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1 **Rapid evolution of leaf physiology in an introduced beach daisy.**

2

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10 **Abstract**

11 Photosynthesis is a key biological process. However, we know little about whether plants change
12 their photosynthetic strategy when introduced to a new range. We located the most likely source
13 population for the South African beach daisy *Arctotheca populifolia* introduced to Australia in the
14 1930s, and ran a common-garden experiment measuring ten physiological and morphological leaf
15 traits associated with photosynthesis. Based on predictions from theory, and higher rainfall in the
16 introduced range, we hypothesised that introduced plants would have a (i) higher photosynthetic rate,
17 (ii) lower water-use efficiency and (iii) higher nitrogen-use efficiency. However, we found that
18 introduced *A. populifolia* had a lower photosynthetic rate, higher water use efficiency and lower
19 nitrogen-use efficiency than did plants from Arniston, South Africa. Subsequent site visits suggested
20 that plants in Arniston may be able to access moisture on a rocky shelf, while introduced plants grow
21 on sandy beaches where water can quickly dissipate. Our unexpected findings highlight that: (1) it is
22 important to compare introduced species to their source population for an accurate assessment of
23 evolutionary change; (2) rainfall is not always a suitable proxy for water availability; and (3)
24 introduced species often undergo evolutionary changes, but without detailed ecological information
25 we may not be able to accurately predict the direction of these changes.

26 **Key-words**

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

29 **Introduction**

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

46 *In situ* measurements, while assessing plant responses in the field, do not allow environmental effects
47 to be separated from any physiological evolution associated with plant adaptation to a new habitat.
48 Excluding environmental effects requires common-garden experiments and such experiments have
49 yielded varying results, including both higher photosynthetic assimilation rates in introduced
50 populations [8, 9] and no difference in photosynthetic assimilation rates between introduced and
51 native populations [10, 11]. None of the surprisingly few common-garden studies comparing the
52 photosynthetic assimilation rate of native and introduced populations of the same species use the

53 actual source population of the introduced populations (likely because most introduced species have
54 large home ranges and/or multiple introductions, making it very difficult to locate the source
55 population). Using the source population as a control could be critical for the most accurate
56 assessment of what evolutionary changes have taken place since introduction, and may explain
57 findings where responses differ between species [12].

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

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

77 separated and genetically distinct from the eastern Australian invasion. STRUCTURE analysis,
78 principal component analysis, and RST all showed that the population of *A. populifolia* from the
79 beach in Arniston (South Africa) was the most closely related to the east Australian populations of
80 the ten South African populations available for analysis [12, 16]. Arniston was also the only one of
81 these ten South African populations that included all of the rare alleles found in the east Australian
82 plants [12]. Finally, log odds analyses indicated that Arniston was $>10^{99}$ times as likely to be the
83 source population than any of the other nine South African populations available for comparison
84 [12]. It is impossible to rule out the possibility that the east Australian populations of *A. populifolia*
85 derived from an unmeasured South African population. However, it seems very likely that Arniston,
86 or a population very similar to it, and therefore likely from the same part of the range, was the source
87 population.

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

100 (V_{cmax}). Calculating J_{max} and V_{cmax} with respect to intercellular CO_2 concentration (C_i) are powerful
101 approaches to identify factors limiting the maximum rate of photosynthetic assimilation.

