

Patterns of phenotypic differentiation among populations were similar in the three regions for all traits measured (Figure 1). Phenotypic differentiation showed a weak decrease with geographical distance that was inconsistent across the data set, never explaining more than 1.9% of the variance among populations. A significant but rather weak decrease with distance of the similarity in MRE among populations was found in North Africa ($r^2 = 0.4\%$, $I = -0.0916 \text{ Dist} + 0.0933$, Mantel test $P = 0.001$, $n = 9453$ pairs of individuals compared among seven populations) and eastern Iberia ($r^2 = 1.9\%$, $I = -0.0724 \text{ Dist} + 0.1296$, Mantel test $P = 0.001$, $n = 4851$ pairs of individuals compared among five populations). Similarity in FRE decreased significantly but weakly with geographic distance in eastern ($r^2 = 0.2\%$, Moran Index = $-0.0282 \text{ Dist} + 0.0497$; Mantel test, $P = 0.001$, $n = 4851$ pairs of individuals compared among five populations) and western Iberia ($r^2 = 0.5\%$, $I = -0.1205 \text{ Dist} + 0.0892$; Mantel test, $P = 0.001$, $n = 9316$

pairs of individuals compared among seven populations) (Figure S2, available online). For MRE in western Iberia, FRE in North Africa and biomass in all three regions, we detected no relationship between distance and similarity.

Discussion

The principal result of our study is clear: whereas within-population genetic diversity is substantially lower in the expanded Iberian range of *M. annua* than in its refugial core in North Africa, there is no corresponding increase in either phenotypic differentiation among populations or in terms of F'_{ST} calculated on the basis of isozyme loci (Figure 1). Note that because populations were grown in a similar controlled environment, it is unlikely that environmental variation affected populations differently in our experiment; therefore the significant phenotypic differentiation detected among populations very likely reflected quantitative genetic variation.

Our results point to a high likelihood that range expansion by hexaploid *M. annua* was relatively recent and that the Strait of Gibraltar represents an effective barrier to gene flow that has prevented the gradual replenishment of genetic variation in the expanded range in Iberia from further south in Africa. Significantly, the similarity in levels of population differentiation between the expanded and core ranges of hexaploid *M. annua* points to corresponding similarities in contemporary gene flow among their respective populations. In other words, contemporary gene flow among populations within the expanded range would appear to have erased the signature of range expansion in terms of genetic differentiation, as predicted by theory (Lande 1992; Austerlitz et al. 1997; Lavergne and Molofsky 2007), whereas the signature in terms of reduced genetic variation has been preserved, presumably by the barrier to contemporary gene flow between core and marginal regions. Range expansion of hexaploid *M. annua* is thus likely to have followed a model similar to that reported by Austerlitz and Garnier-Géré (2003), where the absence of long-distance dispersal maintains regional differences in genetic diversity and the action of dispersal over intermediate distances reduces genetic differentiation among local populations.

During range expansion, successive founder events that accompany the establishment of new populations are typically expected to lead to higher population differentiation in the expanded range than among core populations (Le Corre and Kremer 1998). Differentiation among marginal populations can be reinforced by genetic drift in small isolated populations after expansion (Slatkin 1987; Austerlitz et al. 1997) and by new mutations that appear during range expansion and 'surf' on the wave of colonisation, in other words which diffuse into geographically restricted areas (Excoffier and Ray 2008). Several theoretical models (e.g. Austerlitz et al. 2000; Austerlitz and Garnier-Géré 2003) and empirical studies (e.g. Westerbergh and Saura 1994; McCauley 1994, 1997) indicate that the genetic differentiation that results from several initial

founder events can decrease after homogenization by gene flow. In the case of *Silene latifolia* Poir., for instance (a species that occupies similar habitat to *M. annua*), less than 200 years of gene exchanges among populations were enough to reduce differentiation by about 80% (McCauley 1994, 1997).

Colonisation of the Iberian Peninsula by *M. annua* from North Africa is likely to have occurred after the Pleistocene glaciations through one or multiple events of colonisation (Obbard et al. 2006b). The range expansion route followed by hexaploid *M. annua* matches none of the classical range expansion models defined by Hewitt (1999), because *M. annua* colonisation of Europe finds its origin in North Africa rather than Iberia, Italy, Greece or Turkey. This is probably because documented cases of range expansion for species distributed in Africa and Europe have only recently begun to accumulate, for example the plant *Ecballium elaterium* (L.) A. Rich. (Costich and Meagher 1992). A simple scenario of plant species migration 'out of Africa' implies the colonisation of newly emerged lowlands that connected Africa to Iberia during glaciations of the Pleistocene when sea levels were 120–150 m lower than at present (Ortiz et al. 2007). Subsequently, *M. annua* hexaploid populations probably expanded their range, at the time of, or after, the rise in sea levels that severed genetic links between populations in the expanded range from their source populations in the core. This scenario of range expansion, which includes responses to sea level variation and possible changes in connection and isolation of lands by rivers and mountains, is supported by patterns of plant population genetic structure in several other species, for example *Campanula lusitanica* L. (Cano-Maqueda et al. 2008), *Cistus ladanifer* L. (Guzman and Vargas 2009), *Hypochaeris salzmanniana* DC. (Tremetsberger et al. 2004; Ortiz et al. 2007) and *Quercus suber* L. (Lumaret et al. 2005).

Of course, the process of range expansion by *M. annua* and severance of its marginal populations in Iberia from the core populations in North Africa is unlikely to have been as simple as set out above. It is plausible, for instance, that the colonisation of southern Spain was followed by a complex dynamic process of expansion on the Iberian Peninsula involving a suite of contractions and expansions, as suggested for the plant species *Ferula communis* L. (Perez-Collazos et al. 2009). Northward range expansion in the Iberian Peninsula might in fact have reduced genetic differentiation among northern populations (Gomez and Lunt 2007; Lopez de Heredia et al. 2007; Pico et al. 2008). This latitudinal effect might be explained by the isolation of multiple populations that diverged locally and later entered a phase of contact during later expansion, the so-called 'refugia-within-refugia' model (Gomez and Lunt 2007). Nevertheless, such a process, if it occurred, would still only imply 'refugia-within-refugia' in the expanded range and would not involve further contact between Iberia and Africa.

In principle, certain patterns of genetic and phenotypic differentiation across a species' geographic distribution might be attributable to local adaptation, which is expected

to increase quantitative genetic differentiation at phenotypic traits above the range of genetic differentiation values obtained at neutral marker loci (Merila and Crnokrak 2001; McKay and Latta 2002; Pujol et al. 2008; Whitlock 2008). However, we found no clear environmental gradients in patterns of population phenotypic differentiation in the expanded range of hexaploid *M. annua*; it is difficult to see how our observations could be attributable to local adaptation across the Iberian Peninsula, although local adaptation may have played some role in shaping the evolution of these populations. Given what has been inferred about the history of range expansion in *M. annua* and its recent behaviour, we thus favour demographic processes as the most likely causes of the patterns we have observed, that is migration and drift, including the effects of recurrent genetic bottlenecks, at contrasting spatial scales.

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