Rapid Development of Adaptive, Climate-Driven Clinal Variation in Seed Mass in the Invasive Annual Forb

_Echium plantagineum_ L.

Tara K. Konarzewski¹-², Brad R. Murray¹, Robert C. Godfree²*

¹ Plant Functional Biology and Climate Change Cluster, School of the Environment, University of Technology Sydney, New South Wales, Australia, ²CSIRO Plant Industry, Canberra, Australia

Abstract

We examined adaptive clinal variation in seed mass among populations of an invasive annual species, _Echium plantagineum_, in response to climatic selection. We collected seeds from 34 field populations from a 1,000 km long temperature and rainfall gradient across the species’ introduced range in south-eastern Australia. Seeds were germinated, grown to reproductive age under common glasshouse conditions, and progeny seeds were harvested and weighed. Analyses showed that seed mass was significantly related to climatic factors, with populations sourced from hotter, more arid sites producing heavier seeds than populations from cooler and wetter sites. Seed mass was not related to edaphic factors. We also found that seed mass was significantly related to both longitude and latitude with each degree of longitude west and latitude north increasing seed mass by around 2.5% and 4% on average. There was little evidence that within-population or between-population variation in seed mass varied in a systematic manner across the study region. Our findings provide compelling evidence for development of a strong cline in seed mass across the geographic range of a widespread and highly successful invasive annual forb. Since large seed mass is known to provide reproductive assurance for plants in arid environments, our results support the hypothesis that the fitness and range potential of invasive species can increase as a result of genetic divergence of populations along broad climatic gradients. In _E. plantagineum_ population-level differentiation has occurred in 150 years or less, indicating that the adaptation process can be rapid.

Introduction

Successful invasion of novel environments by exotic plant species requires that species maintain positive population growth and spread in the face of environmental heterogeneity and new selection pressures [1,2]. While many factors determine the demographic characteristics and spatial spread of invading plant populations [3], rapid evolutionary changes in fitness-related traits increase the reproductive output of local populations and often play a fundamental role in the invasion process [1,4,5]. Indeed, significant evolutionary capacity has been identified in many invasive plant species [6–12]. This is perhaps not surprising, since considerable theoretical and empirical evidence supports the notion that the capacity for rapid evolutionary change exists widely in plant populations (e.g., [1,13–15]).

Recently it has been suggested that, at large spatial scales, the spread of invasive populations is mainly determined by evolutionary adaptation and population-level genetic differentiation, while phenotypic plasticity becomes more important where small-scale variation in abiotic conditions impact on population fitness [16]. While the adaptive importance of phenotypic plasticity is well understood [17–21], the capacity for invasive species to undergo adaptive differentiation along broad-scale climatic gradients has been more poorly documented. Adaptive clinal variation in life-history traits has been observed in some invasive species (e.g., [6,9–12,20,22]), and their ability to occupy new climatically distinct envelopes in their introduced range is likely to be a valuable strategy in general [23].

However, not all invasive species display clinal differentiation [24,25], perhaps due to the wide range of genetic, demographic, developmental and environmental factors that influence evolutionary divergence of plant populations in new habitats [1,26–29]. Peripheral populations located in marginal habitats, for example, suffer numerous evolutionary constraints related to population size, gene flow and migration rates [30–32]. Levels of phenotypic plasticity [19], seed dormancy [33], co-variation among fitness traits [29], and pathogen load [34] are also known (among other factors) to limit evolutionary adaptation, and many are especially relevant for invasive plant populations. Given this conflicting evidence, there is a clear need for a more comprehensive understanding of the species, circumstances, and traits in which adaptive clines are likely to develop.

Seed mass is a key fitness-related trait that might be expected to show strong clinal adaptation when the ability of a species to produce seeds of a particular size underpins reproductive success and survival in new environments [35,36]. Seed mass influences
many life-history traits including dispersal ability, seed bank viability and persistence, progeny fitness, flower size and plant longevity [37,38]. Large seed size appears to be especially important in arid zone species probably due to the increased temperature-related metabolic costs and requirements for seedling establishment in arid environments [35,39,40]. Evolution of seed mass in response to environmental gradients is indeed well documented on a local [40–45] and global scale [46,47] although few studies have considered whether such patterns exist among invasive species (but see; [46,48,49]).

