Adaptive divergence in shoot gravitropism creates intrinsic reproductive isolation in an Australian wildflower

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Abstract

Adaptation to the local environment is a major driver of speciation. Yet, it remains largely unknown whether natural selection directly causes intrinsic reproductive isolation (hybrid sterility or inviability) between locally adapted populations. Here, we show that adaptive divergence in shoot gravitropism, the ability of a plant's shoot to bend upwards in response to the downward pull of gravity, contributes to the evolution of intrinsic reproductive isolation in an Australian wildflower, *Senecio lautus*. We find that shoot gravitropism has evolved multiple times in association with plant height between adjacent populations inhabiting contrasting environments, suggesting that these traits have evolved by natural selection. We directly tested this prediction in multi-year reciprocal transplant experiments using hybrid populations. We show that shoot gravitropism and plant height respond to natural selection in the expected direction of the locally adapted population. Remarkably, we find that crossing F11 hybrid individuals with extreme differences in gravitropism significantly reduces their ability to produce seeds, providing strong evidence that this adaptive trait is genetically correlated with intrinsic reproductive isolation. Our results suggest that natural selection can drive the evolution of locally adaptive traits that incidentally create intrinsic reproductive isolation, thus increasing our understanding of the origin and maintenance of new species.

Main

Ever since Darwin's work on the origin of species by natural selection[1], researchers have sought to understand how natural selection creates reproductive barriers between populations[2]. On one hand, many studies have established that adaptation to contrasting environments often reduces migrant and hybrid fitness in the wild, a process commonly known as extrinsic reproductive isolation[3]. These extrinsic barriers to reproduction can dramatically reduce gene flow between populations[4,5]; however, they only act in the local environment of populations and therefore are susceptible to changes in environmental conditions. As a consequence, it is unclear whether the evolution of extrinsic reproductive isolation alone can complete speciation[4]. On the other hand, intrinsic reproductive barriers such as hybrid sterility or inviability, can accumulate regardless of environmental change and are therefore expected to be more stable over time and contribute more reliably to the completion of speciation[2]. One way in which natural selection can simultaneously create both extrinsic and intrinsic reproductive isolation is when the beneficial mutations that drive local adaptation in each population also fail to interact properly between populations through negative epistasis[6] (i.e., Dobzhansky-Muller genetic incompatibilities). Therefore, natural selection could in principle reduce gene flow through multiple extrinsic and intrinsic barriers between populations, and thus genetically link the beginning and completion of speciation[7].

Although several genes that impact plant hybrid sterility and inviability have been identified[8], there is limited evidence supporting local adaptation as the major process responsible for the evolution of these loci. In one notable exception, some intraspecific crosses in *Arabidopsis thaliana* produce dwarf, necrotic, and sterile hybrid individuals as a result of negative epistatic interactions from divergent selection of pathogen resistance genes[9]. In a second example in *Mimulus*, tight genetic linkage between alleles selected for copper tolerance and alleles that cause hybrid mortality has led to divergence between populations growing next to copper mines and those occupying typical *Mimulus* habitats[10]. Although plant-pathogen coevolution and tight linkage between genes performing different functions (e.g., stress tolerance and seed development) could be powerful mechanisms for driving species divergence[11], we need more studies on the genetics of adaptation and speciation to reveal the mechanisms by which natural selection can drive the origin of new species. Here, we introduce a novel example where we take advantage of the repeated evolution of divergent growth

habits – adjacent erect and prostrate populations – to study the contribution of adaptation to the evolution of intrinsic reproductive barriers in an Australian wildflower, *Senecio lautus* (G.Forst. ex Willd)[12,13]. This highly replicated system of evolution not only implies a role for natural selection in the evolution of complex morphological or physiological differences[14,15], but also provides multiple independent opportunities to test whether loci that contribute to local adaptation also create genetic incompatibilities that could lead to speciation.

Previous population genetic studies in *S. lautus* found that different sets of genes related by similar functions were repeatedly differentiated in populations with these contrasting growth habits[16,17]. One of these sets contained genes belonging to the auxin pathway, where genes related to the transport and regulation of this key plant hormone had repeatedly diverged between erect and prostrate populations. In congruence, studies across the plant kingdom have established that the auxin hormone plays a major role in how plants orient themselves with respect to light and gravity cues[18] and in creating variation in height[19,20], branching[21,22] and reproduction[23]. With these three traits differentiating erect and prostrate *S. lautus* populations, the auxin pathway is a compelling candidate to link the molecular basis of adaptation and speciation in *S. lautus*. We reasoned that if divergence in the auxin pathway contributed to the evolution of local adaptation and intrinsic reproductive isolation in *S. lautus*, we would observe the following three predictions. 1) In multiple populations with distinct growth habits, we would consistently detect divergence in auxin-dependent traits, such as differences in the ability of seedlings to alter the direction of growth against the pull of gravity[18,24]. 2) These auxin-dependent traits would respond to natural selection in the local environment. Finally, 3) crosses between hybrid individuals with extreme differences in auxin-dependent traits would be genetically incompatible, thus demonstrating a clear genetic link between local adaptation and intrinsic reproductive isolation.

We test these predictions on multiple coastal populations of *S. lautus* (Fig. 1a, Table S1), where there is evidence of local adaptation and intrinsic reproductive isolation between adjacent Dune and Headland populations[25-29]. Populations inhabiting sand dunes (Dune hereafter) are erect, while populations growing on adjacent rocky headlands (Headland hereafter) are prostrate (Fig. 1b). Erect and prostrate growth habits can also be found in related populations from the alpine regions of

Australia, with a prostrate population inhabiting an exposed alpine meadow and an erect population inhabiting adjacent sheltered alpine gullies (Fig. 1c). Dune populations are continually exposed to high temperatures, high solar radiation, low salinity, and low nutrient sand substrate, whereas Headland populations are exposed to high salinity, high nutrients and powerful winds[28]. These ecotypes are genetically grouped into two major monophyletic clades based on geography (eastern and south-eastern Australia), and adjacent Dune and Headland populations are often sister taxa, suggesting that they have evolved their contrasting growth habits independently multiple times[12,25,30,31]. These Dune and Headland ecotypes also show variable levels of intrinsic reproductive isolation, where hybrid sterility in F1 hybrids is weak[28] (<10% crossing failure, n=65), but strong in F2 hybrids (58% crossing failure, n=163). Genetic incompatibilities are largely removed in one generation as F3 hybrids also have weak hybrid sterility (similar to F1 hybrids)[28]. The inability to produce seed in F2's is therefore likely due to few, yet strong recessive negative epistatic interactions between these ecotypes[32,33]. Furthermore, demographic analyses recently revealed minimal levels of gene flow between adjacent Dune and Headland populations[30], indicating that reproductive barriers (intrinsic or extrinsic) can prevent hybridisation from occurring in the field. Given the presence of reproductive isolation between multiple locally adapted erect and prostrate populations, *S. lautus* provides an excellent system in which to understand whether local adaptation driven by morphological and physiological traits can lead to the accumulation of intrinsic reproductive barriers.

Results

Repeated evolution of auxin-related genes in *Senecio lautus*

We first investigated if auxin-related genes are associated with variation in three growth-habit traits (plant height, main stem angle and the number of branches; Fig. S1) that have evolved repeatedly in *S. lautus*[31]. Specifically, we created four genetic mapping populations representing different origins of erect and prostrate plant architectures by crossing individuals from each of the four pairs of Dune and Headland populations[31] (Fig. S2). Bulk Segregant Analyses (BSA)[34] were used to compare the genomes of pools of plants with extreme values for the three traits in the four mapping populations. In agreement with previous population genetic studies in *S. lautus*[16], we found that highly differentiated genomic regions (candidates) between hybrid individuals with contrasting traits were often not the same across the different mapping populations (Data S1). However, the overlap between candidates

in different populations was higher than expected by chance (number of shared Single Nucleotide Polymorphisms (SNPs) = 2,548; mean for permutations = 1,770; 99% upper CI = 1,897) and we discovered 20 candidate regions shared across all four mapping populations. Out of these 20 repeatedly differentiated candidate regions, 11 contained genes regulating the auxin pathway (Table S2), and four of these 11 candidate regions contained multiple auxin-related genes.

Auxin candidate genes identified in the BSA were also genetically differentiated between adjacent natural populations, and had significant associations with survivorship in reciprocal transplants where we examined shifts in allelic frequency after selection in the wild (Table S2 and Data S1). Additionally, several of these auxin genes displayed differential expression between the shoot apical meristems of erect and prostrate populations, which suggest that they are important for morphogenesis (Fig. S3). The homologs of the BSA auxin candidate genes include genes involved in intracellular auxin transport (Table S2), an essential process for creating auxin concentration gradients that drive a directional growth response to environmental cues such as light (phototropism) or gravity (gravitropism)[24,35]. Some homologs also have pleiotropic effects on reproduction (Table S2), which is consistent with a previous mapping experiment between erect and prostrate populations of *S. lautus*[36], where quantitative trait loci for plant architecture (e.g. plant height) clustered with the total number of flowers an individual produces. Together with previous results, these results strengthen the conjecture that the auxin pathway is important for adaptation to sand dunes and rocky headlands, and that there might be a link between complex adaptations and reproductive barriers between genetically and morphologically divergent populations of *S. lautus*.

Repeated height and gravitropism divergence across *Senecio lautus* populations

The regulation and transport of auxin is a well-established modulator of gravitropism in plants[37,38], and therefore might also contribute to growth habit in *S. lautus*. To test whether plant height predicts gravitropism across *S. lautus* populations, we first quantified plant height and gravitropism in 16 *S. lautus* populations (Fig. 1 and Fig. S5). We defined the gravitropic response as the change in angle of a seedling's stem 24 hours after a 90° rotation[39,40], where 0° is no gravitropic response and 90° is a complete re-orientation. If differences in the auxin pathway have contributed to divergence in growth habit, we expected that prostrate populations would display a smaller gravitropic angle than their

adjacent erect population. We found that plant height in natural environments (field) strongly correlates with estimates of plant height in common garden conditions (glasshouse) ($F_{1,9}$=12.41, P=0.0065), demonstrating a genetic basis of this trait in *S. lautus*. Across these populations, the average height of a population predicted its average gravitropic angle (Fig. 2a; $F_{1,13}$=17.65, P=0.0010), with the average magnitude of gravitropism differing between the two monophyletic clades of this system ($F_{1,13}$=32.58, P<0.0001). Therefore, changes in gravitropism appear to be genetically correlated with divergent growth habit traits such as plant height, and this relationship has evolved at least once in each clade.

To test whether natural selection drove the evolution of differences between locally adapted populations with differences in plant height, we first compared gravitropism between seven adjacent Dune and Headland population pairs (Table S1). Four population pairs showed the expected correlation, where plants from the Headland population exhibited a smaller gravitropic angle and were shorter than plants from their adjacent Dune population (Fig. 2b, c). In two population pairs – Millicent and Point Labatt – plant height did not differ between Dune and Headland population pairs (Fig. 2c), possibly due to their similarity in environmental variables[31]. In the population pair at Stradbroke Island, however, we observed differences in height but not in gravitropism (Fig. 2b), suggesting that similar plant architectures could have evolved through other convergent developmental routes in coastal populations of *S. lautus*. We assessed height and gravitropism in divergent populations from the alpine region of Australia, and also discovered the expected pattern, where the exposed Alpine population in the meadow was shorter and had a smaller gravitropic angle than the population in the sheltered alpine gully (Fig. 2c). These results suggest that natural selection repeatedly acting on auxin-related genes has contributed to differences in gravitropism across coastal and alpine populations in this system.