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

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

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

123 **Methods**

124 STUDY SPECIES

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

131 COLLECTING AND GROWING THE PLANTS

132 We collected seeds from Arniston, South Africa (the most likely source population), and from four
133 introduced populations spanning 600km along the east coast of Australia (Treachery Beach, Wairo
134 Beach, Narooma Beach and Mallacoota Beach; for co-ordinates see Table S1, Electronic
135 Supplementary Material). At each location we collected seeds from multiple seed heads of individual
136 plants, each representing a separate maternal line. The number of individuals collected at these
137 locations ranged from 17 to 46 (Table S1, Electronic Supplementary Material). We then set up a
138 common-garden experiment under controlled conditions in the glasshouse at the University of New
139 South Wales. In order to minimise maternal effects we first used the field-collected seeds to grow
140 and pollinate a generation of parent plants from October 2012 to November 2013 to produce
141 standardised offspring for our experiments. The experimental plants which grew from December
142 2013 to November 2014 were then used for this study. Full sample size and maternal line details are
143 provided in Table S1, Electronic Supplementary Material. Pots were placed haphazardly on
144 glasshouse benches, with positions randomly rotated every 4-6 weeks. Plants were watered by
145 automatic drippers at 5pm daily, and temperatures were controlled between 10-25 °C. Additional
146 experimental details can be found in [12].

147 EXPERIMENTAL MEASUREMENTS

148 For photosynthetic measurements, we randomly selected a subset of plants from our standardised
149 generation of 340 experimental plants. We selected 44 plants spread over the five populations,
150 avoiding siblings in almost all cases (Table S1, Electronic Supplementary Material). We began
151 photosynthetic measurements on 24 February 2014 when plants were approximately twelve weeks
152 old, and took measurements on eight different days from then until 11 April 2014. Measurements
153 were conducted with a portable infrared gas analyser (LiCor-6400XT, Lincoln, Nebraska) on well-
154 watered, non-flowering plants. We measured the youngest fully expanded leaf, and followed
155 standard protocols [34]. All traits were measured on a leaf-area basis in order to make comparable
156 analyses of physiological processes. We took our measurements of photosynthesis under saturating
157 light (photosynthetic photon flux density = $1800 \mu\text{mol m}^{-2}\text{s}^{-1}$), $\text{CO}_2 = 400$ p.p.m. and a leaf
158 temperature of 25°C . We recorded the following parameters: CO_2 assimilation rate (A_{area} ; $\mu\text{mol m}^{-2}$
159 s^{-1}), stomatal conductance (g_s ; $\text{mmol m}^{-2}\text{s}^{-1}$) and intercellular CO_2 concentration (C_i ; $\mu\text{mol m}^{-2}$).
160 Intrinsic water-use efficiency (WUE; $\mu\text{mol CO}_2 \text{mmol}^{-1} \text{H}_2\text{O}$) was calculated as the ratio of A_{area} to
161 g_s . Mass-based assimilation rates can be obtained by multiplying area-based assimilation rates by
162 specific leaf area (SLA). However, our previous study using the whole group of these experimental
163 plants ($n=340$) found no significant difference ($p=0.638$) between the SLA of introduced plants
164 (mean SLA $18.50 \text{m}^2.\text{kg}^{-1}$) and plants from Arniston, South Africa (mean SLA $19.04 \text{m}^2.\text{kg}^{-1}$) [12].
165 Therefore, while we did calculate mass-based assimilation rates (A_{mass} ; Table S2, Electronic
166 Supplementary Material) we do not consider these data in our study as they provide no additional
167 information for our comparisons.

168 For 30 plants (a subset of the 44 plants on which photosynthesis was measured; Table S1, Electronic
169 Supplementary Material), we ran CO_2 response curves using ten CO_2 levels ($\text{CO}_2 = 40, 100, 200,$
170 $300, 400, 550, 1000, 1200, 1500, 1800$ ppm) also at saturating light and 25°C . The equations

171 describing photosynthesis developed by [35] were then fitted to each curve using the ‘fitaci’ function
172 in the plantecophys package in R [36]. From these curves, we calculated the maximum rate of
173 carboxylation (V_{cmax} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$) and the maximum rate of electron transport (J_{max} ; $\mu\text{mol m}^{-2} \text{s}^{-1}$)
174 also using the plantecophys package.

