Rapid evolution of leaf physiology in an introduced beach daisy.

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Abstract

Photosynthesis is a key biological process. However, we know little about whether plants change their photosynthetic strategy when introduced to a new range. We located the most likely source population for the South African beach daisy *Arctotheca populifolia* introduced to Australia in the 1930s, and ran a common-garden experiment measuring ten physiological and morphological leaf traits associated with photosynthesis. Based on predictions from theory, and higher rainfall in the introduced range, we hypothesised that introduced plants would have a (i) higher photosynthetic rate, (ii) lower water-use efficiency and (iii) higher nitrogen-use efficiency. However, we found that introduced *A. populifolia* had a lower photosynthetic rate, higher water use efficiency and lower nitrogen-use efficiency than did plants from Arniston, South Africa. Subsequent site visits suggested that plants in Arniston may be able to access moisture on a rocky shelf, while introduced plants grow on sandy beaches where water can quickly dissipate. Our unexpected findings highlight that: (1) it is important to compare introduced species to their source population for an accurate assessment of evolutionary change; (2) rainfall is not always a suitable proxy for water availability; and (3) introduced species often undergo evolutionary changes, but without detailed ecological information we may not be able to accurately predict the direction of these changes.

Key-words

Introduced species, nitrogen-use efficiency, photosynthesis, rapid evolution, source population, water-use efficiency.
Introduction

Thousands of plant species have been introduced to new ranges and have become naturalised in these areas over the last few hundred years [1]. We now know that introduced plants can evolve in many aspects of their morphology [2, 3], and that this can occur quite commonly [4]. However, much less work has focused on the evolution of physiological changes in introduced plants. For example, carbon assimilation by plants through photosynthesis is one of the most fundamental processes in the natural world, and yet we have scant knowledge of whether photosynthetic capacity in introduced plants can evolve. Comparing plants grown from seeds collected in 1994 to plants grown from seeds collected in 2005 showed that introduced Polygonum cespitosum evolved higher photosynthetic rates over 11 generations [5]. This ‘resurrection’ approach allows researchers to accurately quantify capture recent change in populations that are already established, but it does not allow us to quantify the full extent of change resulting from the introduction of populations in a new range and the selective processes they undergo during early establishment. A global study showed that invasive plant species had higher photosynthetic assimilation rates than co-occurring native species [6]. However, the same group did not find differences in assimilation rates within species measured in both their native and introduced ranges, though there were shifts to faster growth strategies overall for the invasive species studied [7].

In situ measurements, while assessing plant responses in the field, do not allow environmental effects to be separated from any physiological evolution associated with plant adaptation to a new habitat. Excluding environmental effects requires common-garden experiments and such experiments have yielded varying results, including both higher photosynthetic assimilation rates in introduced populations [8, 9] and no difference in photosynthetic assimilation rates between introduced and native populations [10, 11]. None of the surprisingly few common-garden studies comparing the photosynthetic assimilation rate of native and introduced populations of the same species use the
actual source population of the introduced populations (likely because most introduced species have large home ranges and/or multiple introductions, making it very difficult to locate the source population). Using the source population as a control could be critical for the most accurate assessment of what evolutionary changes have taken place since introduction, and may explain findings where responses differ between species [12].

Basing comparisons on plants from various populations in the native range introduces intra-specific variation that may obscure differences between native and introduced plants [12]. For example, two studies on the Mediterranean lineage of common reed (*Phragmites australis*) show different results for maximum photosynthetic assimilation rate ($A_{\text{max}}$) depending on which groups of native and introduced populations were sampled. One study [13] comparing six native populations with six introduced populations found no differences in $A_{\text{max}}$ between native and introduced *P. australis*. Another study [14] comparing four of those six native populations with two of the same and two different introduced populations to [13] found lower $A_{\text{max}}$ in introduced *P. australis*. Genetic techniques can allow the original source population of the introduced plants to be located, and thus offer an opportunity for the most accurate assessment of change since introduction.