Auxin drives gravitropic differences in *Senecio lautus*

To directly test whether synthetic auxin and auxin transport inhibitors would influence gravitropism differently between erect and prostrate populations, we grew Dune (n=90) and Headland (n=98) seeds collected from Lennox Head with synthetic auxin 2,4-D (2,4-Dichlorophenoxyacetic acid), and polar auxin transport inhibitor NPA (naphthylphthalamic acid)[41,42]. Because a gravitropic response

requires an auxin concentration gradient, we reasoned that removing the gradient would reduce the gravitropic angle in Dune individuals (Fig. S4). As expected, addition of synthetic auxin 2,4-D reduced the gravitropic angle more in Dune individuals than in Headland individuals (LR chi-square=18.49, P<0.0001). Similarly, addition of auxin transport inhibitor NPA reduced the gravitropic angle in our experiments more in Dune individuals than in Headland individuals (LR chi-square=21.18, P<0.0001). This difference in hormone response in Dune and Headland individuals supports our hypothesis that differences in the auxin pathway contribute to the different gravitropic responses observed in *S. lautus*.

Local adaptation drives the evolution of height and gravitropism

We next conducted two independent sets of field adaptation experiments to assess the role of natural selection on the evolution of auxin-dependent traits, height and gravitropism. In each field experiment, Dune and Headland parental and hybrid seeds were transplanted into replicated blocks at the sand dune and rocky headland at Lennox Head. In all field experiments, natural selection consistently favoured the local Dune or Headland population over the foreign population (Fig. 3), indicating that our experiment was exposed to the divergent selection that drove local adaptation in each environment.

For the height adaptation experiments, we tested whether differences in height could drive differences in fitness in the rocky headlands. We hypothesised that offspring produced by short hybrid parents would live longer than offspring produced by tall hybrid parents in the rocky headland. We focused on prostrate growth because it is likely the derived trait, given that the majority of *S. lautus* populations have an erect growth habit[16,28]. Our goal was to introgress Dune alleles associated with height onto a Headland genomic background to examine their effect on fitness in the headland environment. Briefly, we crossed Dune and Headland seeds from Lennox Head and then completed two rounds of backcrossing followed by one round of crossing between tall hybrid offspring (tallest 10%) and between short hybrid offspring (shortest 10%; Table S3 and Fig. S6). We transplanted 558 of these seeds (from 28 families) into the rocky headland. As predicted, shorter hybrid parents produced offspring that lived longer in the rocky headland relative to offspring from taller hybrid parents ($F_{1,26.23}$=4.87, P=0.0362). These results suggest that traits genetically correlated with plant height

contributed to early developmental fitness in the rocky headlands and contributed directly or indirectly to the evolution of divergent growth habit.

For the gravitropism adaptation experiments, we tested whether rapid adaptation to contrasting environments can lead to the evolution of gravitropic responses in the direction of the local population. We hypothesised that exposing an advanced recombinant population to multiple rounds of viability selection in the field would drive genetic covariation between fitness and gravitropism. To test this hypothesis, we crossed 23 Dune and 22 Headland individuals from Lennox Head and then maintained three independent genetic lines for eight generations (Table S4). This process disassembled allelic combinations and constructed an F8 recombinant hybrid population[36] (Fig. 4a). We planted 2,403 of these F8 seeds (from 89 families) into both the sand dune and rocky headland (Fig. 4b and Table S5) and conducted family-based truncation selection for three generations. The fittest families in each environment were selected based on the highest germination and survival (top 50%; see Methods for selection details). Full-siblings from these families were crossed (within a genetic line) to produce the next generation. In the F10 generation, we tested whether families that had the greatest number of survivors also produced offspring (F11) with the local gravitropic response under controlled conditions.

In agreement with our hypothesis, F10 families with the greatest number of survivors in the sand dune produced F11 offspring with a higher gravitropic angle (Table 1). We discovered that this relationship between fitness and gravitropism was driven by the fitness of the F10 dam family and not the F10 sire family (Table 1), suggesting maternal effects might contribute to the evolution of gravitropism in the sand dunes. In contrast, in the rocky headland we did not detected an association between the fitness of the F10 families and gravitropism in their offspring. Instead, Headland survivors had a positive association between the number of days until death in a controlled environment (intrinsic viability) and gravitropism, where individuals that died early in development had a smaller gravitropic angle (agravitropic). We are left with the conjecture that there could be intrinsic fitness costs for agravitropic alleles on a hybrid genomic background.

We then tested whether adaptation to contrasting environments can recreate trait correlations observed in nature. As shown in the previous section, there is a strong correlation between gravitropism and height across many natural populations of *S. lautus*. We expect the correlation would be lost in the creation of the hybrid population if the traits are not pleiotropic, but if natural selection for these traits was strong it could be reconstructed after three rounds of selection in the rocky headland and sand dune. There was no genetic correlation between height and gravitropism in the control population grown only in the glasshouse ($F_{1,114.3}$=0.08, P=0.7801, r^2=0.04), but as predicted this genetic correlation was strong after three rounds of selection in the rocky headland ($F_{1,169.5}$=7.09, P=0.0085, r^2=0.27) and weak after selection in the sand dune ($F_{1,151.3}$=3.20, P=0.0756, r^2=0.09). Together, these results indicate that natural selection can act on standing genetic variation and reconstitute genetic architectures and trait combinations favoured in these coastal environments.

The genetics underlying gravitropism in *Senecio lautus*

To better understand the genetics of adaptation, we performed selective genotyping on the tails of the gravitropic distribution of the F11 hybrid populations (Fig. 4c; Dune survivors, Headland survivors and Control). We identified candidate gene regions containing the most extreme allelic differences between individuals with the smallest gravitropic angle (agravitropic tail, <20°; mean of tail = 6.46±1.10°, n=68) and the largest gravitropic angle (gravitropic tail, >56°; mean of tail = 62.03±0.45°, n=77). We found 55 sites (0.2% of all SNPs) across 49 genomic contigs (Data S2) with an allelic difference in the 99.8% quantile ($0.15<p<0.22$). We discovered that these candidate gene regions disproportionally contained homologous genes with predicted gene ontology categories of transport and localisation of molecules within and between cells (Table S6). This is consistent with expectations, as redistribution of auxin is required for a gravitropic response[18]. Five of the 55 sites (11%) are located in gene homologs with functions related to the auxin pathway, including the second (*ENODL1*; early nodulin-like protein 1)[43] and fourth (*ABA3*; molybdenum cofactor sulfurase)[44] most differentiated SNPs between the agravitropic and gravitropic F11 tails.

Auxin-related genes, *ENODL1* and *ABA3*, have likely contributed to the adaptive divergence of gravitropism in *S. lautus*. In both the *ENODL1* and *ABA3* genes, natural selection recreated the expected allelic frequency shift in the F11 tails towards the parent with the same trait (Data S2). For

instance, the alleles favoured in the agravitropic F11 tail were at high frequencies in the Headland natural population (*ENODL1* cytosine allele (C)=0.87 and *ABA3* guanine allele (G)=0.89), with the alternate alleles being favoured in the gravitropic F11 tail and the Dune natural population (*ENODL1* adenine allele (A)=0.69 and *ABA3* A=0.97). *ENODL1* and *ABA3* were in strong linkage disequilibrium in the survivors of the gravitropism adaptation experiment in the rocky headland (Fisher's exact test, n=57, P=0.0008) and sand dune (Fisher's exact test, n=48, P=0.0107), but not in the control population reared in the glasshouse (Fisher's exact test, n=37, P=0.4093), suggesting natural selection has likely reconstituted this favourable allelic combination. *ENODL1* C/C and *ABA3* G/G genotypes were associated with a reduction in gravitropism of 25.10° relative to all other genotype combinations (Fig. 5; $t_{34.30}$=4.86, P<0.0001), suggesting that not sensing the pull of gravity, or reacting to it, is a recessive trait, and these two loci likely contribute to the genetic architecture responsible for the gravitropic difference between the F11 tails, and potentially in nature. *ENODL1* and *ABA3* have gene homologs with functions in not only gravitropism and plant height, but also in salt tolerance and pollen tube reception[43-49], therefore suggesting that evolution of auxin-related genes might not only explain the adaptive evolution of gravitropism, but also the evolution of other known trait differences between Dune and Headland populations. Overall, our results suggest that divergence in auxin-regulated molecular processes contributed to local adaptation to contrasting environments in coastal populations of *S. lautus*.

The consequences of gravitropism divergence for the evolution of intrinsic reproductive isolation

We are now in a position to investigate whether intrinsic reproductive isolation arises as a by-product of local adaptation. We used the F11 hybrids to test whether divergent gravitropic forms are reproductively isolated. If gravitropism was contributing directly to the intrinsic reproductive isolation previously observed between *S. lautus* ecotypes[28,50], we expected to observe increased hybrid sterility between F11 gravitropic and agravitropic individuals. Hybrid sterility was defined as fewer than three seeds produced per flower head with at least three mating attempts for reciprocal crosses. We created two groups of crosses among F11 families: crosses within (agravitropic × agravitropic, n=28; gravitropic × gravitropic, n=37) and crosses between (agravitropic × gravitropic, n=67) the tails of the gravitropic distribution. Consistent with gravitropism contributing to intrinsic reproductive

isolation in *S. lautus*, we found that hybrid sterility was more common (Odd's ratio=4.8x, P=0.0188) in crosses between F11 agravitropic and gravitropic plants (21%) than within each of these tails (5%; LR chi-square=6.86, P=0.0088). Five percent (n=3) of within-tail crosses failed, all of which were in the gravitropic tail. Overall this indicates that gravitropism alleles have linked or pleiotropic effects on intrinsic reproductive isolation in *S. lautus*.

To understand what might be driving this association between gravitropism and intrinsic reproductive isolation, we examined whether genetic line, F11 population or specific individuals were correlated with hybrid sterility. Crosses within (n=77) and between (n=55) the three genetic lines, regardless of their gravitropic response, did not have an effect on hybrid sterility (LR chi-square=1.10, P=0.2937). This indicates that the hybrid sterility found in the F11's is unlikely to be from genetic drift leading to incompatible differences between the genetic lines. Crosses within (n=80) and between (n=52) the three F11 populations (Dune survivors, Headland survivors and Control), again regardless of their gravitropic response, did not have an effect on hybrid sterility (LR chi-square=0.15, P=0.6995), increasing the likelihood that gravitropism, and not another trait driven by selection to one of these environments, caused intrinsic reproductive isolation in this experimental system. Next, we determined whether specific F11 individuals (n=129) were driving this association between gravitropism and hybrid sterility. Thirty-two individuals were a parent in a sterile cross; 26 of these individuals were crossed against a different individual and they successfully produced seeds, indicating that sterility is specific to the parental combination. Finally, we found that crosses were symmetric; only 6 crosses displayed differences in hybrid sterility in the reciprocal cross, indicating the maternal parent did not dictate sterility. Together, these results imply a genetic association between the adaptive divergence of gravitropism and intrinsic reproductive isolation.