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

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

188 DATA ANALYSIS

189 We compared trait values among the five populations using one-way analyses of variance
190 (ANOVAs) with a planned contrast between the Arniston, South Africa, population and the four
191 introduced Australian populations. To account for multiple tests, we applied a Holm-Bonferroni
192 sequential correction [37]. This resulted in one trait changing its significance value (Table S3,
193 Electronic Supplementary Material). Hair density on upper surface of the leaf changed from $p=0.023$

194 (significant) to $p=0.115$ (not significant). This has been reported as such in Results and Figures
195 below; for all other traits the original p-values are reported.

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

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

207 **Results**

208 Contrary to our predictions, the introduced Australian plants had a lower photosynthetic assimilation
209 rate than did plants from Arniston in the South African home range (A_{area} 13% lower, $p=0.006$, Fig.
210 1a). The maximum rate of carboxylation (V_{cmax}) was also significantly lower in the Australian plants
211 compared to the South African plants (12% lower, $p=0.001$, Fig. 1b), but there was no difference in
212 the maximum rate of electron transport (J_{max}) between the two groups ($p=0.948$, Fig. 1c), signalling
213 no change in the photosynthetic capacity for the electron transport chain. The concentration of
214 intercellular CO_2 (C_i) was also significantly lower in the introduced plants ($p=0.002$, Fig. 1d).

215 Surprisingly, the water-use efficiency of the introduced Australian plants was on average 54%
216 greater than that of plants from the South African population ($p < 0.001$, Fig. 2a). Counts of leaf hairs
217 showed that on the lower surface of their leaves, the Australian plants had a remarkable 84% greater
218 leaf hair density than the South African plants ($p < 0.001$), while on the upper surface of their leaves,
219 there were no significant differences (Figs 2b,e). The introduced plants had a much lower stomatal
220 conductance (g_s 41% lower, $p < 0.001$, Fig. 2c) even though there were no differences in stomatal
221 density between the two groups on either upper ($p = 0.423$) or lower ($p = 0.483$) leaf surfaces (Fig. 2d).
222 The mean value of the stomatal ratio between the upper and lower leaf surfaces was 0.93 for both the
223 introduced plants and the plants from Arniston, South Africa ($p = 0.991$, Figure S1 in Electronic
224 Supplementary Material).

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

230 Discussion

231 Even though the beach daisy *A. populifolia* has been in Australia for less than 100 years, the
232 introduced plants have evolved differences in leaf physiology compared to plants from the likely
233 source population growing in Arniston, South Africa. Introduced Australian *A. populifolia* plants had
234 higher water-use efficiency, lower stomatal conductance and higher leaf hair density, most likely due
235 to evolution in response to natural selection following their introduction to Australia. These changes
236 are consistent with exposure to drier conditions [19-22]. Using artificial hair removal on a population
237 in its native range, the leaf hairs of *A. populifolia* have been shown to reduce water loss by

238 decreasing stomatal conductance and transpiration rates [23]. An increase in leaf hair density would
239 therefore result in higher water-use efficiency, which is in agreement with our findings; as is the fact
240 that the introduced plants had a much lower stomatal conductance. In addition, our previous study
241 showed that the introduced plants have leaves that are 27% smaller than those of the plants from
242 Arniston, South Africa [12], another change consistent with adaptation to a drier environment [38,
243 39]. However, the introduced populations experience a much higher rainfall than does the population
244 from Arniston [12]. The apparent responses to drier conditions and records of higher rainfall in the
245 introduced range seemed incongruous, until field visits revealed that the plants in Arniston grow on a
246 rocky shelf which can trap moisture, while the introduced plants grow on sandy beaches from which
247 rain water can quickly drain away (Fig. 4., S. Creer, pers. comm.). Thus, although we cannot be sure
248 without empirical data from the study sites, we suspect that the higher rainfall in the introduced
249 range does not translate into increased water availability for the plants. This finding highlights two
250 important points. First, although precipitation has been a commonly used predictive climatic variable
251 in plant trait ecology for many years [40, 41] it may not always provide the strongest explanatory
252 power for observed trait patterns. For example, mean annual temperature has been shown to be a
253 better predictor of global plant height than mean annual precipitation [42]. Second, although global
254 climate (and other) data are an invaluable resource, it is still essential to visit study sites to obtain
255 local information.