The aim of this study was to test whether, over the past ~150 years since introduction, invasive populations of the annual plant species *Echium plantagineum* L. (Paterson’s curse) have developed adaptive, population-level differentiation in seed mass in response to broad climatic gradients in south-eastern (SE) Australia. *Echium plantagineum* is a genetically diverse [50], globally significant weed [51]. In Australia, it has invaded arid, temperate and coastal environments, costing the meat and wool industry upwards of $125 million annually [52]. We hypothesized that invasive populations of *E. plantagineum* have developed acline in seed mass in response to aridity, with larger seeds prevailing in populations sourced from warmer, drier habitats than those sourced from cooler, wetter temperate and coastal habitats. We also hypothesized that ongoing selection for seed size will have resulted in a narrowing of seed size variation among populations within bioregions and among individual plants within populations in the most arid and unfavourable environments relative to populations from a more favourable core habitat [29,30]. To test these hypotheses, we compared the weights of glasshouse-produced seeds from invasive *E. plantagineum* populations sourced from 34 sites across a very large (1,000 km) temperature and rainfall gradient in SE Australia.

**Methods**

**Study species**

Originally native to Europe and the Mediterranean region, *Echium plantagineum* (Boraginaceae) is an annual forb that was introduced to Australia in around 1850 [51]. It is a globally invasive species that has become successfully established in 30 million hectares of agricultural land in Australia [53,54] (Fig. 1a). *Echium plantagineum* is insect pollinated and can produce up to 10,000 seeds with seed production of up to 30,000 per m². Seeds are dispersed via water, contaminated fodder, garden waste, animal fur and the alimentary tracts of birds or grazing animals [54,55], and while some seeds can remain dormant in the soil for up to ten years [55,56], most germinate more rapidly [51]. Seedlings most effectively colonise bare ground [57] and can have up to ten years [55,56], most germinate more rapidly [51].

**Seed collection and field sites**

*Echium plantagineum* seeds were collected during the 2009 reproductive season (October to December) from a total of 34 sites across seven IBRA (Interim Biogeographic Regionalisation for Australia scientific framework; [58]), bioregions in New South Wales, SE Australia (Fig. 1b). These bioregions are large, geographically distinct areas of land with similar climate, land systems, vegetation and animal communities [58]. The study sites followed a 1000 km long climatic cline which varies from arid (Broken Hill Complex bioregion) to coastal (Sydney Basin and South-East Coast bioregions) and cool temperate (South-East Highlands bioregion; Fig. 1b, Table 1). These bioregions capture a large majority of *E. plantagineum* habitats in SE Australia. Due to similarities between the two coastal bioregions (Sydney Basin and South-East Coast; Fig. 1b) and the limited number of sites containing *E. plantagineum*, these bioregions have been combined to form a single coastal bioregion (COAST) for our analysis. Sites were randomly selected from across each bioregion; all had at least 50 seed-producing plants. Seeds were collected from ten randomly selected individual plants at each site between October 2009 (Broken Hill Complex bioregion) and December 2009 (COAST bioregion), when plants were producing mature seeds. No permits were required for the field collections since *E. plantagineum* is an introduced, invasive species. No collections were made on private land. Seeds were transported to the laboratory (CSIRO Black Mountain Laboratories, Canberra, ACT; Fig. 1b), extracted from the mature fruit using a rubbing board (consisting of two flat rubber pads), and stored in paper bags at room temperature until used in the following glasshouse experiment.

**Common garden glasshouse experiment**

The objective of the glasshouse experiment was to compare the mass of seeds produced by different plants under common growing conditions, thus allowing for a more controlled assessment of the genetic basis of existing variation. Experimental maternal effects were minimised by using seeds from different populations that were equivalent in size, mass, germination time and level of dormancy. Seed choice was facilitated by the fact that field-collected seeds from different bioregions did not differ in mass (TK Konarzewski unpublished data), unlike glasshouse-produced seeds (see below). This probably reflects the dry conditions experienced during the collection year (2009), especially in the most westerly bioregions, since drought stress in reproductive plants can reduce seed mass [59] and cause general divergence of plant traits under field and glasshouse conditions [22,42]. Nonetheless, all populations produced large numbers of viable, fully mature seeds which were adequate for experimental use.

