Divergence in hormone signaling drives local adaptation and intrinsic reproductive isolation

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Abstract

Natural selection is a major driver of both adaptation and speciation. Whether or not the processes driving adaptation and speciation share a molecular basis remains largely unknown. Here, we show that divergence in hormone signaling contributed to the evolution of complex adaptations and intrinsic reproductive isolation in an Australian wildflower, *Senecio lautus*. We provide evidence that differences in the auxin pathway, a hormone signaling pathway required for plant growth and development, has led to the repeated evolution of erect and prostrate forms to contrasting coastal environments. Using multiple hybrid and natural populations, we show that adjacent erect and prostrate populations have repeatedly diverged in auxin-related genes and auxin-dependent phenotypes, such as gravitropism. Analysis of an advanced hybrid population in a multi-year field selection experiment revealed that high fitness families produce offspring with the local gravitropic response. We found that two auxin-related genes explained 45% of the variation in gravitropism in hybrid individuals. Remarkably, crossing hybrid individuals with extreme differences in gravitropism significantly reduces their ability to produce seed, showing gravitropism is genetically correlated with intrinsic reproductive isolation. Together, our results suggest that divergence in hormonal pathways can drive rapid adaptation to contrasting environments and to the origin of new species.

Introduction

Ever since Darwin's work on the origin of new species by natural selection (1), a fundamental question in evolutionary biology remains unanswered: do adaptation and speciation share a common genetic basis? Although several genes that impact speciation have been identified (2), we still remain ignorant as to whether genes driving speciation have evolved as a by-product of local adaptation. One notable exception exists in *Mimulus* populations adapted to copper mines where genetically linked loci for local adaptation and hybrid mortality were found (3). Here, we introduce a novel system and a powerful way to uncover the molecular link between local adaptation and intrinsic reproductive isolation by studying these processes in populations that have repeatedly and independently adapted to contrasting environments. These highly replicated systems imply a role for natural selection in the evolution of complex morphological or physiological differences (4, 5), thus providing a unique opportunity to examine if new species originate via the incidental evolution of intrinsic barriers to reproduction between locally adapted populations.

We examine the genetics of repeated evolution of growth habit and its contribution to the formation of intrinsic reproductive barriers between erect and prostrate populations of an Australian wildflower, *Senecio lautus* (G.Forst. ex Willd) (6, 7). A previous population genetic study in *S. lautus* found that sets of genes related by similar functions were repeatedly differentiated in populations with these contrasting growth habits (8). One of these sets contained genes belonging to the auxin pathway, where genes involved in the polar transport of this key plant hormone had repeatedly diverged between erect and prostrate populations. Transport and regulation of auxin is required to create an auxin-concentration gradient that alters the directional growth of a plant (9) and contributes to variation in height (10, 11), branching (12, 13) and reproduction (14). These traits distinguish the erect and prostrate growth habits of *S. lautus* populations, making the auxin pathway a natural candidate to link the molecular basis of both adaptation and speciation. We reasoned that if divergence in the auxin pathway contributed to the evolution of adaptation and speciation in *S. lautus*, we would make the following three observations. First, we would detect similar patterns of genetic divergence in auxin-related pathways across multiple erect and prostrate populations. Second, these populations would differ in auxin-dependent phenotypes, such as their ability to alter the direction of growth in

relation to gravity (9, 15). Finally, divergence in these auxin-dependent phenotypes would contribute to local adaptation and intrinsic reproductive isolation between populations.

We test these hypotheses primarily on coastal populations of *S. lautus* (Fig. 1a, Table S1), which exhibit strong correlations between growth habit and the environments they occupy (6). 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 a sheltered alpine gully (Fig. 1c). Dune populations are continually exposed to high temperatures and sun radiation, low salinity, and low nutrient sand substrate, whereas Headland populations are exposed to high salinity, high nutrients and powerful winds (16). Neighboring Dune and Headland populations are often sister taxa, group into two major monophyletic clades (eastern and south-eastern) and have evolved their contrasting growth habits independently multiple times (6, 17). These Dune and Headland population pairs are locally adapted (16-19) and their F2 hybrids have low fitness (20), indicating the presence of intrinsic reproductive isolation. Furthermore, performing genetic, physiological, and ecological experimental studies is achievable in this system due to its short life cycle and small vegetative size. Therefore, the evolution of growth habit in *S. lautus* provides an excellent system in which to understand the shared molecular basis of adaptation and intrinsic reproductive isolation.