Discussion

Whether local adaptation can directly drive the formation of intrinsic reproductive isolation is still a topic of debate[5,8]. Genetic incompatibilities are considered to evolve late in the speciation process, often after extrinsic barriers have formed (e.g. low fitness migrants)[4], and genetic drift or natural selection has led to the accumulation of genetic differences[2]. With few studies[9,10] on the genetics of local adaptation and intrinsic reproductive isolation within a species, the role of natural selection in

creating intrinsic reproductive barriers at the start of the speciation process remains unclear. Here, we provide empirical evidence to suggest that natural selection is a major driving force behind the intrinsic reproductive isolation found between recently derived erect and prostrate populations in *S. lautus*. We identified a trait in natural populations that could only result from divergence in certain pathways, thus linking the coordinated function of many genes with the evolution of a divergent trait. Variation in this trait, shoot gravitropism, 1) evolved multiple times between adjacent populations, 2) predicted the height of adult individuals, 3) responded to natural selection in the direction of the local population, and 4) was controlled by genomic regions containing genes in the predicted genetic pathway. Having established gravitropism as a locally adapted trait, we showed that it was genetically correlated with hybrid sterility. This set of experiments demonstrates an elusive link between the genetics of local adaptation and the evolution of intrinsic reproductive isolation, which to our knowledge has only convincingly been shown twice – in *Arabipdopsis*[9] and *Mimulus*[10].

Our results suggest that the evolution of genes related to the auxin pathway could facilitate colonisation of new habitats through concomitant changes in multiple developmental and architectural traits. Transitions from erect to prostrate growth and the associated traits of height, main stem angle and number of branches, are common in plants that colonise coastal headlands[51-54]. This indicates that there are strong selective agents common to headland environments. For example, powerful winds or salty substrates could impose early selective pressures on traits controlled by auxin such as responses to mechanical cues[55] and halotropism[56-58]. As the auxin pathway is common across plant species, local adaptation to headland environments through auxin-related genes could be leading to intrinsic reproductive isolation in other plant species. In *Mimulus*, gibberellin (another key plant hormone), has been recently implicated in local adaptation and also associated with reproductive isolation between coastal and inland populations[59,60]. Similar to *S. lautus* Headland populations, coastal *Mimulus* populations have reduced gibberellin, a prostrate growth form and a higher salt tolerance compared to inland *Mimulus* populations[60,61]. Results from *Mimulus* and our study presented here suggest that divergence in plant hormonal pathways that affect multiple traits during plant development could be a powerful mechanism to link the genetic, ecological and physiological basis of adaptation with speciation in plants.

Previous studies in *S. lautus* support a general link between local adaptation and intrinsic reproductive isolation. Walter, et al. [50] found that additive variance responsible for local adaptation in four ecotypes was also responsible for the loss of genetic variation in an F2 generation that displayed elevated levels of hybrid sterility. This is consistent with the observation that adaptation and intrinsic reproductive isolation are genetically correlated in some *S. lautus* populations. A similar occurrence of hybrid sterility occurred in the F2's that created the F11 generation used here[28], suggesting that genetic incompatibilities are segregating in multiple populations and ecotypes of *S. lautus*. The other generations of the hybrid population (F3-F11), however, are highly fertile, implying that a large proportion of the incompatible alleles between Dune and Headland populations were removed in the F2 generation. Remarkably, we were able to recreate intrinsic reproductive isolation in the F11 hybrid population when we sort F11 families according to the strength of their gravitropic response; crosses between individuals with divergent gravitropism values fail to produce seed. A likely explanation is that incompatibility alleles segregate at low frequencies in the population after the F2 generation, and these incompatibilities are directly associated with gravitropism. Future research will investigate whether the same genetic incompatibilities are causing this intrinsic reproductive isolation throughout the system.

The mechanism by which local adaptation leads to intrinsic reproductive isolation could be pleiotropy (e.g. Bomblies and Weigel [9]) or genetic linkage (e.g. Wright, et al. [10])[2]. The closer a hybrid sterility locus is to a target of selection, the more likely reproductive isolation is to concomitantly evolve. We speculate that divergence in adaptive gravitropism and hybrid sterility could result from pleiotropic effects in *ENODL* genes. *ENODL1* was not only a strong gravitropism candidate gene in this hybrid population but members of the ENODL family display functions related to pollen tube reception. For example, *Arabidopsis* mutants of *ENODL* failed to arrest growth and rupture the entering pollen tube[49]. This suggests a female-specific role in which *ENODL* is used to communicate with the male pollen tube to enable reproduction. Consistent with this mechanism, the fitness of the dam family and not the sire family explained gravitropism in our selection experiment. However, one finding that does not support a dam-sire interaction for the source of intrinsic reproductive isolation in these hybrids is the symmetric reciprocal crosses. Dam-sire interactions are more likely to cause differences in hybrid sterility depending on the maternal parent[62]. Instead, symmetric reciprocal crosses are consistent with

the presence of negative nuclear genetic interactions, which is observed in the genetics of reproductive isolation in F2 hybrids[28,50]. Future genetic studies will focus on isolating the loci causing intrinsic reproductive isolation in *S. lautus* so we can reveal the molecular mechanisms by which intrinsic barriers evolve as a by-product of local adaptation.

Overall, our study illustrates a powerful strategy to explore the genetic basis of complex adaptation and speciation in natural systems that challenges the common gene-centric paradigm and moves towards identifying sets of genes related by function. We postulate that the evolution of hormonal pathways in plants provides a simple mechanism for the rapid formation of integrated traits that facilitate colonisation of new habitats, and to the incidental evolution of intrinsic reproductive isolation.

Materials and Methods

Reference genome

Headland individuals from Lennox Head were used for the creation of an Illumina *S. lautus* reference genome. Firstly, we collected seeds from the Headland at Lennox Head (Table S1) and germinated seeds from two individuals by cutting 1mm off the micropyle side of the seed and placing in petri dishes with dampened filter paper. The seeds were placed in darkness for two days to allow for roots to grow and then transferred to a 12-hour light cycle in a constant temperature room at 25°C for seven days to allow for shoots to grow. Seedlings were then transferred into large individual pots with standard potting mix and grown in a glasshouse. These two individuals were crossed by rubbing flower heads together and collecting the seeds produced. Siblings from the seeds produced were grown and crossed by rubbing flower heads together to produce a family of individuals capable of selfing (self-fertilisation is rare in *S. lautus*). One generation of selfing was completed to increase homozygosity. We extracted DNA from leaf tissue of one individual using a modified CTAB protocol[63].

A draft genome of *S. lautus* was de novo assembled using second-generation short reads and AllPaths-LG 6.1.2 using default settings. We utilised a series of eight read libraries (Table S7). The reads were trimmed to remove residual adapter sequences and low-quality bases (minimum quality 15). The final assembly was ~843mb long and consisted of 96,372 scaffolds with an N50 of 21K. Although 843mb is much shorter than the expected haploid size of 1.38GB[64] of the full genome, the

BUSCO gene content completeness of 84% (5% fragmented and 11% missing) suggests that this assembly is primarily missing intergenic repetitive DNA sequences, which are notoriously difficult to assemble. This Illumina assembly was used for mapping of all F11 samples.

A PacBio *S. lautus* reference genome was then created in an attempt to overcome the problem caused by repetitive DNA. The same individual described above was put in darkness for three days before leaf samples were collected and sent to BGI for DNA extraction and sequencing. The genome was de novo assembled using next-generation PacBio long reads (56 SMRT cells) and CANU 1.6[65] with default settings. A total of 11,481,638 filtered subreads (5,072,756 from RSII sequencer and 6,408,882 from Sequel sequencer) were used with a mean read length of ~8K base pairs and an expected genome coverage of ~60x. The final assembly consisted of 60,947 contigs with an N50 of 34K. The BUSCO gene content completeness was 86% (4% fragmented and 10% missing).

Genetic mapping of convergent traits

Creation of the Bulk Segregant Analysis populations

To identify the genomic regions associated to growth habit in *S. lautus*, we created recombinant populations by crossing individuals from four pairs of parapatric populations (Fig. S2) that represent independent origins of the erect and prostrate phenotypes[12,16,31,66]. We crossed individuals from contrasting populations growing in the following locations: (1) headland (prostrate) and dune (erect) from Lennox Head (NSW), (2) headland (prostrate) and dune (erect) from Coffs Harbour (NSW), (3) headland (prostrate) and muttonbird island (erect) from Coffs Harbour, and (4) exposed (prostrate) and sheltered (erect) alpine sites from Mt Kosciuszko (NSW) and Falls Creek (VIC), respectively. Using a random-mating-equal-contributions breeding design[67], we crossed multiple individuals from pairs of adjacent populations to create F1's (2 crosses per plant on average) and conducted intercrosses (2 crosses per plant on average) for eight generations between Lennox Head populations (F8-LH), four generations between Alpine populations (F4-A) and three generations between both Coffs Harbour populations (F3-CHD and F3-CHI) (Fig. S2).

We measured three phenotypic traits that distinguish erect and prostrate architectures and are possibly regulated by auxin: height, angle of the main stem, and number of tertiary branches. Height

(vegetative) was measured as the vertical distance from the soil to the highest point of the plant that has vegetative leaves (flowers and stems are not included). The angle of the main stem was measured with respect to the soil. Higher values of this angle indicate a more erect stem. To evaluate the number of tertiary branches, we collected three basal secondary branches (i.e. branches that originate from the main stem) and counted the number of branches arising from these. Trait distributions and population statistics are provided in Data S3.

Genotyping of the Bulk Segregant Analysis populations

We genotyped the four populations described above by pooling individuals from extremes of the three traits. More specifically, young leaves were collected, and DNA was extracted following a modified version of the CTAB protocol[63]. The DNA was quantified with the PicoGreen reagent (Invitrogen, Carlsbad, CA) and pools of recombinant individuals were created as described in Roda, et al. [31]. For each recombinant population we pooled DNA from plants from the tails of the distribution (upper and lower 10%) of the three convergent traits (height, angle of the main stem and number of tertiary branches). We thus produced 24 pools of DNA (3 traits x 4 populations x 2 tails; Data S4). Pooled DNA was cleaned using the Wizard PCR-cleanup kit (Promega, Sydney, Australia). Libraries of Restriction-site Associated DNA (RAD) tags[68] were created and sequenced at Floragenex (University of Oregon High Throughput Sequencing Facility) following the methodology described in Roda, et al. [31] but sequencing depth and coverage was increased to 50x depth and 2m reads / sample.