Ten seeds from each of ten plants from each site (3400 seeds in total) were germinated in the laboratory on moist filter paper in petri dishes under dark conditions at room temperature. After the radicle had emerged, one similar sized embryo (based on radicle length) from each plant (340 in total) was transplanted into small biodegradable pots (JiffyPot® in a temperature controlled glasshouse at the CSIRO Black Mountain site. After ten days the pots were planted into 10 cm pots of standard potting mix, and then four weeks prior to the commencement of the experiment, plants were again transplanted into 20 cm pots containing standard, high nutrient compost potting soil (consisting of a mix of calcium carbonate lime, dolomite lime, blood and bone, and NPK fertiliser; pH = 6.5). Pots were arranged in a randomised block design with five blocks each consisting of three benches; two plants from each of the 34 study sites were randomly placed in each block. Plants were grown from April to December 2010 under a photoperiod governed by natural sunlight and a targeted day/night temperature regime of 25/15°C with an average of 20°C. Temperatures were logged from August to October and followed the targeted regime reasonably closely, with daily averages of 16–20°C, although spot temperatures as high as 27°C and as low as 12°C were observed. Of the 340 plants used in the experiment, two died and 34 plants did not flower within the duration of the experiment (250 days); these were removed from further consideration. After 27 weeks sufficient flowers were produced to allow pollination. Pots were fertilised with Aquasol® Soluble Fertiliser (Yates, Australia) fortnightly or as required.
Suitable plants for open pollination were defined as plants with ten or more open receptive healthy flowers which were identified by the shape and size of the flower and the length and maturity of the stigma. Between October and November 2010 plants from each site were transported together but separately from plants from other sites to a pollination chamber (a small naturally-lit glasshouse) to ensure that cross pollination occurred among plants that originated from the same site. Between six and ten plants, with suitable numbers of flowers, were available for each site. Plants were stored in a separate, insect-free glasshouse for 24 hours prior to placement in the pollination chamber and previously mature flowers were removed to ensure that only newly developed flowers were pollinated. Pollination was performed by European honey bees (*Apis mellifera*) with an exposure period of

**Figure 1. Distribution of Echium plantagineum in Australia and location of collection sites across south-eastern Australia.** (a) Herbarium records from the periods 1889–1910, 1911–1960 and 1961–2010 based on data obtained from Australia’s Virtual Herbarium (2009 Council of Heads of Australasian Herbaria Inc) show the pattern of spread since introduction. (b) Distribution of study sites (depicted by black diamonds) across south-eastern Australia, grouped by IBRA bioregion (coloured). BHC = Broken Hill Complex, MDD = Murray Darling Depression, RIV = Riverina, NSS = NSW (New South Wales) South-Western Slopes, SEH = South-Eastern Highlands, SB = Sydney Basin, SEC = South-East Corner. Note that for all analyses SB and SEC were combined into a single coastal bioregion (COAST). The location of CSIRO Black Mountain Laboratories (S 35.27', E 149.12') where the glasshouse experiment was conducted is indicated.

doi:10.1371/journal.pone.0049000.g001
### Table 1. Environmental characteristics of the six bioregions from the Interim Biogeographic Regionalisation for Australia Framework (IBRA) sampled within the study region.

| Bioregion | Elevation (m) | Precipitation (mm) | Tmax (°C) | Tmin (°C) | Tav (°C) | PET (mm) | AWB (mm) | Aridity Index | pH (CaCl₂) | Nitrogen % | Carbon % |
|-----------|---------------|--------------------|-----------|-----------|----------|----------|---------|--------------|------------|------------|----------|
| BHC       | 366           | 109                | 13.6      | 9.7       | 11.6     | 395.6    | 348.6   | 0.93         | 8.1        | 0.21       | 8.1      |
| MDD       | 109           | 202                | 14.1      | 7.1       | 11.1     | 395.6    | 348.6   | 0.93         | 8.1        | 0.21       | 8.1      |
| RIV       | 156           | 273                | 17.1      | 11.1      | 14.1     | 395.6    | 348.6   | 0.93         | 8.1        | 0.21       | 8.1      |
| NSS       | 353           | 383                | 13.5      | 8.1       | 10.7     | 395.6    | 348.6   | 0.93         | 8.1        | 0.21       | 8.1      |
| SEH       | 882           | 358                | 18.9      | 13.0      | 16.0     | 395.6    | 348.6   | 0.93         | 8.1        | 0.21       | 8.1      |
| COAST     | 178           | 358                | 18.9      | 13.0      | 16.0     | 395.6    | 348.6   | 0.93         | 8.1        | 0.21       | 8.1      |

1BHC = Broken Hill Complex; MDD = Murray Darling Depression; RIV = Riverina, NSS = NSW South-Western Slopes, SEH = South-Eastern Highlands, COAST = combined Sydney Basin and South-East Corner bioregions. Bioregion means are averages of site-level data within each bioregion.

Mean climatic data (1910–2010) were determined for the May to October growing season (see methods).

Tmax = maximum temperature, Tmin = minimum temperature, Tav = average temperature, PET = potential evapotranspiration, atmospheric water balance (AWB) = precipitation – PET [64], Aridity Index = precipitation/PET [65].

Seed observations and measurements were recorded for all plants that flowered throughout the entire study period. Two measurements were recorded for each plant: seed mass (g) and the number of seeds produced. Seed mass was measured using a Cahn electrobalance (Model: 1600, Cahn Electronics, USA) to an accuracy of ±0.01 mg. Once fully mature, the seed mass (g) per plant was recorded using a Cahn electrobalance (Model: 1600, Cahn Electronics, USA) to an accuracy of ±0.01 mg. The number of seeds produced per plant was recorded as a count. The relationship between seed mass and number of seeds produced was determined for each site using the linear regression equation.