In previous work, we compared microsatellite data from 10 populations spanning the entire native range of the beach daisy *Arctotheca populifolia* (which has a narrow distribution spanning ~ 2200km along the coast of South Africa [15]) with data from four populations spanning the introduced range of *A. populifolia* in eastern Australia [16]. We do not know the number of individuals introduced to Australia, but the fact that the eastern Australian *A. populifolia* have only 1% of the genetic diversity of *A. populifolia* in the native range is consistent with the east Australian populations deriving from a small number of founding individuals [16]. There are no native Australian *Arctotheca* with which *A. populifolia* could have hybridised after arrival [17]. Molecular data show that there was a separate introduction of *A. populifolia* to western and southern Australia [16], but these plants are spatially


separated and genetically distinct from the eastern Australian invasion. STRUCTURE analysis, principal component analysis, and RST all showed that the population of *A. populifolia* from the beach in Arniston (South Africa) was the most closely related to the east Australian populations of the ten South African populations available for analysis [12, 16]. Arniston was also the only one of these ten South African populations that included all of the rare alleles found in the east Australian plants [12]. Finally, log odds analyses indicated that Arniston was >10⁹⁹ times as likely to be the source population than any of the other nine South African populations available for comparison [12]. It is impossible to rule out the possibility that the east Australian populations of *A. populifolia* derived from an unmeasured South African population. However, it seems very likely that Arniston, or a population very similar to it, and therefore likely from the same part of the range, was the source population.

We set up a common-garden glasshouse experiment, and measured ten physiological and morphological traits related to photosynthesis, thus providing the first study to test introduced plants for differences in photosynthetic capacity relative to plants from the most likely source population. Our hypothesis was that introduced *A. populifolia* would have a higher rate of photosynthetic assimilation than would plants from the likely source population. This prediction was based on the results of previous studies [8, 9], and the Evolution of Increased Competitive Ability hypothesis [18] which predicts increased growth in introduced populations due to a reduced allocation to defence - including evidence of increased photosynthesis in conjunction with decreased cell-wall defence (Feng et al. 2009). In addition to testing this hypothesis, we investigated mechanisms that might underlie any observed changes. Photosynthesis involves light-dependent and -independent reactions, where the first are constrained by the capacity for electron transport to support Ribulose 1,5-bisphosphate (RuBP) regeneration (*J*ₘₐₓ), and the latter by the maximum rate of carboxylation
Calculating $J_{\text{max}}$ and $V_{\text{cmax}}$ with respect to intercellular CO$_2$ concentration ($C_i$) are powerful approaches to identify factors limiting the maximum rate of photosynthetic assimilation.

Even though the Arniston, South Africa and introduced east Australian populations of *A. populifolia* grow at similar latitudes and temperatures, the introduced populations experience rainfall that is two to three times higher than that experienced by the population in Arniston, South Africa [12]. Since water relations regulate stomatal behaviour [19] and plants in wet environments tend to have low water-use efficiency compared to plants in dry environments [20, 21], our second hypothesis was that higher rainfall in the introduced range would lead to lower water-use efficiency in the introduced plants. To investigate the mechanisms underlying any observed changes, we measured stomata [22] and leaf hair densities [23], in addition to stomatal conductance and water use efficiency.

Soil nitrogen (N) is often low on beach dunes [24, 25], the habitat of *A. populifolia*. The majority of N in leaves occurs as a component of the Rubisco enzyme [26]; both $V_{\text{cmax}}$ and $J_{\text{max}}$ are positively correlated with leaf N in a range of plant functional types [26-29]. Limited soil nitrogen could therefore contribute to differences in photosynthetic capacity among populations. We hypothesise that the generally low nitrogen of the beach environment combined with our prediction of higher rates of photosynthetic assimilation in the introduced range would result in increased photosynthetic nitrogen-use efficiency (PNUE) in introduced plants. This prediction is consistent with previous studies on congeneric species which showed that introduced plants had higher PNUE than their native counterparts [30, 31].

In summary, we tested three hypotheses: That introduced populations of *A. populifolia* in Australia will have higher rates of photosynthetic assimilation (H1), lower water-use efficiency (H2) and higher photosynthetic nitrogen-use efficiency (H3) than their likely source population from Arniston, South Africa.
**Methods**

**STUDY SPECIES**

*Arctotheca populifolia* (P.J. Bergius) Norlindh (Asteraceae) is a semi-succulent, perennial herb which is native to South Africa and grows on the foredunes of sandy beaches [32]. It was introduced to eastern Australia in the 1930s and can now be found along the southern half of the Australian coastline from Geraldton in the west to Arakoon in the east [33]. *A. populifolia* plants typically produce seeds in their first year of growth, so there might have been as many as ~80 generations since the South African and east Australian populations diverged.