The Bulk Segregant Analysis

We identified genomic regions associated to convergent traits by conducting pairwise comparisons between contrasting pools from the same trait and population (3 traits x 4 populations = 12 comparisons). To identify Single Nucleotide Polymorphisms (SNPs), determine allelic frequencies and calculate genetic differentiation between pairs of pools, RADseq data was processed using the PoPoolation2 pipeline[69]. Illumina reads were mapped to the PacBio genome draft of *S. lautus* using BWA 0.7.15[70] with default parameters. We then used SAMtools 1.5[71] to create an mpileup file for each pair of pools (Phred quality score of 20). Finally we used the PoPoolation2 pipeline 1201[69] to extract variable positions (minimum count of 2, minimum coverage of 15, and maximum coverage of 800), estimate allelic frequency differences[72] between pools and calculate Fisher Exact tests (FET) of

allelic differentiation[73-75]. We thus obtained an average number of SNPs per comparison of 39,161 ± 7,244 SNPs located in 10,826 ± 1,405 genomic contigs. Candidate SNPs for each comparison were based on two conditions; located in the upper 5 percentiles of the genome-wide distribution of allele frequency differences and having Bonferroni-corrected p-values lower than 5% in the FET. Genomic contigs containing at least one candidate SNP were considered candidates (Table S2 and Data S5).

To determine if candidates from the BSA of the three traits show footprints of natural selection we re-analysed previous pool-seq samples of natural populations[16,31] and survivors from reciprocal transplants[36] (Table S2). We used the pipeline described above to identify candidate SNPs and contigs for pairwise comparisons between either adjacent populations[16,31] or survivors from transplants to different environments[36]. To identify SNPs differentiated in natural populations we performed 12 pairwise genomic comparisons between DNA pools from adjacent Dune and Headland populations (Data S4). To identify loci putatively under natural selection in the sand dune and rocky headland we performed comparisons between pools of survivors from reciprocal transplants using F8 populations (6 comparisons; Data S6). Outlier SNPs and contigs were defined as described previously for both types of pairwise comparisons.

Permutation analyses

We ran a permutation analysis in R v3.6.0[76] to determine if the number of candidates from BSA shared between populations and traits is greater than expected by chance. Specifically, we randomly re-assigned outliers to SNP positions for each of the 12 comparisons (3 traits x 4 populations) and calculated the number of positions shared between at least two comparisons. We repeated this process 10,000 times and calculated 99% confidence intervals.

Differential gene expression between erect and prostrate natural populations

Plant material and RNA extraction

To obtain plant tissue to evaluate gene expression, we used mature plants derived from seeds collected in the field from three erect natural populations: Alpine (A03), Dune (D01), and Tableland (T01), and from one prostrate natural population, Headland (H01). Field-collected seeds were grown (see above for details) under natural light conditions and temperature was controlled to 22°C during

the night (6:00pm-6:00am) and 25°C during the day (6:00am-6:00pm). Plants were randomly crossed within each natural population, and 96 seeds of each of the four natural populations were germinated as described above and grown in 48-cell trays in a controlled temperature room at 25°C with a 12-hour light cycle. We sampled the shoot apical meristem (SAM) along with the youngest three leaf primordia (LP 1, 2 and 3) when plants were 7 weeks old.

cDNA library construction

For each natural population, SAM-LP from each plant was dissected under a dissecting microscope and conserved in RNAlater solution (Ambion, Inc., Austin, USA). Total RNA was extracted using TRIzol[77]. Total RNA was quantified using a NanoDrop (Thermo Fisher Scientific, Waltham, USA) and quality was checked using a 2100 Bioanalyzer (Agilent technologies, USA) at the Australian Genome Research Facility. Only samples with an RNA integrity number[78] > 6.5 and a ratio of the 28S and 18S ribosomal bands > 1.0 were further processed. 0.5 µg of total RNA were used to construct sequencing libraries with the TruSeq RNA Sample Preparation Kits, and sequenced using 100bp pair-end sequencing with Illumina HiSeq 2000 at BGI.

Gene expression quantification

We used the new tuxedo suite, composed of HISAT, StringTie and Ballgown, to identify differentially expressed genes between the prostrate population, H01, and each of the three erect populations, A03, D01 and T01. We first filtered RNAseq raw reads by removing sequencing adaptors, reads with more than 10% unknown bases and more than 50% bases with quality lower than five. Clean reads of each population were mapped to the PacBio *S. lautus* genome draft using HISAT2 2.0.0[79] to align pair-end reads, and Samtools 1.5[71] to obtain sorted BAM files. Stringtie 1.3.4d[80] was used to 1) assemble mapped reads of each population to transcripts, 2) merge the transcripts of the four populations into a non-redundant set of transcripts contained in the four populations, and 3) quantify transcript abundances in each of the four populations. Finally, we used the Ballgown[81] R package to obtain the FPKM (fragments per kilobase of exon per million reads mapped) by gene. We calculated the relative expression between erect (A03, D01 and T01) and prostrate (H01) samples as Log2(FPKM erect/ FPKM prostrate) (Data S7).

Gene annotation

To identify auxin genes associated to the evolution of erect and prostrate phenotypes, we annotated candidate loci from the BSA using databases from *Arabidopsis thaliana*, which is the plant model where most studies of auxin signalling have been conducted, and has the best genome annotation among plants. We first conducted a BLASTx of the *S. lautus* genes, as predicted from transcriptome data, against the TAIR protein database (https://www.arabidopsis.org/) using standalone BLAST 2.6.0, and a minimum *e-value* of 1e-6 (Data S8). For genomic contigs showing associations to adaptive traits in the BSA we also searched for genes not detected in the transcriptome by doing a BLASTx of the contigs against the TAIR protein database. We then retrieved the function of significant hits using the DAVID server[82,83] (Data S9). Auxin genes were identified by searching for the term "auxin" in this annotation. We refined our functional description of these auxin genes by searching for the terms "biosynthesis", "branch", "efflux", "gravitropism", "influx", "morphogenesis", "polar", "protein modification", "reproduction", "transcription factor", "transport", and "tropism" (Table S2). We also searched in the TAIR database for literature references associated to the function of these *Arabidopsis* homologs. Given their large number, these references are not provided in the manuscript.

Phenotyping of natural populations

Height measurements

We measured height as described above in all 16 populations in the glasshouse and 12 of the populations in their native field environment (Data S10 and Data S11). In the field, we measured height in 32 individuals evenly across the range of each population. In the controlled conditions of the glasshouse, we sampled an average of 14 individuals per population after plants reached maturity.

For both the glasshouse and field measurements, we used a linear model to determine whether Dune populations were taller than their adjacent Headland pair: $y_{ijk} = P_i + E_{j(i)} + e_{k(ij)}$, where pair ($P_i$) is an adjacent Dune and Headland population at the same locality and ecotype ($E_{j(i)}$) is Dune or Headland and is nested within pair. All factors are fixed effects and $e_{k(ij)}$ is the residual error. Population height (y_{ijk}) for each pair was compared using a one-tailed t-test. The Alpine populations were also included with the prediction that the sheltered Alpine population (A03) would be taller than the wind exposed Alpine population (A07). All statistical results reported here were produced in JMP 13 (SAS 2015).

Gravitropism measurements

Gravitropism was measured *in vitro* on agar plates in a dark growth cabinet using seeds from all 16 natural populations. For each population, 2-4 seeds per family were grown for ~40 maternal families (1,278 seeds in total). The seeds from a population were combined in a falcon tube and were first sterilised with a quick rinse in 70% EtOH, followed by four 10 minute inversions in a sterilising solution of 6% sodium hypochlorite and 1% Tween 20. Seeds were then rinsed three times with distilled water and vertically orientated on Murashiga and Skoog (MS) agar plates containing 0.15% MS, 0.05% 2-(*N*-morpholino)ethanesulfonic acid (MES), 0.15% sucrose and 1% agar. We placed eight seeds on each plate and incubated the plates at 21°C in the dark to avoid any light effects. After seven days, all plates were rotated clockwise by 90° and a photograph of each plate was taken 24 hours after rotation. The photographs were imported into ImageJ[84] to determine gravitropism by measuring the angle to which the stem reorientated to the horizontal[85-87].

Overall there was a 63.8% germination success but we excluded seedlings that were shorter than 5mm, contacted the edge of the plate, or germinated after rotation. This left a total of 736 seedlings across all 16 populations (57.6% of the total number of seeds planted; Data S10). To test the hypothesis that Dune populations would have a stronger gravitropic response in their stem than their adjacent Headland pair, we used a mixed linear model: $y_{ijkl} = P_i + E_{j(i)} + A_k + e_{l(ijk)}$, where pair ($P_i$) is an adjacent Dune and Headland population at the same locality, ecotype ($E_{j(i)}$) is Dune or Headland and is nested within pair, and agar plate (A_k) is the MS plate that the seeds were grown on. Agar plate was fitted as a random effect while the rest were fixed effects, and $e_{l(ijk)}$ is the residual error. Gravitropism (y_{ijkl}) measures were averaged for each population and compared between each population pair using a one-tailed t-test. We then tested the correlation between height and gravitropism by performing a linear regression with mean height against mean gravitropism for all 16 populations, where populations were grouped into their respective clades (eastern and south-eastern). All statistical results reported here were produced in JMP 13 (SAS 2015).

Synthetic auxin and auxin transport inhibitor experiments

We tested if gravitropism is governed by auxins in *S. lautus* by evaluating gravitropic responses in seedlings treated with chemicals affecting auxin signalling. Specifically we used 2,4-Dichlorophenoxyacetic acid (2,4-D), a carrier-dependent synthetic auxin[41,88] and Naphthylphthalamic acid (NPA) an efflux inhibitor[42]. We collected seeds from Lennox Head sand dune and rocky headland and grew them on MS plates containing the different chemicals at concentrations of 0 µM, 0.5 µM, 5 µM and 50µM. For this we used the following stock solutions: 2,4-D: 1mM in Ethanol and NPA: 10mM in dimethyl sulfoxide (DMSO). We created 1ml dilutions of stock solutions (in ethanol or DMSO), which were dissolved in 500 ml of media. Plates were incubated, rotated, photographed and gravitropism was measured in ImageJ[84], as outlined above.

Approximately 40 seeds from different maternal families were planted for each population and chemical concentration. Seeds were excluded from analyses following the same protocols as above, leaving a total of 188 seedlings to quantify the gravitropic response. The 50µM concentration of 2,4-D treatment was excluded, as less than six seeds germinated under this high concentration (Data S12). For each chemical we used a mixed linear model using the lmer function of lme4 package in R v3.1.3[76]: $y_{ijkl} = A_k + E_i + C_j + E_i \times C_j + e_{l(ijk)}$, where agar plate ($A_k$) is the MS plate that the seeds were grown on, ecotype (E_i) is Dune or Headland and concentration (C_j) are the 4 different concentrations of the chemical. Agar plate was fitted as a random effect, while ecotype, concentration and their interaction ($E_i \times C_j$) were fixed effects, and $e_{l(ijk)}$ is the residual error. Gravitropism (y_{ijkl}) was compared using a Type II Wald chi-square test.

Field experiments

All field experiments were conducted at the sand dune and rocky headland at Lennox Head (NSW) in the same location where native *S. lautus* grow. We tracked each individual by gluing each seed to a toothpick with nondrip superglue and placing them 1-2mm under the ground within a grid cell (Fig. 3b) that was randomly assigned (for details see Walter, et al. [28,]Walter [89]). 50% shade cloth was suspended 15cm above all plots to replicate the shade given by surrounding vegetation and was later replaced with very light bird netting. Seeds were watered twice a day to keep the soil moist and replicate ideal germination conditions to maximise the number of seeds in the experiment. Once germination plateaued for all genotypes watering was gradually ceased.

