Supplementary material

Title: Rapid evolution of leaf physiology in an introduced beach daisy.

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Table S1. Latitude and longitude for the source population and the four introduced populations; sample size (with maternal line information in brackets) for each stage of the experiment.

Table S2. Calculations of mass-based assimilation rates (A_{mass}) by using area-based assimilation rates (A_{area}) multiplied by the mean specific leaf area (SLA) values from the whole group of experimental plants.

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).

Fig. S1. Stomatal ratios (stomata count on upper surface/stomata count on lower surface) in source and introduced plants as mean values (+/- standard error).

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

Appendix S1. Raw data.

Table S1. Latitude and longitude for the most likely South African source population and the four Australian introduced populations; sample size (with maternal line information in brackets) for each stage of the experiment.

	Source population	**Introduced populations**			
	Arniston, South Africa	Treachery beach, Aus	Wairo beach, Aus	Narooma, Aus	Mallacoota, Aus
Latitude	-34.6579	-32.4468	-35.4423	-36.2238	-37.5688
Longitude	20.2329	152.5202	150.4089	150.1401	149.7621
Number of individuals sampled at each beach	46	17	38	24	45
Number of parent plants planted in the glasshouse	143	41	68	39	70
Number of parent plants producing seeds	36	20	53	26	51
Number of resulting experimental plants (number of distinct maternal lines)	123 (20)	40 (13)	68 (31)	70 (12)	39 (33)
Plants randomly selected for photosynthetic measurements (number of distinct maternal lines)	11 (9)	6 (6)	9 (9)	8 (8)	10 (10)
Subset of plants randomly selected to run CO_2 curves (number of distinct maternal lines)	8 (8)	5 (5)	6 (6)	5 (5)	6 (6)

Table S2. Calculations of mass-based assimilation rates (A_{mass}) by using area-based assimilation rates (A_{area}) multiplied by the mean specific leaf area (SLA) values from the whole group of experimental plants [1]. 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.

	A_{area} ($\mu mol\ m^{-2}\ s^{-1}$)	Mean SLA, n=340 ($m^2\ kg^{-1}$)	A_{mass} ($nmol\ g^{-1}\ s^{-1}$)
South African source plants	29.71	19.04	565.8
Australian introduced plants	25.96	18.50	480.2
p-value	p=0.006	p=0.638	p=0.001

Table S3. A contrast for each trait between the South African population and the four Australian introduced populations using one-way analyses of variance (ANOVAs) with a planned contrast. Data shown are the t statistic (t) and p-values. The adjusted p-values and resulting significance outcomes are those obtained after calculating a Holm-Bonferroni sequential correction to account for multiple tests [2, 3]. Only one trait (hair density on upper leaf surface, italicised) changed its significance.

Trait	**t**	**p-value**	**Adjusted p-values**	**Significance outcome**
Maximum rate of carboxylation (V_{cmax})	19.265	0.001	0.012	SIG
Hair density on lower leaf surface	7.809	0.001	0.012	SIG
Stomatal conductance (g_s)	-5.298	0.001	0.012	SIG
Water-use efficiency	3.975	0.001	0.012	SIG
Intercellular CO_2 (C_i)	-3.396	0.002	0.016	SIG
Photosynthetic nitrogen-use efficiency	-3.369	0.002	0.016	SIG
CO_2 assimilation rate (A_{area})	-2.918	0.006	0.036	SIG
Hair density on upper leaf surface	*-2.359*	*0.023*	*0.115*	*NON SIG*
Nitrogen per leaf area	0.888	0.382	1.000	NON SIG
Stomatal density on upper leaf surface	0.810	0.423	1.000	NON SIG
Stomatal density on lower leaf surface	0.708	0.483	1.000	NON SIG
Maximum rate of electron transport (J_{max})	-0.065	0.948	1.000	NON SIG

Table S4. A contrast for each trait among only the four introduced populations in Australia using one-way analyses of variance (ANOVAs). Data shown are the mean-square (MS), the *F* statistic (F) and p-values. The adjusted p-values and resulting significance outcomes are those obtained after computing a Holm-Bonferroni sequential correction to account for multiple tests [2, 3]. Only one trait (stomatal density on bottom of leaf, italicised) changed its significance.