256 The fact that the introduced Australian plants have a lower photosynthetic nitrogen-use efficiency
257 (PNUE) is not surprising once we consider the increase in water-use efficiency. Plants optimise how
258 they obtain and use both water and nitrogen in the process of carbon assimilation [43] and there is a
259 trade-off between these two resources. When CO₂ diffuses into open stomata for photosynthetic
260 assimilation (with enzymes that require nitrogen), water is unavoidably lost due to transpiration.
261 Plants that are able to conserve water with high water-use efficiency tend to have low photosynthetic

262 nitrogen-use efficiency [20, 44-46]. Differences in how introduced plants and plants from Arniston,
263 South Africa allocate their leaf nitrogen could also contribute to the lower PNUE in the introduced
264 plants. The bulk of leaf nitrogen is either allocated to cell walls or to photosynthetic proteins, with a
265 trade-off occurring between these allocations [47, 48]. Plants investing more mass in cell walls can
266 have less nitrogen to invest in photosynthetic proteins, and can also show reduced CO₂ diffusion to
267 areas of carboxylation due to thicker mesophyll cell walls [48]. Increased allocation to cell walls
268 might have evolved in introduced *A. populifolia* to provide the rigidity and strength needed by the
269 mesophyll structures to maintain photosynthetic capacity in habitats with lower water availability
270 [48]. An increase in the mass of cell walls could then lead to decreased photosynthetic nitrogen-use
271 efficiency [47-49] as per our results. Further support for this idea is that plants with thicker
272 mesophyll cell walls show reduced CO₂ diffusion to areas of carboxylation [42] which is also in
273 agreement with our findings.

274 Finally, the introduced *A. populifolia* plants in Australia have a lower photosynthetic assimilation
275 rate than plants from Arniston, South Africa. This finding is contrary to our predictions based on
276 previous studies and the EICA hypothesis which predicts increased growth in introduced
277 populations, including an increase in photosynthesis [47]. The lower photosynthetic assimilation rate
278 occurred via a decrease in the maximum rate of carboxylation (V_{cmax}). Since V_{cmax} is limited by the
279 supply of CO₂, and since CO₂ must diffuse from the air outside the leaf to the site of carboxylation
280 inside the leaf [50], it seems likely that the lower stomatal conductance of the introduced plants is
281 limiting the supply of CO₂ and thus contributing to their lower photosynthetic assimilation rate,
282 shown by the lower concentration of intercellular CO₂ (C_i) in the introduced plants (Fig. 1d). The
283 relationship between low stomatal conductance and low photosynthetic assimilation rate is well
284 understood [43, 51]. The artificial hair removal experiments which have previously shown that leaf

285 hairs in *A. populifolia* decrease intercellular CO₂ concentrations (Ripley, Pammenter & Smith 1999),
286 indicate a link between the lower photosynthetic capacity and increased water use efficiency.

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

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

306 In summary, evidence indicates that introduced *A. populifolia* plants have rapidly evolved
307 physiological changes consistent with exposure to drier conditions. A promising future direction for
308 this work would be to test whether these trait changes result in a direct selective advantage for *A.*

309 *populifolia* experiencing drier conditions in the introduced Australian range. The physiological
310 changes we observed have simultaneously led to increased water-use efficiency, decreased
311 photosynthetic nitrogen-use efficiency and a lower photosynthetic assimilation rate in the introduced
312 range. This outcome is the opposite to our predictions and highlights three important points: (1) it is
313 important to compare introduced species to their original source population for the most accurate
314 assessment of evolutionary change; (2) rainfall may not always be a suitable proxy for water
315 availability; and (3) introduced species often undergo evolutionary changes, but without detailed
316 ecological information we may not be able to accurately predict the direction of these changes [52].