Height adaptation experiments

We created genetic lines that aimed to isolate height differences on a common genomic background (Fig. 1). Firstly, Lennox Head Dune and Headland seeds were grown and crossed to create an F1 generation. Secondly, we backcrossed to Headland parental plants for two generations to produce a BC2F1 generation. We then grew and crossed the tallest BC2F1 individuals (n=16, tallest 10% of the population) among one another, and the shortest individuals (n=18, shortest 10% of the population) among one another. See Table S3 for the number of individuals and families that contributed to every generation to create these BC2F2 genetic lines. The BC2F2 seeds were planted into the rocky headland at Lennox Head in October 2016 (Australian spring). We planted five replicate plots, where each plot (1.08x0.33m) consisted of the same 12 families with four (occasionally three) individuals per family in each plot, totalling 1,116 BC2F2 seeds. Germination and mortality were recorded every day for 49 days, then every 3-4 days until day 79 and then weekly for the remainder of the experiment, until day 159 (Data S13).

We implemented a mixed linear model to test the hypothesis that individuals with short parents will have higher fitness in the headland environment: $y_{ijkl} = H_i + F_j + B_k + e_{l(ijk)}$, where parental height (H_i) was the average height of the parents measured in the glasshouse, family (F_j) was individuals with the same parents, and block (B_k) is the five replicate plots across the rocky headland. Parental height is a fixed effect, and family and block are random effects, and $e_{l(ijk)}$ was the residual error. Offspring fitness (y_{ijk}) was the total number of days alive in the rocky headland from planting. All statistical results reported here were produced in JMP 13 (SAS 2015).

Gravitropism adaptation experiments

We created an advanced recombinant population (F8) from 23 Dune and 22 Headland Lennox Head individuals using a North Carolina 2 breeding design[67,90] as described in Roda, et al. [36]. Briefly, we replicated the construction of the F8 using three independent replicate crossing lines (A, B and C), all derived from the same base population. See Table S4 for the number of families per replicate genetic line for every generation. F8 seeds were planted into the sand dune and rocky headlands in 2012 as described above. The fittest families (top 50%) within an environment (and genetic line) were selected

using Aster modelling[91,92] implemented with the 'Aster' package in R[76]. The fitness components included germination and survival success, where germination was the total number of individuals that germinated in each family and survival success was the total number of individuals per family that survived to day 85 in the F8 generation. One full-sibling from the 101 selected families (50 Dune survivor and 51 Headland survivor families) and 44 families randomly selected from all F8 families for the control population were then grown in the glasshouse. Each individual was randomly assigned as a dam or sire and after reaching maturity crossed twice in a full-sibling, half-sibling crossing design. This maintained ~100 full-sibling families for each population (Dune survivors, Headland survivors and Control). The same field experiment and selection procedure was conducted on the F9 (in 2013) and F10 generations (in 2014), for a total of three rounds of selection, where survival success was measured at day 138 in the F9 generation, and day 232 in the F10 generation. See Table S5 for the number of seeds and families planted per genetic line and environment for the three transplant experiments.

F11 gravitropism measurements

We measured gravitropism, in the F11 generation described above, as the angle of the stem after a 90° rotation of a seedling. This included 39 Dune families, 37 Headland families and 25 inbred control families with 12 individuals per family (1,212 seeds in total). These families were germinated in three separate batches ~7 days apart. Briefly, we germinated the F11 seeds by cutting 1mm off the micropyle side of the seed and placing in petri dishes with dampened filter paper. The seeds were placed in darkness for two days to enable roots to grow and then transferred to light for 4 days in order for shoots to grow. Seedlings were then transferred into small square pots with standard potting mix in a constant temperature room at 25°C with a 12-hour light cycle. After one week of growing in the pot, the plants were rotated by 90° and a photograph of each individual was taken 12 hours after rotation. The photographs were imported into ImageJ[84] to measure gravitropism as described above. Data S14 contains gravitropism measures and dam and sire fitness values.

Gravitropism tests of selection

We implemented a linear model to test the hypothesis that high fitness dune families will produce gravitropic plants and high fitness headland families will produce agravitropic plants. Independent

models were used for the sand dune and rocky headland to test the effect of gravitropism on fitness in each environment: $y_{ijklmn} = B_i + V_j + G_{k(i)} + D_{l(ik)} + S_{m(ik)} + e_{n(ijklm)}$, where temporal block ($B_i$) is the three time points in which the F11 seeds were grown (~seven days apart); intrinsic viability (V_j) is the number of days until the death of F11 plants in controlled conditions; and genetic line, which consists of the three independent genetic lines (A, B and C), is nested within temporal block ($G_{k(i)}$). Dam fitness was nested in genetic line and temporal block ($D_{l(ik)}$) and sire fitness was also nested in genetic line and temporal block ($S_{m(ik)}$). Dam and sire fitness are the F10 family fitness values for the individuals that were crossed to create the F11 offspring where gravitropism was measured. All factors were included as fixed effects and $e_{n(ijklm)}$ was the residual error. Genetic line C was removed from analyses as it has little variation in fitness values which means it did not converge. Shapiro-Wilk W test shows the residuals from the model are normally distributed for both the sand dune (W=0.98, P=0.3879) and rocky headland (W=0.98, P=0.2776). The linear model was performed in JMP 13 (SAS 2015).

Genetic association between height and gravitropism

We tested the genetic association between height and gravitropism after segregation in an advanced recombinant population. We implemented a mixed linear model for the three F11 populations (Dune survivors, Headland survivors and a Control population) that accounts for family variation: $y_{ijk} = H_i + F_j + e_{k(ij)}$, where gravitropism ($y_{ijk}$) is the angle of the growth response 12 hours after a 90° rotation, height (H_i) is the vertical distance from the soil to the top of the vegetative leaves, measured after maturity in the glasshouse and family (F_j) is a random effect that consists of individuals that have the same dam and sire. The mixed linear model was performed in JMP 13 (SAS 2015).

Genotyping of F11 gravitropism tails

To isolate gravitropism candidate genes, we genotyped 77 gravitropic (>56°) and 68 agravitropic (<20°) F11 individuals (Data S14). We extracted DNA from leaf tissue using a modified CTAB protocol[63] and quantified the DNA using the PicoGreen reagent (Invitrogen, Carlsbad, CA). To determine which parent the alleles were derived from, we included 39 Dune parentals (D01) and 41 Headland parentals (H01). Leaves from the Lennox Head Dune and Headland natural populations were collected directly from the field and the same DNA extraction protocol was followed. To increase accuracy of genotyping, each F11 individual was duplicated in independent wells and libraries of

Restriction-site Associated DNA (RAD) tags were created at Floragenex following the protocol from Baird, et al. [68] but using the PstI restriction enzyme. We sequenced 380 samples on four lanes of an Illumina HiSeq 4000 with 91bp single end reads at Floragenex. A total of 1.39 billion reads with a mean of 3.62 million reads per sample were produced. Reads were aligned to the reference genome using Bowtie 1.1.1[93] and a FASTQ quality score of above 20. We then used SAMtools 0.1.16[71] to create an mpileup file of all samples with a minimum Phred quality score of 10, minimum sequencing depth per sample of 6x and minimum percent of population genotyped of 75%. This produced 26.8K variable positions (224K variants before filtering) with a minimum distance between variants of 50bp. The gravitropism candidate gene set consisted of SNPs in the 99.9% quantile of the distribution of differentiated SNPs between the gravitropism tails. The region of the scaffold containing the SNP was annotated using the BLASTx NCBI database[94].

Senecio lautus gravitropism candidate genes

In the gravitropism candidate gene set, we tested for overrepresentation of gene function and linkage disequilibrium between gravitropism loci. A statistical overrepresentation test was performed in PANTHER (http://pantherdb.org/) using the TAIR identification for 32 unique gravitropism candidate genes matched to a reference list of 27,502 *Arabidopsis thaliana* genes. To calculate linkage disequilibrium between loci, a likelihood-ratio chi-square test was performed in JMP 13 (SAS 2015) with each F11 population independently (Dune survivors, Headland survivors and Control); Data S15).

Intrinsic reproductive isolation

We tested whether hybrid fertility was associated with gravitropism by randomly crossing within and between the F11 gravitropic and agravitropic groups in a controlled temperature room and recording successful and failed crosses. To maximise sample size, all three replicate genetic lines (A, B and C) were used across all three environments (Dune survivors, Headland survivors and Control). A total of 138 crosses were completed (Data S16), 71 crosses between the tails and 67 within the tails (agravitropic tail = 29 and gravitropic tail = 38). Crosses were completed by rubbing multiple flower heads of two individuals together and collecting the seeds produced from both plants. To remove genetic incompatibilities that might be caused by relatedness, crosses within the same family were

not performed. Hybrid sterility (a failed cross) was considered when, in the presence of pollen, less than three seeds were produced per flower head from both plants (reciprocal crosses) with three mating attempts. Six crosses displayed differences in sterility in the reciprocal cross (asymmetry), with one successful and one failed cross when using the same parents, and so were removed from the analysis (4 crosses between the tails and 2 within the tails).

If gravitropism was contributing to divergence in *S. lautus* then we expected to observe an increase in hybrid sterility in crosses between gravitropic and agravitropic individuals. We performed a linear model in JMP 14 (SAS. 2018) to determine whether there was a significant association between cross type (within vs between gravitropism tails) and hybrid sterility, while accounting for genetic line and F11 population: $y_{ijkl} = T_i + G_j + E_k + e_{l(ijk)}$, where tails ($T_i$) is either crosses within or between gravitropism tails, genetic line (G_j) are the three independent genetic lines (A, B and C) and population (P_k) are the three F11 populations (Dune survivors, Headland survivors and Control). All factors were included as fixed effects and $e_{l(ijk)}$ was the residual error. Hybrid fertility (y_{ijkl}) was compared using a likelihood-ratio chi-square test.

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Author Contributions

MJW, FR and DO conceived the projects and experiments. MJW conducted height adaptation experiments, reared and phenotyped various glasshouse populations and performed reproductive isolation experiment. FR created the BSA mapping populations and performed the BSA analyses. FR and MJW performed physiological and molecular biology experiments. GMW reared and phenotyped the natural populations in the glasshouse with help from MJW and MEJ. GMW conducted the field experiment for the gravitropism adaptation experiments with assistance from MJW, MEJ, FR and DO. DMB performed expression analyses. MJW, MEJ and HLN phenotyped natural populations. MEJ and MJW extracted the DNA from the F11's and RN and JW called the genotypes. MEJ reared the plants and prepared the Illumina libraries for SA to assemble the *S. lautus* genomes. CB guided the physiological experiments. MJW, FR, DO wrote the paper with input from all authors. DO is mentor and supervisor for the research program.

References

1 Darwin, C. *On the origins of species by means of natural selection*. Vol. 247 (1859).

2 Coyne, J. A. & Orr, H. A. *Speciation*. (Sinauer Associates, 2004).

3 Rundle, H. D. & Whitlock, M. C. A genetic interpretation of ecologically dependent isolation. *Evolution* **55**, 198-201 (2001).

4 Seehausen, O. *et al.* Genomics and the origin of species. *Nat. Rev. Genet.* **15**, 176-192, doi:10.1038/nrg3644 (2014).

5 Baack, E., Melo, M. C., Rieseberg, L. H. & Ortiz-Barrientos, D. The origins of reproductive isolation in plants. *New Phytol.* **207**, 968-984, doi:10.1111/nph.13424 (2015).