Trait	**MS**	**F**	**p-value**	**Adjusted p-values**	**Significance outcome**
Stomatal density on bottom of leaf	*3338*	*4.207*	*0.013*	*0.156*	*NON SIG*
Hair density on top of leaf	192.4	2.581	0.072	0.792	NON SIG
Photosynthetic nitrogen-use efficiency	10.52	1.883	0.171	1.000	NON SIG
Nitrogen per leaf area	0.284	1.689	0.205	1.000	NON SIG
Stomatal density on top of leaf	2952	1.554	0.221	1.000	NON SIG
Maximum rate of carboxylation (V_{cmax})	119.0	0.528	0.669	1.000	NON SIG
Intercellular CO_2 (C_i)	123.3	0.489	0.692	1.000	NON SIG
Maximum rate of electron transport (J_{max})	220.6	0.446	0.723	1.000	NON SIG
Hair density on bottom of leaf	194.0	0.434	0.731	1.000	NON SIG
Water-use efficiency	42.53	0.336	0.800	1.000	NON SIG
CO_2 assimilation rate (A_{area})	4.574	0.312	0.816	1.000	NON SIG
Stomatal conductance (g_s)	0.015	0.237	0.870	1.000	NON SIG

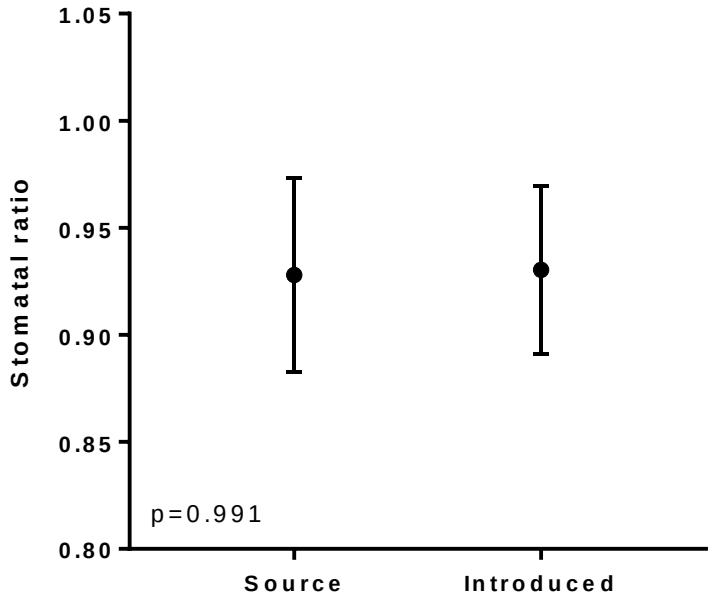
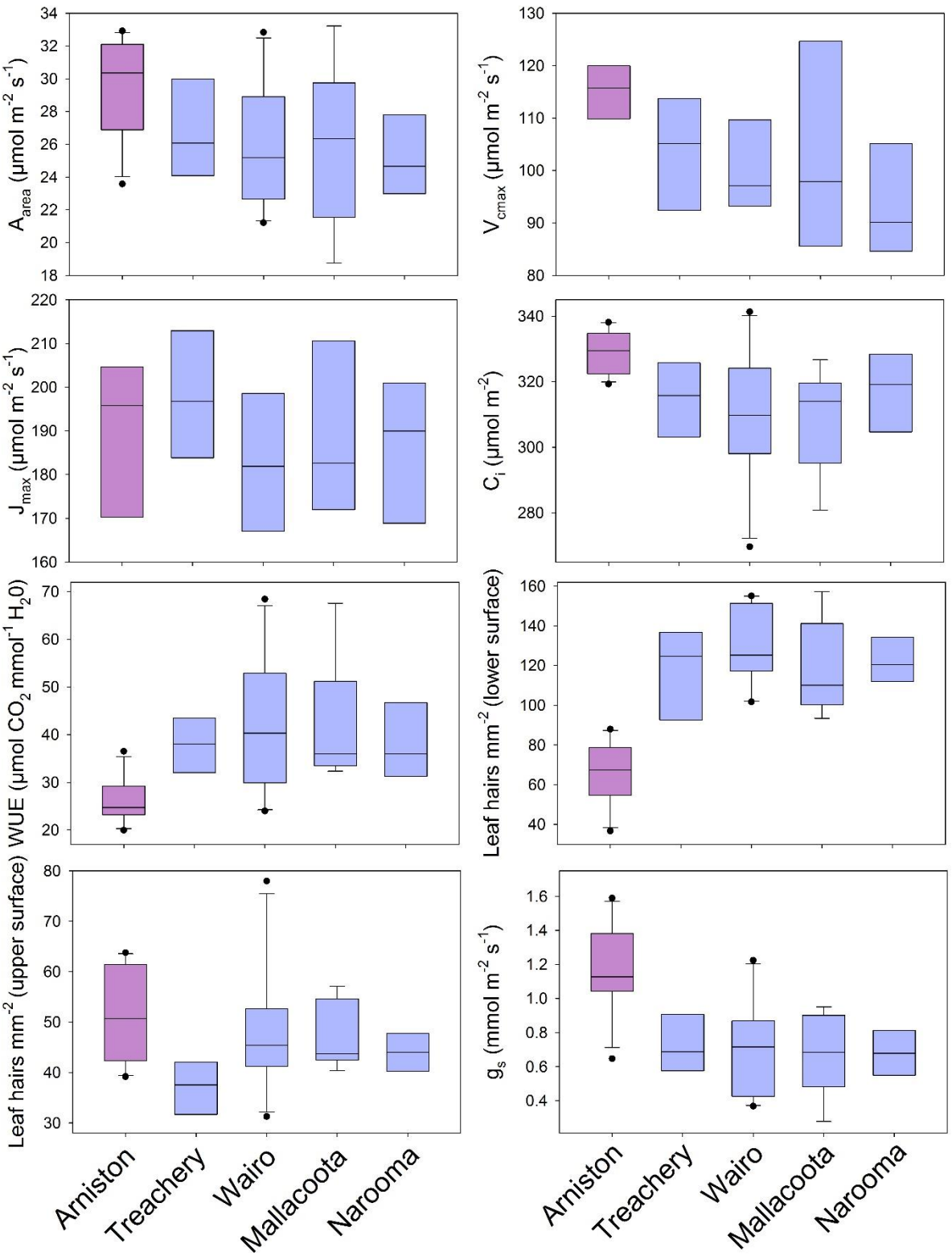


Fig. S1. Stomatal ratios (stomata count on upper surface/stomata count on lower surface) in source and introduced plants as mean values (+/- standard error). The p-value is from a planned contrast between the most likely South African source population and the four Australian introduced populations following 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-axis has been truncated.


A_{area} (µmol m^{-2} s^{-1})
V_{cmax} (µmol m^{-2} s^{-1})
J_{max} (µmol m^{-2} s^{-1})
C_i (µmol m^{-2})
WUE (µmol CO_2 $mmol^{-1}$ H_20)
Leaf hairs mm^{-2} (lower surface)
Leaf hairs mm^{-2} (upper surface)
g_s (mmol m^{-2} s^{-1})
Arniston
Treachery
Wairo
Mallacoota
Narooma