**COLLECTING AND GROWING THE PLANTS**

We collected seeds from Arniston, South Africa (the most likely source population), and from four introduced populations spanning 600km along the east coast of Australia (Treachery Beach, Wairo Beach, Narooma Beach and Mallacoota Beach; for co-ordinates see Table S1, Electronic Supplementary Material). At each location we collected seeds from multiple seed heads of individual plants, each representing a separate maternal line. The number of individuals collected at these locations ranged from 17 to 46 (Table S1, Electronic Supplementary Material). We then set up a common-garden experiment under controlled conditions in the glasshouse at the University of New South Wales. In order to minimise maternal effects we first used the field-collected seeds to grow and pollinate a generation of parent plants from October 2012 to November 2013 to produce standardised offspring for our experiments. The experimental plants which grew from December 2013 to November 2014 were then used for this study. Full sample size and maternal line details are provided in Table S1, Electronic Supplementary Material. Pots were placed haphazardly on glasshouse benches, with positions randomly rotated every 4-6 weeks. Plants were watered by automatic drippers at 5pm daily, and temperatures were controlled between 10-25 °C. Additional experimental details can be found in [12].
For photosynthetic measurements, we randomly selected a subset of plants from our standardised generation of 340 experimental plants. We selected 44 plants spread over the five populations, avoiding siblings in almost all cases (Table S1, Electronic Supplementary Material). We began photosynthetic measurements on 24 February 2014 when plants were approximately twelve weeks old, and took measurements on eight different days from then until 11 April 2014. Measurements were conducted with a portable infrared gas analyser (LiCor-6400XT, Lincoln, Nebraska) on well-watered, non-flowering plants. We measured the youngest fully expanded leaf, and followed standard protocols [34]. All traits were measured on a leaf-area basis in order to make comparable analyses of physiological processes. We took our measurements of photosynthesis under saturating light (photosynthetic photon flux density = 1800 μmol m⁻²s⁻¹), CO₂ = 400 p.p.m. and a leaf temperature of 25 °C. We recorded the following parameters: CO₂ assimilation rate (A_area; μmol m⁻²s⁻¹), stomatal conductance (gₛ; mmol m⁻²s⁻¹) and intercellular CO₂ concentration (Cᵢ; μmol m⁻²). Intrinsic water-use efficiency (WUE; μmol CO₂ mmol⁻¹ H₂O) was calculated as the ratio of A_area to gₛ. Mass-based assimilation rates can be obtained by multiplying area-based assimilation rates by specific leaf area (SLA). However, our previous study using the whole group of these experimental plants (n=340) found no significant difference (p=0.638) between the SLA of introduced plants (mean SLA 18.50 m².kg⁻¹) and plants from Arniston, South Africa (mean SLA 19.04 m².kg⁻¹) [12]. Therefore, while we did calculate mass-based assimilation rates (A_mass; Table S2, Electronic Supplementary Material) we do not consider these data in our study as they provide no additional information for our comparisons.

For 30 plants (a subset of the 44 plants on which photosynthesis was measured; Table S1, Electronic Supplementary Material), we ran CO₂ response curves using ten CO₂ levels (CO₂ = 40, 100, 200, 300, 400, 550, 1000, 1200, 1500, 1800 ppm) also at saturating light and 25 °C. The equations
describing photosynthesis developed by [35] were then fitted to each curve using the ‘fitaci’ function in the plantecophys package in R [36]. From these curves, we calculated the maximum rate of carboxylation ($V_{\text{cmax}}$, $\mu$mol m$^{-2}$ s$^{-1}$) and the maximum rate of electron transport ($J_{\text{max}}$, $\mu$mol m$^{-2}$ s$^{-1}$) also using the plantecophys package.

To make counts of stomata and leaf hairs we made epidermal impressions from the upper (adaxial) and lower (abaxial) surfaces of one leaf from each plant (n=44) using clear nail polish. The imprints were observed under an Olympus CX41 microscope at x100 magnification for stomata and x40 for leaf hairs, and images were captured with an attached digital camera (QImaging MicroPublisher 3.3 RTV). We viewed the images in Adobe Photoshop version 14.0 (Adobe Systems Inc., San Jose, CA, USA) and used the Eyedropper Tool to count the number of stomata and hairs present in each image. We used a stage micrometer to calculate the area of the leaf we were viewing and convert our count data to densities.