6 Schluter, D. & Conte, G. L. Genetics and ecological speciation. *Proc. Natl. Acad. Sci. USA* **106**, 9955 (2009).

7 Lowry, D. B., Rockwood, R. C. & Willis, J. H. Ecological reproductive isolation of coast and inland races of *Mimulus guttatus*. *Evolution* **62**, 2196-2214, doi:10.1111/j.1558-5646.2008.00457.x (2008).

8 Fishman, L. & Sweigart, A. L. When two rights make a wrong: The evolutionary genetics of plant hybrid incompatibilities. *Annu. Rev. Plant Biol.*, doi:10.1146/annurev-arplant-042817-040113 (2018).

9 Bomblies, K. & Weigel, D. Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species. *Nat. Rev. Genet.* **8**, 382, doi:10.1038/nrg2082 (2007).

10 Wright, K. M., Lloyd, D., Lowry, D. B., Macnair, M. R. & Willis, J. H. Indirect evolution of hybrid lethality due to linkage with selected locus in *Mimulus guttatus*. *PLoS Biol.* **11**, e1001497, doi:10.1371/journal.pbio.1001497 (2013).

11 Kirkpatrick, M. & Barton, N. Chromosome inversions, local adaptation and speciation. *Genetics* **173**, 419 (2006).

12 Radford, I. J., Cousens, R. D. & Michael, P. W. Morphological and genetic variation in the *Senecio pinnatifolius* complex: are variants worthy of taxonomic recognition? *Aust. Syst. Bot.* **17**, 29-48, doi:10.1071/sb03013 (2004).

13 Thompson, I. *Taxonomic studies of Australian Senecio (Asteraceae): 5. The S. lautus/S. lautus complex.*, 23-76 (Royal Botanic Gardens, 2005).

14 Stinchcombe, J. R. & Hoekstra, H. E. Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. *Heredity* **100**, 158-170 (2007).

15 Barrett, R. D. H. & Schluter, D. Adaptation from standing genetic variation. *Trends Ecol. Evol.* **23**, 38-44, doi:10.1016/j.tree.2007.09.008 (2008).

16 Roda, F. *et al.* Convergence and divergence during the adaptation to similar environments by an Australian groundsel. *Evolution* **67**, 2515-2529, doi:10.1111/evo.12136 (2013).

17 James, M. E., Wilkinson, M. J., North, H. L., Engelstädter, J. & Ortiz-Barrientos, D. A framework to quantify phenotypic and genotypic parallel evolution. *bioRxiv*, doi:10.1101/2020.02.05.936450 (2020).

18 Friml, J. Auxin transport—shaping the plant. *Curr. Opin. Plant Biol.* **6**, 7-12 (2003).

19 Xu, M., Zhu, L., Shou, H. & Wu, P. A PIN1 family gene, OsPIN1, involved in auxin-dependent adventitious root emergence and tillering in rice. *Plant Cell Physiol.* **46**, 1674-1681, doi:10.1093/pcp/pci183 (2005).

20 Wallace, J. G. *et al.* Genome-wide association for plant height and flowering time across 15 tropical maize populations under managed drought stress and well-watered conditions in Sub-Saharan Africa. *Crop Sci.* **56**, 2365-2378, doi:10.2135/cropsci2015.10.0632 (2016).

21 Gallavotti, A. The role of auxin in shaping shoot architecture. *J. Exp. Bot.* **64**, 2593-2608, doi:10.1093/jxb/ert141 (2013).

22 Domagalska, M. a. & Leyser, O. Signal integration in the control of shoot branching. *Nat. Rev. Mol. Cell Biol.* **12**, 211-221, doi:10.1038/nrm3088 (2011).

23 Sundberg, E. & Østergaard, L. Distinct and dynamic auxin activities during reproductive development. *Cold Spring Harb. Perspect. Biol.* **1**, a001628-a001628, doi:10.1101/cshperspect.a001628 (2009).
24 Vanneste, S. & Friml, J. Auxin: a trigger for change in plant development. *Cell* **136**, 1005-1016, doi:10.1016/j.cell.2009.03.001 (2009).
25 Melo, M. C., Grealy, A., Brittain, B., Walter, G. M. & Ortiz-Barrientos, D. Strong extrinsic reproductive isolation between parapatric populations of an Australian groundsel. *New Phytol.* **203**, 323-334, doi:10.1111/nph.12779 (2014).
26 Richards, T. J. & Ortiz-Barrientos, D. Immigrant inviability produces a strong barrier to gene flow between parapatric ecotypes of *Senecio lautus*. *Evolution* **70**, 1239-1248, doi:10.1111/evo.12936 (2016).
27 Richards, T. J., Walter, G. M., McGuigan, K. & Ortiz-Barrientos, D. Divergent natural selection drives the evolution of reproductive isolation in an Australian wildflower. *Evolution* **70**, 1993-2003, doi:10.1111/evo.12994 (2016).
28 Walter, G. M. *et al.* Diversification across a heterogeneous landscape. *Evolution* **70**, 1979-1992, doi:10.1111/evo.13009 (2016).
29 Walter, G. M., Wilkinson, M. J., Aguirre, J. D., Blows, M. W. & Ortiz-Barrientos, D. Environmentally induced development costs underlie fitness tradeoffs. *Ecology* **99**, 1391-1401, doi:doi:10.1002/ecy.2234 (2018).
30 James, M. E., Arenas-Castro, H., Groh, J. S., Engelstädter, J. & Ortiz-Barrientos, D. Highly replicated evolution of parapatric ecotypes. *bioRxiv*, 2020.2002.2005.936401, doi:10.1101/2020.02.05.936401 (2020).
31 Roda, F. *et al.* Genomic evidence for the parallel evolution of coastal forms in the *Senecio lautus* complex. *Mol. Ecol.* **22**, 2941-2952, doi:10.1111/mec.12311 (2013).
32 Fishman, L. & Willis, J. H. Evidence for Dobzhansky-Muller incompatibilites contributing to the sterility of hybrids between *Mimulus guttatus* and *M. nasutus*. *Evolution* **55**, 1932-1942 (2001).
33 Stelkens, R. B., Schmid, C. & Seehausen, O. Hybrid breakdown in cichlid fish. *PLOS One* **10**, e0127207, doi:10.1371/journal.pone.0127207 (2015).
34 Michelmore, R. W., Paran, I. & Kesseli, R. V. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA* **88**, 9828-9832, doi:10.1073/pnas.88.21.9828 (1991).
35 Petrasek, J. & Friml, J. Auxin transport routes in plant development. *Development* **136**, 2675-2688, doi:10.1242/dev.030353 (2009).
36 Roda, F., Walter, G. M., Nipper, R. & Ortiz-Barrientos, D. Genomic clustering of adaptive loci during parallel evolution of an Australian wildflower. *Mol. Ecol.* **26**, 3687–3699, doi:10.1111/mec.14150 (2017).
37 Tan, L. *et al.* Control of a key transition from prostrate to erect growth in rice domestication. *Nat. Genet.* **40**, 1360-1364, doi:10.1038/ng.197 (2008).
38 Li, P. *et al.* LAZY1 controls rice shoot gravitropism through regulating polar auxin transport. *Cell Res.* **17**, 402-410, doi:10.1038/cr.2007.38 (2007).
39 Lopez, D., Tocquard, K., Venisse, J.-S., Legué, V. & Roeckel-Drevet, P. Gravity sensing, a largely misunderstood trigger of plant orientated growth. *Front. Plant Sci.* **5**, doi:10.3389/fpls.2014.00610 (2014).
40 Blancaflor, E. B. Regulation of plant gravity sensing and signaling by the actin cytoskeleton. *Am. J. Bot.* **100**, 143-152, doi:10.3732/ajb.1200283 (2013).
41 Yamamoto, M. & Yamamoto, K. T. Differential effects of 1-naphthaleneacetic acid, indole-3-acetic acid and 2,4-dichlorophenoxyacetic acid on the gravitropic response of roots in an auxin-resistant mutant of *Arabidopsis*, *auxl*. *Plant Cell Physiol.* **39**, 660-664 (1998).

42 Ottenschläger, I. *et al.* Gravity-regulated differential auxin transport from columella to lateral root cap cells. *Proc. Natl. Acad. Sci. USA* **100**, 2987-2991, doi:10.1073/pnas.0437936100 (2003).
43 Ranocha, P. *et al.* Walls are thin 1 (WAT1), an *Arabidopsis* homolog of *Medicago truncatula* NODULIN21, is a tonoplast-localized protein required for secondary wall formation in fibers. *The Plant Journal* **63**, 469-483, doi:10.1111/j.1365-313X.2010.04256.x (2010).
44 Promchuea, S., Zhu, Y., Chen, Z., Zhang, J. & Gong, Z. ARF2 coordinates with PLETHORAs and PINs to orchestrate ABA-mediated root meristem activity in *Arabidopsis*. *J. Integr. Plant Biol.* **59**, 30-43, doi:10.1111/jipb.12506 (2017).
45 Wu, H., Shen, Y., Hu, Y., Tan, S. & Lin, Z. A phytocyanin-related early nodulin-like gene, BcBCP1, cloned from *Boea crassifolia* enhances osmotic tolerance in transgenic tobacco. *J. Plant Physiol.* **168**, 935-943, doi:10.1016/j.jplph.2010.09.019 (2011).
46 Ma, H., Zhao, H., Liu, Z. & Zhao, J. The phytocyanin gene family in rice (Oryza sativa L.): Genome-wide identification, classification and transcriptional analysis. *PLOS One* **6**, e25184, doi:10.1371/journal.pone.0025184 (2011).
47 Garcia de la Garma, J. *et al.* New insights into plant salt acclimation: the roles of vesicle trafficking and reactive oxygen species signalling in mitochondria and the endomembrane system. *New Phytol.* **205**, 216-239, doi:10.1111/nph.12997 (2015).
48 Mittler, R. & Blumwald, E. The roles of ROS and ABA in systemic acquired acclimation. *The Plant Cell* **27**, 64-70 (2015).
49 Hou, Y. *et al.* Maternal ENODLs are required for pollen tube reception in *Arabidopsis*. *Curr. Biol.* **26**, 2343-2350, doi:10.1016/j.cub.2016.06.053 (2016).
50 Walter, G. M. *et al.* Loss of ecologically important genetic variation in late generation hybrids reveals links between adaptation and speciation. *Evolution Letters* **4**, 302-316, doi:10.1002/evl3.187 (2020).
51 Beeftink, W., Rozema, J. & Huiskes, A. *Ecology of coastal vegetation*. Vol. 6 (Springer, 1985).
52 Auld, T. & Morrison, D. Genetic determination of erect and prostrate growth habit in five shrubs from windswept headlands in the Sydney region. *Aust. J. Bot.* **40**, 1-11 (1992).
53 Morrison, D. & Rupp, A. Patterns of morphological variation within *Acacia suaveolens* (Mimosaceae). *Aust. Syst. Bot.*, 1013-1027 (1995).
54 Crutsinger, G. M., Strauss, S. Y. & Rudgers, J. a. Genetic variation within a dominant shrub species determines plant species colonization in a coastal dune ecosystem. *Ecology* **91**, 1237-1243 (2010).
55 Li, T. *et al.* Calcium signals are necessary to establish auxin transporter polarity in a plant stem cell niche. *Nature Communications* **10**, 726, doi:10.1038/s41467-019-08575-6 (2019).
56 Naser, V. & Shani, E. Auxin response under osmotic stress. *Plant Mol. Biol.* **91**, 661-672, doi:10.1007/s11103-016-0476-5 (2016).
57 van den Berg, T., Korver, R. A., Testerink, C. & ten Tusscher, K. H. Modeling halotropism: a key role for root tip architecture and reflux loop remodeling in redistributing auxin. *Development* **143**, 3350-3362 (2016).
58 Galvan-Ampudia, C. S. *et al.* Halotropism is a response of plant roots to avoid a saline environment. *Curr. Biol.* **23**, 2044-2050 (2013).
59 Lowry, D. B. & Willis, J. H. A widespread chromosomal inversion polymorphism contributes to a major life-history transition, local adaptation, and reproductive isolation. *PLoS Biol.* **8**, e1000500, doi:10.1371/journal.pbio.1000500 (2010).
60 Lowry, D. B., Popovic, D., Brennan, D. J. & Holeski, L. M. Mechanisms of a locally adaptive shift in allocation among growth, reproduction, and herbivore resistance in *Mimulus guttatus**. *Evolution* **73**, 1168-1181, doi:10.1111/evo.13699 (2019).
61 Lowry, D. B., Hall, M. C., Salt, D. E. & Willis, J. H. Genetic and physiological basis of adaptive salt tolerance divergence between coastal and inland *Mimulus guttatus*. *New Phytol.* **183**, 776-788, doi:10.1111/j.1469-8137.2009.02901.x (2009).