51

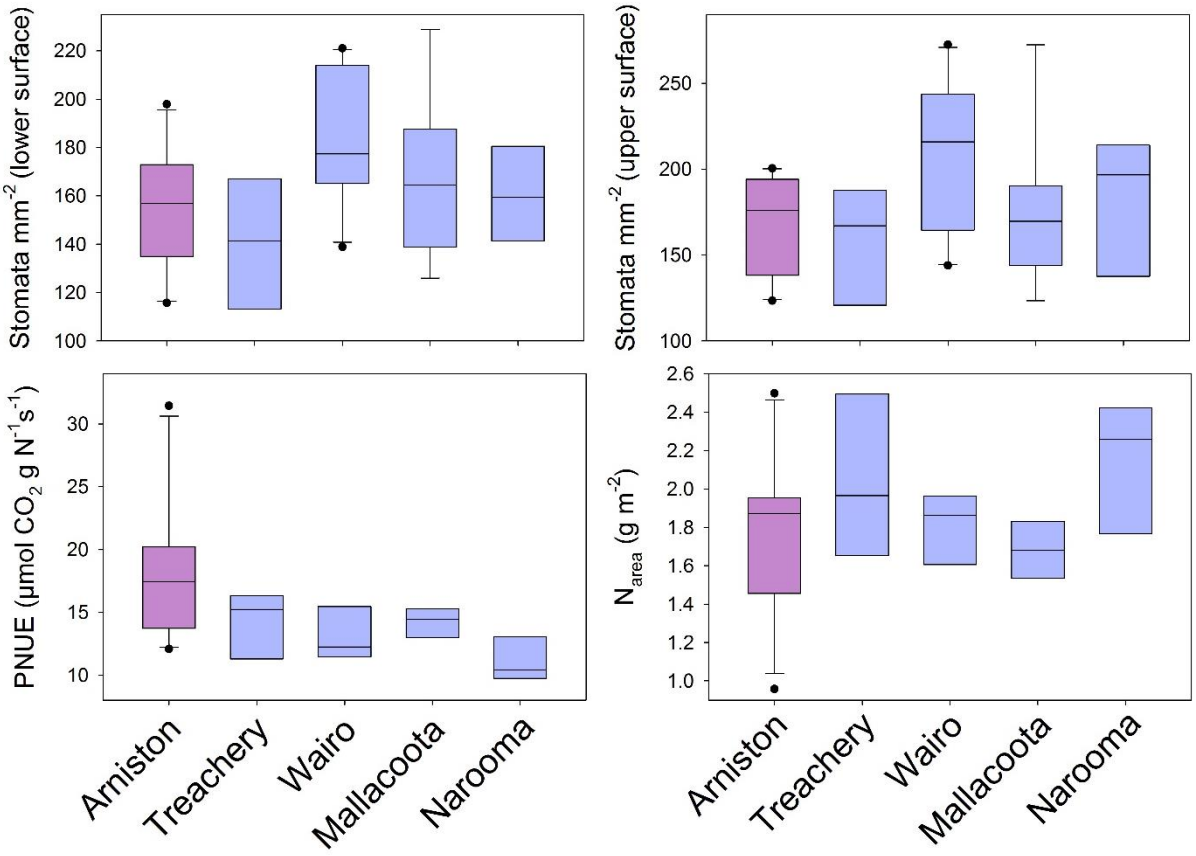


Fig. S2. Boxplots showing the distribution of trait data within each of the five populations, for each of the traits presented in Figures 1-3. The South African population (Arniston) is shown in pink, while the four Australian populations (Treachery, Wairo, Mallacoota and Narooma) are in blue. None of the differences between Australian populations are significant (Table S4). Boxes span from the 25th to 75th percentile with the median marked as a horizontal line. Whiskers span from the 5th to 95th percentiles, and outliers are shown as dots. The y-axes have been truncated.

References

[1] Brandenburger, C.R., Sherwin, W.B., Creer, S.M., Buitenwerf, R., Poore, A.G., Frankham, R., Finnerty, P.B. & Moles, A.T. 2019 Rapid reshaping: the evolution of morphological changes in an introduced beach daisy. *Proceedings of the Royal Society B.* (doi:10.1098/rspb.2018.1713).

[2] Holm, S. 1979 A simple sequentially rejective multiple test procedure. *Scand J Stat* **6**, 65-70.

[3] Gaetano, J. 2013 Holm-Bonferroni sequential correction: an EXCEL calculator (1.1) Microsoft Excel workbook.