We measured leaf nitrogen using a 17 LECO TruSpec CN Analyser at the Solid State and Elemental Analysis Unit at UNSW, using one dried leaf from each of 33 plants. To convert $N_{\text{mass}}$ to $N_{\text{area}}$ we multiplied $N_{\text{mass}}$ by leaf mass per area (LMA) using leaf area and dry mass values previously recorded for those leaves [12]. Photosynthetic nitrogen use efficiency (PNUE; $\mu$mol CO$_2$ g$^{-1}$ N s$^{-1}$) was calculated as the ratio of photosynthetic assimilation rate ($A_{\text{area}}$) to leaf nitrogen ($N_{\text{area}}$).

**DATA ANALYSIS**

We compared trait values among the five populations using one-way analyses of variance (ANOVAs) with a planned contrast between the Arniston, South Africa, population and the four introduced Australian populations. To account for multiple tests, we applied a Holm-Bonferroni sequential correction [37]. This resulted in one trait changing its significance value (Table S3, Electronic Supplementary Material). Hair density on upper surface of the leaf changed from $p=0.023$
(significant) to $p=0.115$ (not significant). This has been reported as such in Results and Figures below; for all other traits the original $p$-values are reported.

Given that the Australian populations experience a range of environments across $5^\circ$ of latitude, we checked for variation in the introduced range by comparing traits among just the four Australian populations using one-way ANOVAs. After a Holm-Bonferroni sequential correction, one trait changed its significance value. Stomatal density on the bottom of the leaf changed from $p=0.013$ (significant) to $p=0.156$ (not significant). All the other traits also showed no significant differences among the four Australian populations (Table S4, Electronic Supplementary Material). We have therefore presented only the contrast between South African and Australian plants in the figures in the main manuscript. Boxplots showing the data distribution within each of the five populations can be found in Figure S2.

All analyses were performed using SPSS version 22.0 (IBM Corp, Armonk, NY, USA), and a Holm-Bonferroni sequential correction calculator (Gaetano 2013).

**Results**

Contrary to our predictions, the introduced Australian plants had a lower photosynthetic assimilation rate than did plants from Arniston in the South African home range ($A_{area}$ 13% lower, $p=0.006$, Fig. 1a). The maximum rate of carboxylation ($V_{cmax}$) was also significantly lower in the Australian plants compared to the South African plants (12% lower, $p=0.001$, Fig. 1b), but there was no difference in the maximum rate of electron transport ($J_{max}$) between the two groups ($p=0.948$, Fig. 1c), signalling no change in the photosynthetic capacity for the electron transport chain. The concentration of intercellular CO$_2$ ($C_i$) was also significantly lower in the introduced plants ($p=0.002$, Fig. 1d).
Surprisingly, the water-use efficiency of the introduced Australian plants was on average 54% greater than that of plants from the South African population (p<0.001, Fig. 2a). Counts of leaf hairs showed that on the lower surface of their leaves, the Australian plants had a remarkable 84% greater leaf hair density than the South African plants (p<0.001), while on the upper surface of their leaves, there were no significant differences (Figs 2b,e). The introduced plants had a much lower stomatal conductance (gs 41% lower, p<0.001, Fig. 2c) even though there were no differences in stomatal density between the two groups on either upper (p=0.423) or lower (p=0.483) leaf surfaces (Fig. 2d). The mean value of the stomatal ratio between the upper and lower leaf surfaces was 0.93 for both the introduced plants and the plants from Arniston, South Africa (p=0.991, Figure S1 in Electronic Supplementary Material).

The introduced Australian plants were only 73% as efficient at using nitrogen as the South African plants (p<0.002, Fig. 3a). This is due to the fact that even though there was no difference in the amount of leaf nitrogen per unit leaf area (N_area) between introduced plants and plants from Arniston, South Africa (p=0.382, Fig. 3b), the introduced plants still had a lower photosynthetic assimilation rate.