941 62 Turelli, M. & Moyle, L. C. Asymmetric Postmating Isolation: Darwin's Corollary to Haldane's
942 Rule. *Genetics* **176**, 1059, doi:10.1534/genetics.106.065979 (2007).
943 63 Clarke, J. D. Cetyltrimethyl ammonium bromide (CTAB) DNA miniprep for plant DNA
944 isolation. *Cold Spring Harb. Protoc.* **2009**, pdb.prot5177, doi:10.1101/pdb.prot5177 (2009).
945 64 Liu, H. *Developing genomic resources for an emerging ecological model species Senecio*
946 *lautus* PhD Thesis thesis, The University of Queensland, (2015).
947 65 Koren, S. *et al.* Canu: scalable and accurate long-read assembly via adaptive k-mer weighting
948 and repeat separation. *Genome Res.* **27**, 722-736, doi:10.1101/gr.215087.116 (2017).
949 66 Ali, S. *Senecio lautus* complex in Australia. I. Taxonomic considerations and discussion of
950 some of the related taxa from New Zealand. *Aust. J. Bot.* **12**, 282-291,
951 doi:10.1071/BT9640282 (1964).
952 67 Rockman, M. V. & Kruglyak, L. Breeding designs for recombinant inbred advanced intercross
953 lines. *Genetics* **179**, 1069-1078, doi:10.1534/genetics.107.083873 (2008).
954 68 Baird, N. A. *et al.* Rapid SNP discovery and genetic mapping using sequenced RAD markers.
955 *PLOS One* **3**, 1-7, doi:10.1371/journal.pone.0003376 (2008).
956 69 Kofler, R., Pandey, R. V. & Schlotterer, C. PoPoolation2: identifying differentiation between
957 populations using sequencing of pooled DNA samples (Pool-Seq). *Bioinformatics* **27**, 3435-
958 3436, doi:10.1093/bioinformatics/btr589 (2011).
959 70 Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows–Wheeler transform.
960 *Bioinformatics* **25**, 1754-1760 (2009).
961 71 Li, H. *et al.* The sequence alignment/map format and SAMtools. *Bioinformatics* **25**, 2078-
962 2079 (2009).
963 72 Rosenzweig, B. K., Pease, J. B., Besansky, N. J. & Hahn, M. W. Powerful methods for
964 detecting introgressed regions from population genomic data. *Mol. Ecol.* **25**, 2387-2397
965 (2016).
966 73 Benfey, P. N. & Mitchell-Olds, T. From Genotype to Phenotype: Systems Biology Meets
967 Natural Variation. *Science* **320**, 495-497, doi:10.1126/science.1153716 (2008).
968 74 Futschik, A. & Schlötterer, C. The next generation of molecular markers from massively
969 parallel sequencing of pooled DNA samples. *Genetics* **186**, 207-218,
970 doi:10.1534/genetics.110.114397 (2010).
971 75 Magwene, P. M., Willis, J. H. & Kelly, J. K. The statistics of bulk segregant analysis using next
972 generation sequencing. *PLoS Comp. Biol.* **7**, e1002255, doi:10.1371/journal.pcbi.1002255
973 (2011).
974 76 R Core Team. R: A language and environment for statistical computing. *R Foundation for*
975 *Statistical Computing, Vienna, Austria* (2013).
976 77 Simms, D., Cizdziel, P. E. & Chomczynski, P. J. F. TRIzol: A new reagent for optimal single-step
977 isolation of RNA. **15**, 532-535 (1993).
978 78 Schroeder, A. *et al.* The RIN: an RNA integrity number for assigning integrity values to RNA
979 measurements. *BMC Mol. Biol.* **7**, 3 (2006).
980 79 Kim, D., Langmead, B. & Salzberg, S. L. HISAT: a fast spliced aligner with low memory
981 requirements. *Nat. Methods* **12**, 357-360, doi:10.1038/nmeth.3317 (2015).
982 80 Pertea, M. *et al.* StringTie enables improved reconstruction of a transcriptome from RNA-seq
983 reads. *Nat. Biotechnol.* **33**, 290-295, doi:10.1038/nbt.3122 (2015).
984 81 Pertea, M., Kim, D., Pertea, G. M., Leek, J. T. & Salzberg, S. L. Transcript-level expression
985 analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nat. Protoc* **11**, 1650-
986 1667, doi:10.1038/nprot.2016.095 (2016).
987 82 Huang da, W., Sherman, B. T. & Lempicki, R. A. Systematic and integrative analysis of large
988 gene lists using DAVID bioinformatics resources. *Nat. Protoc* **4**, 44-57,
989 doi:10.1038/nprot.2008.211 (2009).

83 Huang da, W., Sherman, B. T. & Lempicki, R. A. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res.* **37**, 1-13, doi:10.1093/nar/gkn923 (2009).

84 Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **9**, 671-675 (2012).

85 Sang, D. *et al.* Strigolactones regulate rice tiller angle by attenuating shoot gravitropism through inhibiting auxin biosynthesis. *Proc. Natl. Acad. Sci. USA* **111**, 11199-11204, doi:10.1073/pnas.1411859111 (2014).

86 Rigo, G. *et al.* Inactivation of plasma membrane-localized CDPK-RELATED KINASE5 decelerates PIN2 exocytosis and root gravitropic response in *Arabidopsis*. *The Plant Cell* **25**, 1592-1608, doi:10.1105/tpc.113.110452 (2013).

87 Rashotte, A. M., Brady, S. R., Reed, R. C., Ante, S. J. & Muday, G. K. Basipetal auxin transport is required for gravitropism in roots of *Arabidopsis*. *Plant Physiol.* **122**, 481-490, doi:10.1104/pp.122.2.481 (2000).

88 Yang, Y., Hammes, U. Z., Taylor, C. G., Schachtman, D. P. & Nielsen, E. High-affinity auxin transport by the AUX1 influx carrier protein. *Curr. Biol.* **16**, 1123-1127, doi:10.1016/j.cub.2006.04.029 (2006).

89 Walter, G. M. *The genetic and ecological basis of diversification* PhD Thesis thesis, The University of Queensland, (2016).

90 Lynch, M. & Walsh, B. *Genetics and analysis of quantitative traits*. (Sinaeur Associates Inc, 1998).

91 Geyer, C. J., Wagenius, S. & Shaw, R. G. Aster models for life history analysis. *Biometrika* **94**, 415-426 (2007).

92 Shaw, R. G., Geyer, C. J., Wagenius, S., Hangelbroek, H. H. & Etterson, J. R. Unifying life-history analyses for inference of fitness and population growth. *The American Naturalist* **172**, E35-E47, doi:10.1086/588063 (2008).

93 Langmead, B., Trapnell, C., Pop, M. & Salzberg, S. L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biology* **10**, R25, doi:10.1186/gb-2009-10-3-r25 (2009).

94 Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment search tool. *J. Mol. Biol.* **215**, 403-410, doi:10.1016/s0022-2836(05)80360-2 (1990).

Figures and Tables

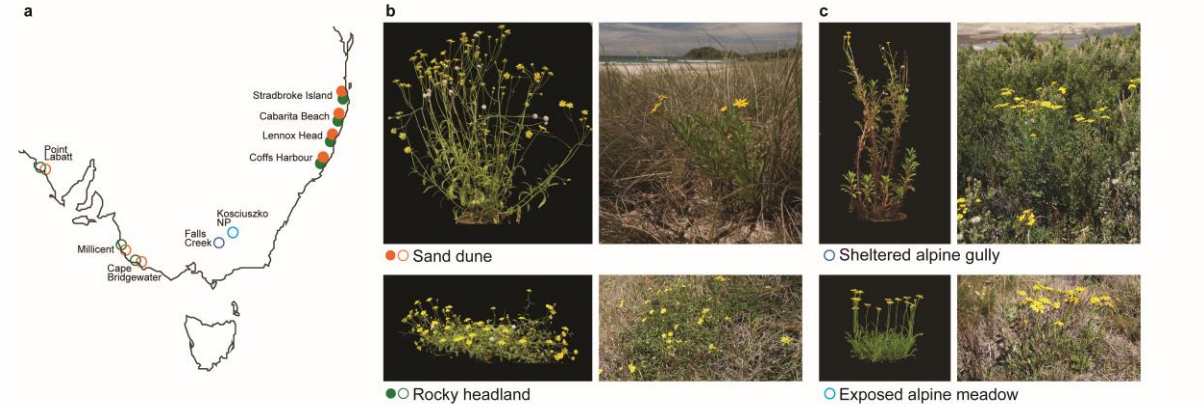

Fig. 1. Sample locations and growth habit differences between adjacent *Senecio lautus* populations. a, Map of Australia showing locations of the 16 populations used in this study. The seven coastal localities have a Dune (orange) and Headland (green) population occurring adjacent to each other. The populations are split into two monophyletic clades[31], the eastern clade (closed circles) and the south-eastern clade (open circles). **b,** *Senecio lautus* native to the sand dunes have an erect growth habit and *S. lautus* native to the rocky headlands have a prostrate growth habit. **c,** Alpine populations of *S. lautus* include a sheltered alpine gully and a wind exposed alpine meadow, containing individuals with an erect and prostrate growth habit, respectively.