Statistical analyses

The primary data set consisted of mean seed mass for 189 seed-producing plants across 34 study sites (the remainder either died, produced no flowers, or produced no viable seeds). We constructed the final data set for analysis by removing data for the parental plants (n = 45) that produced fewer than three seeds since in most cases the seeds produced were very small due to early abortion or senescence of the fertilised flowers. For one site we retained data

24 hours. Plants were changed at night when the bees returned to their hive to reduce the risk of cross pollination between sites. All plants were then moved back to the glasshouse (which was also insect free) to complete their development. Seeds were collected from all plants after five weeks following seed maturation and placed into paper bags for storage at room temperature. Seed production was only observed in flowers that were exposed to bee pollination.

Seed measurements

Seed mass per individual plant was determined as the weight of ten viable seeds dried for one week at a temperature of 80°C, expressed as seed weight (g) per 100 seeds. Seed viability was determined by visual inspection and lightly pressing on either side of the seed with forceps. Experience with germination of field collected seeds indicated that seeds were viable if the seed coat did not crack or deform under light pressure (i.e., the seed was filled)
from a single plant that produced two healthy, viable seeds because it was the only datum available for that site. The final data set thus consisted of mean seed mass for 144 seed-producing plants. While we report the results of analyses conducted on the final data set, because the presence of small or aborted seeds may reflect varying levels of self incompatibility or inbreeding depression [66], we conducted all analyses on both data sets. This decision had no impact on interpretation of the results, although the exclusion of smaller seeds did slightly reduce among-site variation in seed mass.

Linear mixed model analysis was used to relate seed mass to bioregion (fixed predictor variable), site within bioregion (random) and block (fixed). Effects of fixed variables were tested using standard F-tests and the effect of the random variable was tested using the Wald Z test [67]. The final seed mass data set was square-root transformed according to $y = \sqrt{x}$ to meet model assumptions. Post-hoc tests were performed on bioregion means using the Tukey-Kramer adjustment for multiple testing [68].

We also quantified the direct relationships between seed mass and specific environmental characteristics of maternal field site using linear regression. We first used principal component analysis (PCA) to reduce the ten correlated environmental variables describing the sites (six climatic, four soil variables; see Table 2) to two components that, combined, accounted for 82% of the total variance. The first component (PC1) accounted for 58% of the variation in the data and was strongly associated with climatic variables (Table 2). Scores on PC1 decrease from the arid (BHC) to mesic (SEH) bioregions, with coastal, slopes and Riverina regions having intermediate scores (Fig. 3a). The second component (PC2) accounted for 24% of the variation and primarily reflected site-level soil characteristics (Table 2). PC2 primarily distinguished between coastal and highland bioregions (Fig. 3b), with low-elevation coastal areas having higher soil fertility (Table 2). The relationship between soil pH and PC1 (Table 2; Fig. 3a) is indicative of the general tendency for soil acidity to increase from the western to eastern parts of the study area [69], although soil pH also loaded on PC2.

Both PC1 and PC2 were related to mean site-level seed mass using linear regression analysis. Finally, we used individual linear regression analyses [67,70] to directly assess the impact of latitude and longitude on mean site-level seed mass since these relationships have been previously assessed for a number of species in Australia (e.g., [71]). Mean growing season (May–October) precipitation, temperature, and aridity index were also used in individual regression analyses in order to directly determine the relationships between these variables and seed mass. No data transformations were required for the regression analyses.

We next tested whether site-level seed mass variance differed across bioregions. First, we performed Levene’s test of homogeneity on non-transformed data to determine whether variance among populations differed among bioregions. We then performed a one-way analysis of variance to determine whether mean among population seed mass variance differed across bioregions. Data from two sites were excluded because plants produced insufficient seeds to determine variance. Finally, we used simple linear regression to determine whether among population seed mass variance was related to longitude, latitude and scores on PC1 and PC2. All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Carey, NC, USA).

Results

Seed mass varied significantly among bioregions ($F_{5,28} = 3.42$, $P = 0.02$, Fig. 4a,b), but not among sites within bioregion (WALD $Z = 1.20$, $P = 0.12$). Seed mass did not vary significantly across blocks ($F_{4,106} = 0.77$, $P = 0.54$). There was an overall pattern for seeds sourced from populations found in drier bioregions (especially the Broken Hill Complex and Murray Darling

![Figure 2. Climatological data for the May–October growing season for bioregions in the study area.](http://www.longpaddock.qld.gov.au/silo/). (a) Total precipitation (mm). (b) Mean temperature ($^\circ$C). (c) Aridity Index. Abbreviations denote the individual bioregions, See Fig. 1 for full names. Bioregions are arranged along the x axis from most westerly (BHC) to most easterly (COAST). doi:10.1371/journal.pone.0049000.g002}
at the population level, seed mass was significantly related to climatic and soil-related factors, indicating that climate and soil conditions play an important role in seed mass variation. Specifically, seed mass decreases with increasing latitude and longitude, as well as with increased aridity (as measured by the aridity index). Additionally, soil pH and soil electrical conductivity also negatively affect seed mass.