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323 **Authors' contributions**

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

327 **Data Accessibility**

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

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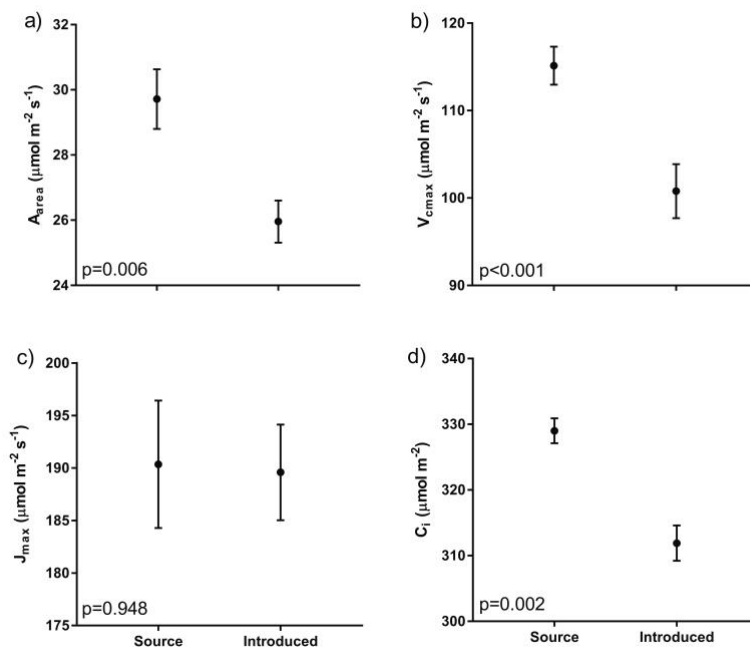
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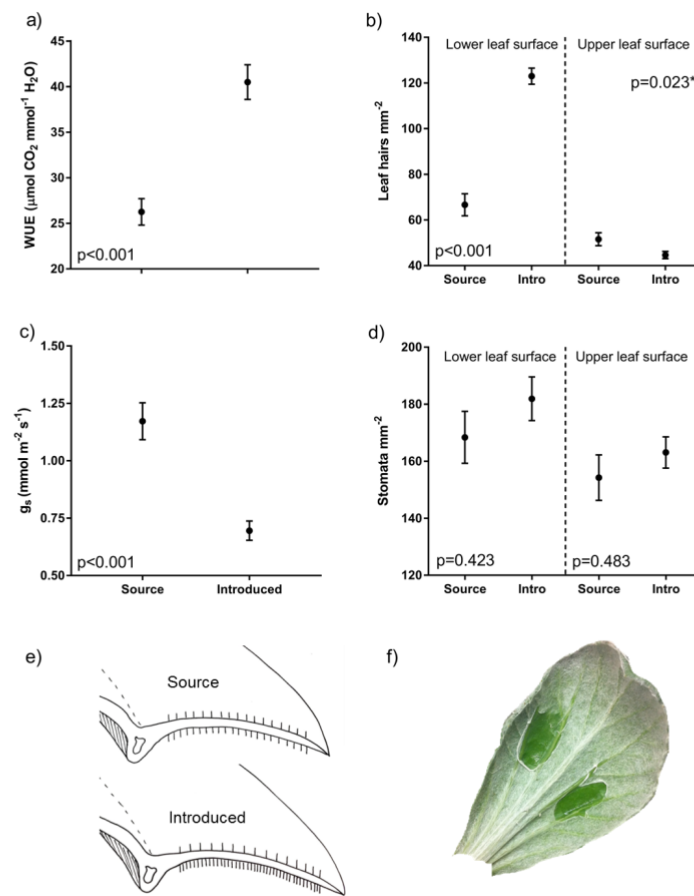
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461 **Figures**

462

463 **Fig. 1.** a) Photosynthetic assimilation rate (A_{area}), b) maximum rate of carboxylation (V_{cmax}), c)
 464 maximum rate of electron transport (J_{max}) and d) concentration of intercellular CO_2 (C_i) comparing
 465 source and introduced plants showing mean values (\pm standard error). The p-values for each trait
 466 are from a planned contrast between the most likely South African source population and the four
 467 Australian introduced populations following a one-way analysis of variance (ANOVA). This type of
 468 analysis takes into account the defined comparison of plants from one South African population with
 469 plants from four Australian populations. The y-axes have been truncated.