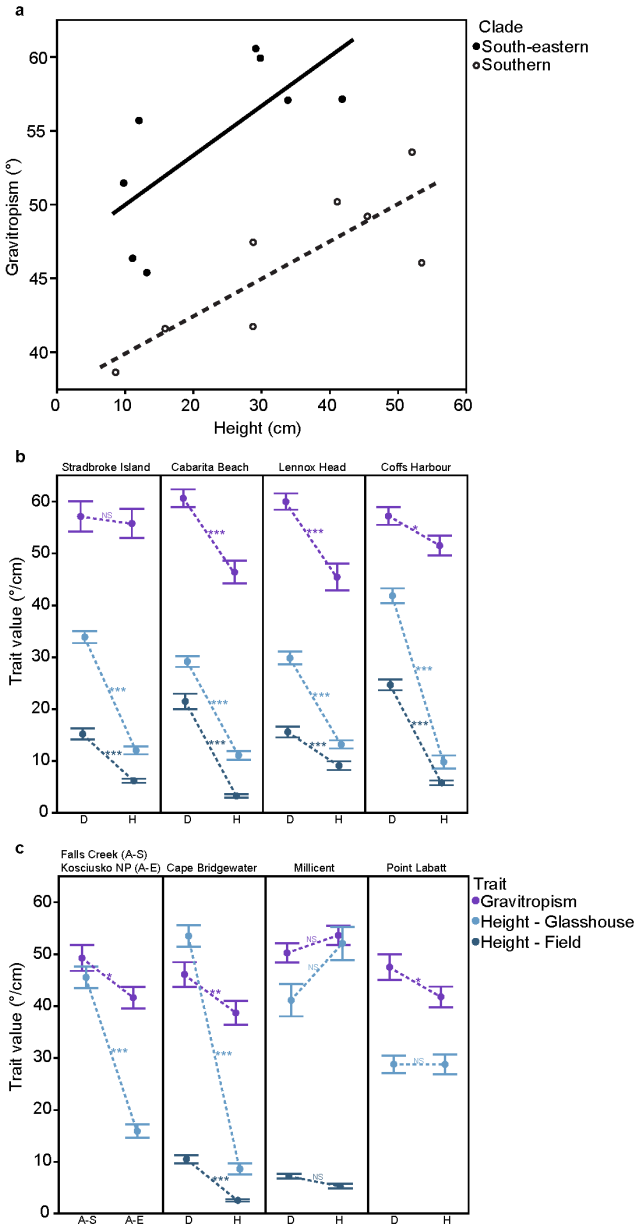

Fig. 2. Gravitropism and height variation across 16 *Senecio lautus* populations. a, The correlation between gravitropism and height across *S. lautus* populations split into their monophyletic clades – see Fig. 1 for details. Each point in the graph represents a population mean where height was measured in the glasshouse and gravitropism was measured 24 hours after a 90° rotation. **b**,**c**, Divergence in gravitropism, height in the glasshouse and height in the field between adjacent *S. lautus* populations (D = Dune, H = Headland, A-S=Alpine Sheltered and A-E = Alpine Exposed). **b**, eastern clade and **c**, south-eastern clade. Height in the field for Falls Creek, Kosciusko NP and Point Labatt were not measured. Data are mean ± s.e.m.; one tailed Student's t-test (Table S8), *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, NS not significant.

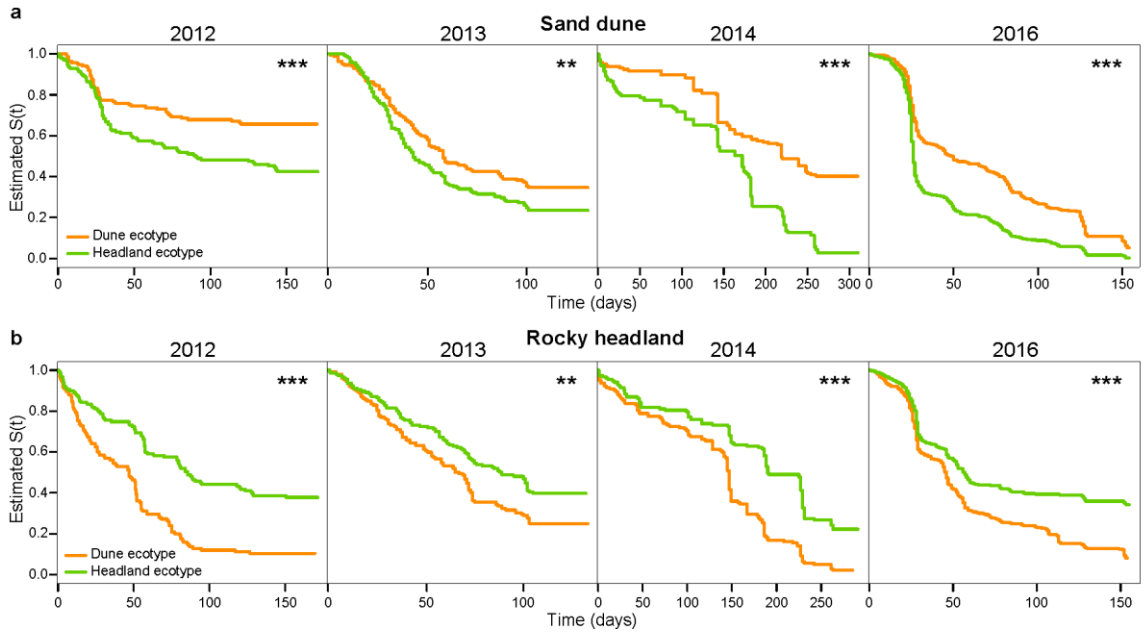

Fig. 3. Parental survival curves in the height (2016) and gravitropism (2012-2014) adaptation experiments at the sand dune and rocky headland at Lennox Head. a,**b**, Survival (Estimated S(t)) over the length of the field experiments is shown for the Lennox Head Dune population (orange) and the Lennox Head Headland population (green) for four independent field selection experiments at the **a**, sand dune and **b**, rocky headland. Asterisks indicate a significant difference in mortality risk between the Dune and Headland ecotypes (**P ≤ 0.01, ***P ≤ 0.001).

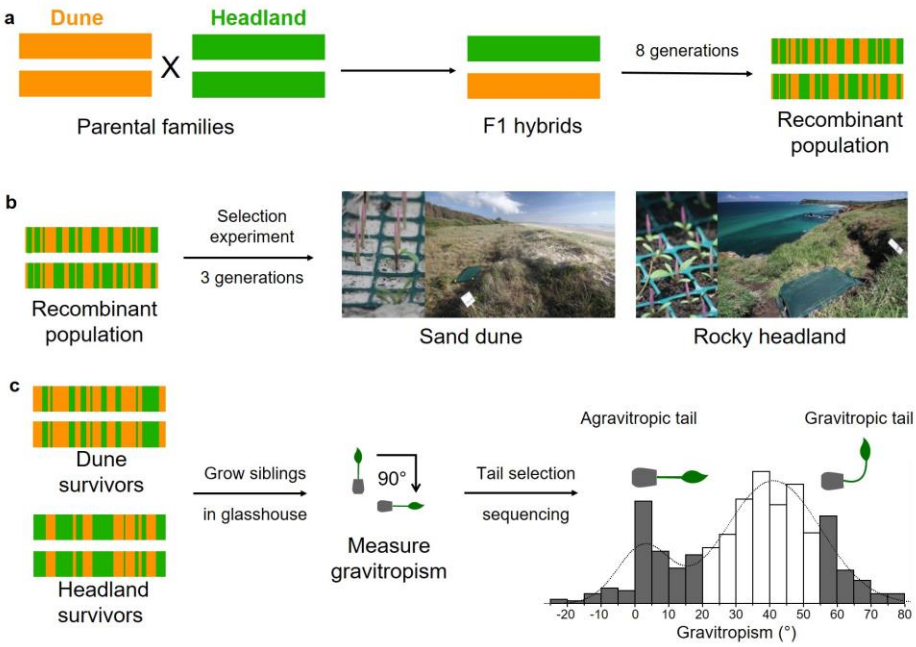

Fig. 4. The creation of the recombinant hybrid generation, the design of the gravitropism adaptation experiments and sequencing of the tails of the gravitropic distribution. a, 23 parental Dune and 22 parental Headland individuals from Lennox Head were crossed randomly and with equal contribution for eight generations. **b**, Seeds from this F8 recombinant population were glued to toothpicks and transplanted into the sand dune and rocky headland at Lennox Head. Among-family based selection occurred for three generations (F8, F9 and F10), where full-siblings from the fittest families were grown in the glasshouse and crossed amongst their respective genetic lines (A, B and C) and their environment (Dune survivors or Headland survivors). An inbred control was kept in the glasshouse and underwent the same crossing scheme but free from viability selection. **c**, Gravitropism was measured in the F11 recombinant population by re-orientating the plant by 90°. Here, agravitropic plants are define as individuals with gravitropic angles <20°, while gravitropic plants have gravitropic angles >56° as they reorient their growth and subsequently grow upright. Individuals in the tails of the gravitropism distribution were sequenced on four lanes of the Illumina HiSeq 4000 platform.

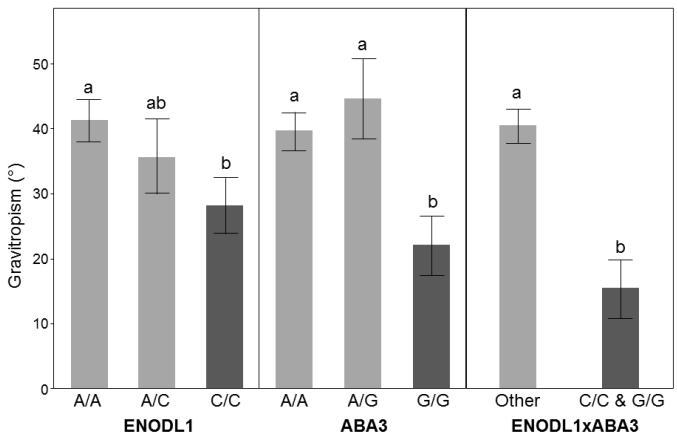

Fig. 5. Association between *ENODL1* and *ABA3* alleles and gravitropism in *Senecio lautus.* The average gravitropism angle is shown for each allelic combination, independently and when the agravitropic alleles (dark grey) are combined. Different letters denote significant differences between genotypes (t>1.98, P<0.05).

Table 1. General linear model for the effect of dam and sire on gravitropism (°) after a field selection experiment on a recombinant hybrid Dune and Headland population. Field selection experiments were performed on F8, F9 and F10 recombinant hybrid generations to achieve three rounds of selection in the sand dune and rocky headland at Lennox Head (see Fig. 4 for the experimental design). Dam and sire fitness are the F10 family fitness values for the individuals that were crossed to create the F11 offspring where gravitropism was measured. Intrinsic viability is the number of days until death of the F11 generation in the controlled temperature room. This experiment was conducted three times (temporal block) with three independent genetic lines.

	Dune				Headland			
Source	DF	SS	F-Ratio	P-value	DF	SS	F-Ratio	P-value
Dam family fitness	6	8515.77	4.779	**<0.001**	6	1884.31	0.701	0.650
Sire family fitness	6	1806.62	1.014	0.424	5	2315.36	1.033	0.405
Intrinsic viability	1	260.85	0.878	0.352	1	5209.38	11.624	**0.001**
Genetic lines	3	1135.49	1.275	0.290	3	3357.19	2.497	0.067
Temporal block	2	193.14	0.325	0.724	2	2234.96	2.494	0.090

DF, degrees of freedom; SS, sum of squares.