**Table 2. Component loadings of ten variables based on principal components analysis of site-level climatic and soil data.**

| Variable                  | PC 1²  | PC 2²  |
|---------------------------|--------|--------|
| Atmospheric water balance | –0.972 | 0.047  |
| Potential evapotranspiration | 0.957 | 0.065  |
| Precipitation             | –0.931 | 0.175  |
| Maximum temperature       | 0.924  | 0.290  |
| Average temperature       | 0.923  | 0.317  |
| Minimum temperature       | 0.895  | 0.345  |
| Soil pH                   | 0.763  | –0.406 |
| Soil nitrogen             | –0.070 | 0.957  |
| Soil carbon               | 0.062  | 0.891  |
| Soil electrical conductivity | 0.100 | 0.474  |

Climatic data for the May to October growing season (1910–2010) (see methods). Potential evapotranspiration (PET); atmospheric water balance (AWB) = precipitation - PET.

Component loadings above 0.400 or below –0.400 are in bold.

**Discussion**

The results of this study support the hypothesis that relatively recent climatic adaptation has resulted in the development of a cline in *Echium plantagineum* seed mass in arid SE Australia. Our data indicate that *E. plantagineum* seed mass declines by around 25% across the 1,000 km west-east gradient spanning the study area, with smaller seeds being produced by populations from cooler, wetter environments (e.g., COAST bioregion) than warmer, drier environments (e.g., Broken Hill Complex bioregion). Seed mass correspondingly declines by close to 2.5% (on average) per degree of longitude, and broadly increases with site aridity,
which is highest in the west of the study region. We also found that seed mass declines with increasing latitude, although the strength of the association is lower than for longitude (Fig. 5b,c).

These data suggest that selection pressure, associated with aridity, has acted on invasive populations of *E. plantagineum* to increase seed size in arid relative to mesic habitats. This supports the general argument that seed mass plays an important role in maintaining population fitness in arid-adapted species [35,39], and that seed-related traits have the capacity to undergo evolutionary shifts that increase the invasive potential of newly introduced plant populations [45]. Adaptive variation in seed mass in response to broad environmental gradients has been well documented [40–45,47]; but this study is one of the first to document such a change in an invasive species (see [46,48,49] for other examples).

Interestingly, the decline in seed mass of 2.5% per degree of longitude and 4% per degree of latitude in *E. plantagineum* is remarkably similar to that reported by [71] for native perennial *Glycine* species in Australia. They attributed the presence of larger seeds in inland areas and at low latitudes to the increased metabolic costs of high temperature and increase in availability of...
photosynthate in these environments [40], which is a plausible explanation for existence of the same pattern in *E. plantagineum*. The high degree of spatial convergence of *Echium* and *Glycine* strongly supports the view that, in Australia, both native and exotic species experience similar climatic selection pressures and have the capacity to develop adaptive clinal variation in seed mass in response. However, while seed mass evolution is likely to have increased the overall distribution, abundance and fitness of *E. plantagineum*, other factors, such as overall seed size [45], broad differences in other life history traits, or the effects of disease [34] are also likely to play a key role in determining the performance of this and other invasive species relative to sympatric native species.

We also hypothesized that variation in seed mass should decrease in populations subjected to the strongest directional selection pressure (see [29]), which in this case is more arid areas where seed mass is known to be a key determinant of fitness [35,39]. However, in contrast to mean seed mass, we did not find a clear pattern in seed mass variation across the study region. Variation in seed mass among plants at the individual site scale was unrelated to longitude, latitude, or variation in climate and soil (i.e., PC1), and did not differ across bioregions (Fig. 4c). This is consistent with other studies that have shown *E. plantagineum* populations in Australia to be extremely genetically diverse, with geographically isolated peripheral populations as diverse as those located in core habitat [50,72]. It is worth noting, however, that the magnitude of variation across different plants within each study site was relatively low (coefficients of variation mainly in the 10–30% range; data not shown), and could reflect experimental error. Another possibility is that other processes, including gene flow and resulting migration-selection balance [30,31,73], genetic drift in peripheral populations with small effective population sizes [74], increased local genetic diversity arising from genotypic admixture [30] or “genetic rescue” [31] could have resulted in the lack of clinal development in variation for seed mass.