470

471 **Fig. 2.** a) Water-use efficiency (WUE), b) leaf hair density, c) stomatal conductance (g_s), and d)

472 stomatal density comparing source and introduced plants showing mean values (+/- standard error).

473 The p-values for each trait are from a planned contrast between the most likely South African source

474 population and the four Australian introduced populations following a one-way analysis of variance

475 (ANOVA). This type of analysis takes into account the defined comparison of plants from one South

476 African population with plants from four Australian populations. Fig. 2e) is a diagrammatic

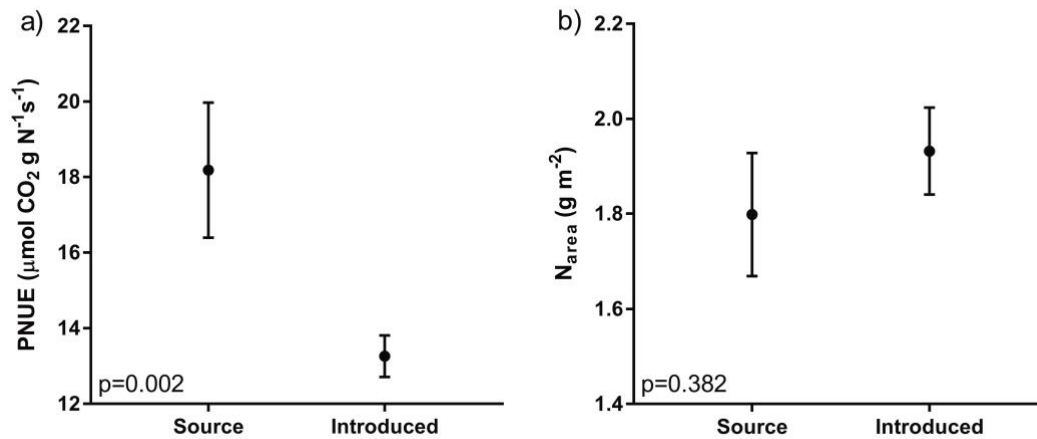
477 representation of the differences in leaf hair density between source and introduced plants, and Fig.

478 2f) shows the underside of a leaf where strips of leaf hairs have been removed with nail polish peels.

479 The y-axes have been truncated. *After applying a Holm-Bonferroni sequential correction, there was

480 no significant difference in leaf hair density on top of the leaves.

481



482

483 **Fig. 3.** a) Photosynthetic nitrogen-use efficiency (PNUE) and b) nitrogen per leaf area (N_{area})
 484 comparing source and introduced plants showing mean values (\pm standard error). The p-values for
 485 each trait are from a planned contrast between the most likely South African source population and
 486 the four Australian introduced populations following a one-way analysis of variance (ANOVA). This
 487 type of analysis takes into account the defined comparison of plants from one South African
 488 population with plants from four Australian populations. The y-axes have been truncated.



489

490 **Fig. 4.** The most likely source population in Arniston, South Africa (photograph on the left), where
 491 *A. populifolia* grows on a rocky shelf; an introduced population at Treachery Beach, Australia (one

492 of our sampled populations; photograph on the right) where *A. populifolia* grows on an exposed
493 sandy beach. Photographs taken by S. Creer (Arniston) and C. Brandenburger (Treachery Beach).

494 **Electronic Supplementary Material**

495

496 **Table S1.** Latitude and longitude for the source population and the four introduced populations;
497 sample size with maternal line information for each stage of the experiment.

498 **Table S2.** Calculations of mass-based photosynthetic assimilation rates (A_{mass}).

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

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

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

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

506

507 **Appendix S1.** Raw data.