**Discussion**

Even though the beach daisy *A. populifolia* has been in Australia for less than 100 years, the introduced plants have evolved differences in leaf physiology compared to plants from the likely source population growing in Arniston, South Africa. Introduced Australian *A. populifolia* plants had higher water-use efficiency, lower stomatal conductance and higher leaf hair density, most likely due to evolution in response to natural selection following their introduction to Australia. These changes are consistent with exposure to drier conditions [19-22]. Using artificial hair removal on a population in its native range, the leaf hairs of *A. populifolia* have been shown to reduce water loss by
decreasing stomatal conductance and transpiration rates [23]. An increase in leaf hair density would therefore result in higher water-use efficiency, which is in agreement with our findings; as is the fact that the introduced plants had a much lower stomatal conductance. In addition, our previous study showed that the introduced plants have leaves that are 27% smaller than those of the plants from Arniston, South Africa [12], another change consistent with adaptation to a drier environment [38, 39]. However, the introduced populations experience a much higher rainfall than does the population from Arniston [12]. The apparent responses to drier conditions and records of higher rainfall in the introduced range seemed incongruous, until field visits revealed that the plants in Arniston grow on a rocky shelf which can trap moisture, while the introduced plants grow on sandy beaches from which rain water can quickly drain away (Fig. 4., S. Creer, pers. comm.). Thus, although we cannot be sure without empirical data from the study sites, we suspect that the higher rainfall in the introduced range does not translate into increased water availability for the plants. This finding highlights two important points. First, although precipitation has been a commonly used predictive climatic variable in plant trait ecology for many years [40, 41] it may not always provide the strongest explanatory power for observed trait patterns. For example, mean annual temperature has been shown to be a better predictor of global plant height than mean annual precipitation [42]. Second, although global climate (and other) data are an invaluable resource, it is still essential to visit study sites to obtain local information.

The fact that the introduced Australian plants have a lower photosynthetic nitrogen-use efficiency (PNUE) is not surprising once we consider the increase in water-use efficiency. Plants optimise how they obtain and use both water and nitrogen in the process of carbon assimilation [43] and there is a trade-off between these two resources. When CO\textsubscript{2} diffuses into open stomata for photosynthetic assimilation (with enzymes that require nitrogen), water is unavoidably lost due to transpiration. Plants that are able to conserve water with high water-use efficiency tend to have low photosynthetic
nitrogen-use efficiency [20, 44-46]. Differences in how introduced plants and plants from Arniston, South Africa allocate their leaf nitrogen could also contribute to the lower PNUE in the introduced plants. The bulk of leaf nitrogen is either allocated to cell walls or to photosynthetic proteins, with a trade-off occurring between these allocations [47, 48]. Plants investing more mass in cell walls can have less nitrogen to invest in photosynthetic proteins, and can also show reduced CO₂ diffusion to areas of carboxylation due to thicker mesophyll cell walls [48]. Increased allocation to cell walls might have evolved in introduced *A. populifolia* to provide the rigidity and strength needed by the mesophyll structures to maintain photosynthetic capacity in habitats with lower water availability [48]. An increase in the mass of cell walls could then lead to decreased photosynthetic nitrogen-use efficiency [47-49] as per our results. Further support for this idea is that plants with thicker mesophyll cell walls show reduced CO₂ diffusion to areas of carboxylation [42] which is also in agreement with our findings.

Finally, the introduced *A. populifolia* plants in Australia have a lower photosynthetic assimilation rate than plants from Arniston, South Africa. This finding is contrary to our predictions based on previous studies and the EICA hypothesis which predicts increased growth in introduced populations, including an increase in photosynthesis [47]. The lower photosynthetic assimilation rate occurred via a decrease in the maximum rate of carboxylation (*V*<sub>max</sub>). Since *V*<sub>max</sub> is limited by the supply of CO₂, and since CO₂ must diffuse from the air outside the leaf to the site of carboxylation inside the leaf [50], it seems likely that the lower stomatal conductance of the introduced plants is limiting the supply of CO₂ and thus contributing to their lower photosynthetic assimilation rate, shown by the lower concentration of intercellular CO₂ (*C*<sub>i</sub>) in the introduced plants (Fig. 1d). The relationship between low stomatal conductance and low photosynthetic assimilation rate is well understood [43, 51]. The artificial hair removal experiments which have previously shown that leaf
hairs in *A. populifolia* decrease intercellular CO$_2$ concentrations (Ripley, Pammenter & Smith 1999), indicate a link between the lower photosynthetic capacity and increased water use efficiency.

The east Australian and South African *A. populifolia* now differ in many ways. It seems likely that many of these differences result from evolution in response to novel selective pressures encountered by the plants in their introduced range. However, there are other possibilities. For example, because the founding population was probably very small (see above), differences could be partly due to initial random chance (founder effect). The founding individuals may also have been deliberately selected for characteristics such as being larger or more vigorous, having dune stabilising properties or having showy flowers. Some of these characteristics could be associated with the traits we have investigated; meaning that our results may have been affected by selection or introduction bias.