Variation among sites in seed mass also did not differ among bioregions, but there was a suggestion that sites had greater similarity in mean seed size (i.e., lower variance) at either ends of the cline than in the centre (Fig. 4a). This effect was more evident in sites means determined from the full data set. The overall relationship between seed mass variance and clinal location (e.g., longitude) appears to be curvilinear, with a peak in the NSS bioregion, and if true, could reflect the effects of directional selection operating more strongly on heritable genetic variation in marginal than in core populations. Indeed, the observed pattern differs from that predicted under simple genetic drift, i.e., greater differentiation among small, isolated (peripheral), populations than larger, more interconnected (core) populations [8]. However, the reduced variance observed in marginal populations could also reflect the generally lower levels of climatic variation across survey sites in the most westerly and easterly bioregions (Broken Hill Complex and COAST bioregions respectively) compared with those towards the centre of *E. plantagineum*’s distribution. Clearly, further data are required to resolve whether convergence in seed mass among range-edge populations has occurred as a result of selection, other demographic or evolutionary processes, or simply as a sampling artefact.

From an evolutionary standpoint, the development of clinal population-level variation in fitness-related traits observed in invasive species both here and globally (e.g., [11,12,16,22,36,75,76]; although see [25] for a contrasting example) can arise via several different mechanisms. Clinal differentiation can occur via adaptive radiation, the evolution of diversity within a rapidly expanding lineage or during range expansion as a restricted number of founder genotypes incrementally diverge over time [9,16]. This process can be facilitated by an admixture of populations sourced from different parts of the native range and generations of novel genotypes for selection to act upon [11,16,34]. Alternatively, a broad base of genotypes may be introduced across the species range, mean trait shifts occurring as a result of selective filtering of pre-adapted or climatically matched genotypes [9,16]. While the latter process is sometimes not seen as adaptive evolution [9], it does still involve incremental improvement in population fitness via natural selection of phenotypes with heritable traits, which is a condition necessary for evolutionary change [77].

In *E. plantagineum*, both processes have probably taken place, as is the case in invasions more generally [16]. Early introductions of *E. plantagineum* are thought to have come from a variety of areas in the native range, including England, Morocco and France, with multiple introductions occurring across eastern Australia in the mid- to late 1800’s [51]. Between 1910 and 2010 populations expanded and merged (Fig. 1a), and during this time significant mixing of genotypes has been likely. Populations in Australia are extremely genetically diverse, recombinants are ubiquitous [50], and overall levels of genetic diversity are similar to that observed in the native range [72]. The breeding system of *E. plantagineum* has also diverged in Australian and native range populations [66] with Australian populations being self compatible and able to outcross [50]. These lines of evidence suggest that the clinal development observed in *E. plantagineum* in this study can be at least in part be explained by evolutionary adaptive radiation and not simply by fitness optimisation of populations via filtering of pre-adapted genotypes.

The rate at which adaptive variation in seed mass exhibited by *E. plantagineum* in Australia has developed is noteworthy. Mean differences in seed mass of around 25% have occurred in, at the very most, 150 generations, which is towards the lower end of the evolutionary rates observed, in traits related to invasiveness, elsewhere [5,6,9,10,16,78-81]. Despite this short timeframe, geographic patterns in seed mass observed in *E. plantagineum* have apparently converged with those observed in other native Australian forbs.

The results of this study have significant long-term management implications for *E. plantagineum* and other invasive species globally. Predicted global climate change is expected to favour species that have the capacity to rapidly adapt to new conditions [36], and these are the same characteristics that facilitate the invasion of new environments [82]. Our study supports the view that the fitness and range potential of invasive species can rapidly increase as a result of genetic divergence of populations along broad climatic and geographic gradients, and that selection for seed mass can play in important role in this process.

**Acknowledgments**

We thank David Marshall for technical assistance, Carol Elliott for assistance with hand pollination techniques, and Michael Konarzewski and Claire Morgan for giving generously of their time to help with fieldwork. We also thank four anonymous reviewers for helpful comments on earlier versions of the manuscript.

**Author Contributions**

Conceived and designed the experiments: TK RG BM. Performed the experiments: TK. Analyzed the data: TK RG. Contributed reagents/materials/analysis tools: TK RG. Wrote the paper: TK RG BM. Collected seed from Echium populations TK RG.
References

1. Barrett SC, Colautti RI, Eckert G (2008) Plant reproductive systems and evolution during biological invasion. Molecular Ecology 17: 373–383.

2. Monty A, Lebeau J, Meerts P, Mahy G (2009) An explicit test for the contribution of environmental maternal effects to rapid clinal differentiation in an invasive plant. Journal of Evolutionary Biology 22: 5: 917–926.

3. Arim M, Abades SR, Neil PE, Lima M, Marquet PA (2006) Spread dynamics of invasive plants. Proceedings of the National Academy of Sciences of the United States of America 103: 374–378.

4. Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecology Letters 7: 1225–1241.

5. Ridley CE, Ellstrand NC (2010) Rapid evolution of morphology and adaptive life history in the invasive California wild radish (Raphanus sativus) and the implications for management. Evolutionary Applications 3: 1: 64–76.

6. Weber E, Schmid B (1998) Latitudinal population differentiation in two species of Solado (Asteraceae) introduced into Europe. American Journal of Botany 85: 11: 1010–1021.

7. Rezzuk DN, Ghalmur CB (2001) The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. Genetic 112: 113: 103–196.

8. Lee CE (2002) Evolutionary genetics of invasive species. Trends in Ecology and Evolution 17: 306–391.

9. Moron JL, Vila M, Bonmarre R, Elmdorf B, Beardle P (2004) Rapid evolution of an invasive plant. Ecological Monographs 74: 1: 261–280.

10. Leger EA, Rice KJ (2007) Assessing the speed and predictability of local adaptation in an invasive ornamental plant. Ecology Letters 10: 701–709.

11. Etterson JR, Delf DE, Craig TP, Ando Y, Ohgushi T (2008) Parallel patterns of clonal variation in Solado altissimo in its native range in central USA and its invasive range in Japan. Botony 86: 1: 91–97.

12. Monty A, Mahy G (2009) Clinical differentiation during invasion: Senecio inaequidens (Asteraceae) along altitudinal gradients in Europe. Population Ecology 159: 305–315.

13. Snawdon RW, Davies TM (1982) Rapid divergence of plant populations in response to recent changes in soil conditions. Evolution 36: 289–297.

14. Linkhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. Annual Review of Ecology and Systematics 27: 237–277.

15. Mealor BA, Hild AL (2007) Post-invasion evolution of native plant populations: a test of biological resilience. Oikos 116: 1493–1500.

16. Montague JL, Barrett SCH, Eckert CG (2003) Re-establishment of clinal variation in flowering time among introduced populations of purple loosestrife (Lythrum salicaria, Lythraceae). Journal of Evolutionary Biology 21: 1: 234–245.

17. Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics 13: 115–155.

18. Schlichting CD (1986) The evolution of phenotypic plasticity in plants. Annual Review of Ecology, Evolution and Systematics 17: 667–69.

19. DeWitt TJ, Sih A, Wilson DS (1992) Costs and limits of phenotypic plasticity. Journal of Evolutionary Biology 21: 1: 234–245.

20. Hollis JD, Sih A, Lively CM (1983) Polymorphism of natural populations of Panus halophilus Mill. in Morocco as revealed by morphological characters. Euphytica 119: 309–316.

21. Moles AT, Ackerly DD, Wilson DS (1998) Evidence of climatic niche shift during biological invasion. Ecology 79: 1110–1121.

22. Leishman MR, Westoby M (1994) The role of seed size in seedling establishment in dry soil conditions - Experimental evidence from a semi-arid species. Journal of Ecology 82: 249–258.

23. Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. Annual Review of Ecology and Systematics 27: 237–277.

24. Leger EA, Rice KJ (2007) Assessing the speed and predictability of local adaptation in an invasive ornamental plant. Ecology Letters 10: 701–709.

25. Rayment GE, Higginson FR (1992) Australian laboratory handbook of soils and water chemical methods. Inkata Press.

26. Leishman MR, Wright JF, Moles AT, Westoby M (2000) The evolutionary ecology of seed size. In: Fenner M, editor. Seeds: The Ecology of Regeneration in Plant Communities. Wallingford, UK: 31–57.

27. Murray BR, Brown AHD, Dickson CR, Crowther MS (2004) Geographical gradients in seed mass in relation to climate. Journal of Biogeography 31: 379–387.

28. Aizen MA, Woodcock H (1992) Latitudinal trends in acorn size in eastern North American species of Quercus. Canadian Journal of Botany 70: 1218–1222.

29. Witon AA, Gross KL (1993) Latitudinal variation in seed weight and flower number in Prunella vulgaris. Oecologia 93: 53–62.

30. Boulić A, Bausta M, Hirsch G (2002) Polymorphism of natural populations of Echium plantagineum L. in its native range. Botany 86: 1: 91–97.

31. Vaughton G, Ramsey M (1998) Sources and consequences of seed mass variation in Banksia marginata (Proteaceae). Journal of Ecology 86: 4: 563–573.

32. Brown JH, Valone TJ, Kachman SD (2000) Constraints of seed size on seedling establishment. Invasions 1: 105–135.

33. Mason RAB, Cooke JJ, Moles AT, Leishman MR (2008) Reproductive output of invasive versus native plants. Global Ecology and Biogeography 17: 633–640.