Unfortunately, we do not know why *A. populifolia* was introduced to eastern Australia and so we have no information as to whether any characteristics were deliberately selected for the purpose of introduction. Another possibility is that the populations might have undergone genetic drift since their introduction. Further it is possible that plants in the home range have changed since the founding individuals were introduced to Australia in the 1930s, perhaps as a result of land use change, climate change, or pressure from introduced species. We do not have the historical data required to tease these possibilities apart. However, we have some indication of the relative magnitude of change in native vs introduced populations from a study of herbarium specimens in New Zealand [4]. This study showed that 16 out of 23 (70%) introduced species had undergone significant morphological change since their arrival in New Zealand, while only one of five native New Zealand species had undergone change over the same time period.

In summary, evidence indicates that introduced *A. populifolia* plants have rapidly evolved physiological changes consistent with exposure to drier conditions. A promising future direction for this work would be to test whether these trait changes result in a direct selective advantage for *A.*
*populifolia* experiencing drier conditions in the introduced Australian range. The physiological changes we observed have simultaneously led to increased water-use efficiency, decreased photosynthetic nitrogen-use efficiency and a lower photosynthetic assimilation rate in the introduced range. This outcome is the opposite to our predictions and highlights three important points: (1) it is important to compare introduced species to their original source population for the most accurate assessment of evolutionary change; (2) rainfall may not always be a suitable proxy for water availability; and (3) introduced species often undergo evolutionary changes, but without detailed ecological information we may not be able to accurately predict the direction of these changes [52].

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**Authors’ contributions**

ATM, CRB, WBS and JC conceived the ideas and designed the methods; CRB grew the plants; JC and CRB collected the data; JC and CRB analysed the data; CRB led the writing of the manuscript. All authors contributed critically to the drafts.

**Data Accessibility**

Our raw data are available in Electronic Supplementary Material Appendix S1.
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Figures

**Fig. 1.** a) Photosynthetic assimilation rate (A<sub>area</sub>), b) maximum rate of carboxylation (V<sub>max</sub>), c) maximum rate of electron transport (J<sub>max</sub>) and d) concentration of intercellular CO<sub>2</sub> (C<sub>i</sub>) comparing source and introduced plants showing mean values (+/- standard error). The p-values for each trait are from a planned contrast between the most likely South African source population and the four Australian introduced populations following a one-way analysis of variance (ANOVA). This type of analysis takes into account the defined comparison of plants from one South African population with plants from four Australian populations. The y-axes have been truncated.
Fig. 2. a) Water-use efficiency (WUE), b) leaf hair density, c) stomatal conductance ($g_s$), and d) stomatal density comparing source and introduced plants showing mean values (+/- standard error).

The p-values for each trait are from a planned contrast between the most likely South African source population and the four Australian introduced populations following a one-way analysis of variance (ANOVA). This type of analysis takes into account the defined comparison of plants from one South African population with plants from four Australian populations. Fig. 2e) is a diagrammatic representation of the differences in leaf hair density between source and introduced plants, and Fig. 2f) shows the underside of a leaf where strips of leaf hairs have been removed with nail polish peels. The y-axes have been truncated. *After applying a Holm-Bonferroni sequential correction, there was no significant difference in leaf hair density on top of the leaves.
Fig. 3. a) Photosynthetic nitrogen-use efficiency (PNUE) and b) nitrogen per leaf area (N\textsubscript{area}) comparing source and introduced plants showing mean values (+/- standard error). The p-values for each trait are from a planned contrast between the most likely South African source population and the four Australian introduced populations following a one-way analysis of variance (ANOVA). This type of analysis takes into account the defined comparison of plants from one South African population with plants from four Australian populations. The y-axes have been truncated.

Fig. 4. The most likely source population in Arniston, South Africa (photograph on the left), where *A. populifolia* grows on a rocky shelf; an introduced population at Treachery Beach, Australia (one
of our sampled populations; photograph on the right) where *A. populifolia* grows on an exposed sandy beach. Photographs taken by S. Creer (Arniston) and C. Brandenburger (Treachery Beach).
Table S1. Latitude and longitude for the source population and the four introduced populations; sample size with maternal line information for each stage of the experiment.

Table S2. Calculations of mass-based photosynthetic assimilation rates ($A_{\text{mass}}$).

Table S3. A contrast for each trait between the South African source population and the four Australian introduced populations using one-way analyses of variance (ANOVAs) with a planned contrast.

Table S4. A contrast for each trait among only the four introduced populations in Australia using one-way analyses of variance (ANOVAs).

Figure S1. Mean ratio of stomata on the upper and lower leaf surfaces.

Figure S2. Graphs showing distribution of data within each population for all traits in Figures 1-3.

Appendix S1. Raw data.