34. VanDonk L, Abadi D, Friedle D, Looman HWE, Verheyen K, et al. (2005) Expansion of invasive species: is there a characteristic seed size? Oecologia 144: 1–11.

35. Leishman MR, Westoby M (1994) The role of seed size in seedling establishment in dry soil conditions - Experimental evidence from a semi-arid species. Journal of Ecology 82: 249–258.

36. Morris RAB, Cooke JJ, Moles AT, Leishman MR (2008) Reproductive output of invasive versus native plants. Global Ecology and Biogeography 17: 633–640.

37. Vaughton G, Ramsey M (1998) Sources and consequences of seed mass variation in Banksia marginata (Proteaceae). Journal of Ecology 86: 4: 563–573.

38. Guo Q, Brown JH, Valone TJ, Kachman SD (2000) Constraints of seed size on seedling establishment. Invasions 1: 105–135.

39. Leishman MR, Westoby M (1994) The role of seed size in seedling establishment in dry soil conditions - Experimental evidence from a semi-arid species. Journal of Ecology 82: 249–258.

40. Morris RAB, Cooke JJ, Moles AT, Leishman MR (2008) Reproductive output of invasive versus native plants. Global Ecology and Biogeography 17: 633–640.

41. Aizen MA, Woodcock H (1992) Latitudinal trends in acorn size in eastern North American species of Quercus. Canadian Journal of Botany 70: 1218–1222.
64. Rasmusson EM (1968) Atmospheric water vapor transport and the water balance of north America 11. Large-scale water balance investigations. Monthly Weather Review 96: 720–734.
65. Lioubimtseva E, Adams JM (2004) Possible implications of increased carbon dioxide levels and climate change for desert ecosystems. Environmental Management 33: 4: 5389–5404.
66. Petanidou T, Godfree RC, Song DS, Kantza A, Dupont YL, et al. (2011) Self-compatibility and plant invasiveness: Comparing species in native an invasive ranges. Perspectives in Plant Ecology, Evolution and Systematics 14: 1: 3–12.
67. Dytham C (2011) Choosing and Using Statistics: A Biologist’s Guide. Wiley-Blackwell. West Sussex, UK.
68. Tukey JW (1953) The problem of multiple comparisons. New Jersey: Department of Statistics, Princeton University.
69. Scott BJ, Radley AM, Conyers MK (2000). Management of soil acidity in long-term pastures of south-eastern Australia: a review. Australian Journal of Experimental Agriculture 40: 1173–1198.
70. Sokal RR, Rohlf FJ (1995) Introduction to Biostatistics 2nd ed. Dover publications, Mineola, New York.
71. Murray BR, Brown AHD, Grace JP (2003) Geographic gradients in seed size among and within perennial Australian Glycine species. Australian Journal of Botany 51: 47–57.
72. Burdon JJ, Brown AHD (1986) Population genetics of Echium plantagineum L., target weed for biological control. Australian Journal of Biological Sciences 39: 369–378.
73. Phillips PC (1996) Maintenance of polygenic variation via a migration-selection balance under uniform selection. Evolution 50: 1334–1339.
74. Vucetich JA, Waite TA (2003) Spatial patterns of demography and genetic processes across the species’ range: Null hypotheses for landscape conservation genetics. Conservation Genetics 4: 639–645.
75. Maron JL, Elmendorf S, Vila M (2007) Contrasting plant physiological adaptation to climate in the native and introduced range of Hypericum perforatum. Evolution 61: 1912–1924.
76. Ettersson JR (2004) Evolutionary potential of Chamaecrista fasciculata in relation to climate change. I. Clinal patterns of selection along an environmental gradient in the great plains. Evolution 58: 1446–1450.
77. Hoffinan AA, Merila J (1999) Heritable variation and evolution under favourable and unfavourable conditions. Trends in Ecology and Evolution 14: 96–101.
78. Franks SJ, Sim S, Weis AE (2007) Rapid evolution of flowering time by an annual plant in response to climate fluctuation. Proceedings of the National Academy of Science 104: 4: 1278–1282.
79. Friedman JM, Roelle JE, Gaskin JF, Pepper AE, Manhar JR (2008) Latitudinal variation in cold hardiness in introduced Tamarix and native Populus. Evolutionary Applications 1: 589–607.
80. Whitney KD, Gabler CA (2008) Rapid evolution in introduced species, ‘invasive traits’ and recipient communities: challenging for predicting invasive potential. Diversity and Distributions 14: 569–580.
81. Thompson JN (1998) Rapid evolution as an ecological process. Trends in Ecology and Evolution 13: 329–332.
82. Bone E, Farres A (2001) Trends and rates of microevolution in plants. Genetica 112–113: 165–182.