Results

Repeated evolution of auxin-related genes in *S. lautus*

To investigate whether there are similar patterns of genetic divergence in auxin-related pathways across erect and prostrate populations, we assessed the genes that govern variation in three traits that distinguish growth habit in *S. lautus* (plant vegetative height, main stem angle and the number of tertiary branches; Fig. S1). We performed a Bulk Segregant Analysis (BSA) (21) in four independent mapping populations that represent replicates for the evolution of the erect and prostrate morphologies in *S. lautus* (22) (Fig. S2). For each mapping population, we pooled individuals from the tails of each of the three trait distributions and created reduced representation libraries for

Genotyping-by-Sequencing. In agreement with a previous study in *S. lautus* (8), we found that highly differentiated genomic regions (candidates) between extreme forms were often not the same across mapping populations (Data S1). Only 20 candidate regions were shared across all four mapping populations: 14 candidates (out of 2,648) for main stem angle, six candidates for branch number (out of 1,782) and zero candidates for height (out of 1,198), suggesting repeated evolution at the gene level is limited. However, we found that 11 out of these 20 candidate regions contained genes regulating the auxin pathway (Table S2), implying evolution might have repeatedly occurred in hormone signaling pathways. Four of these 11 candidate regions contained multiple auxin-related genes, which could indicate the existence of pleiotropy or clusters of genes controlling variation in multiple adaptive traits. Auxin candidate genes from the BSA also showed footprints of natural selection such as genetic differentiation between adjacent populations, and significant associations with survivorship in reciprocal transplants (Table S2 and Data S1). This suggests that the auxin pathway might be important for adaptation to sand dunes and rocky headlands. Several of these auxin genes also displayed differential expression between shoot apical meristems of erect and prostrate populations (Fig. S3), providing further evidence for their involvement in mediating plant architecture differences. The homologs of BSA auxin candidate genes include genes largely 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) (15, 23). Some homologs also have pleiotropic effects on reproduction (Table S2), which is consistent with a previous mapping experiment where quantitative trait loci were the same for plant architecture and hybrid fecundity between erect and prostrate populations of *S. lautus* (24). This indicates that a genetic link between complex adaptations and reproductive barriers between genetically and morphologically divergent populations might exist in *S. lautus*.

The physiological basis of repeated evolution in *S. lautus*

Since we identified a multitude of auxin related genes associated with erect and prostrate phenotypes, and that the regulation and transport of auxin is a well-established modulator of gravitropism in plants, we predicted that the evolution of divergent growth habits in many plants may be a direct consequence of changes in the auxin pathway (25, 26). If this is true in *S. lautus* then plant height should predict gravitropism across populations, and differences in gravitropism would have

occurred repeatedly between locally adapted populations with contrasting morphologies. Additionally, synthetic auxin and auxin transport inhibitors should influence gravitropism differently between these erect and prostrate populations.

To test whether plant height predicts gravitropism across populations, we first quantified plant height and gravitropism in 16 *S. lautus* populations (Fig. 1 and Fig. S4). We defined the gravitropic response as the change in angle of a seedlings stem 24 hours after a 90° rotation (27, 28), where 0° is no gravitropic response and 90° is a complete re-orientation in relation to the new gravity vector. If differences in the auxin pathway have contributed to divergence in growth habit, we expect that prostrate populations will display a smaller gravitropic angle than their adjacent erect population. First, we show that height has a genetic basis in *S. lautus* by confirming that plant height in natural environments (field) and plant height in common garden conditions (glasshouse) are strongly correlated ($F_{1,9}$=12.41, p=0.0065). Next, we found that the average height of a population predicted its average gravitropic angle (Fig. 2a; $F_{1,13}$=17.65, p=0.0010), where the average magnitude of gravitropism differed between the two monophyletic clades ($F_{1,13}$=32.58, p<0.0001). These results suggest that changes in gravitropism are genetically correlated with divergent growth habit traits such as plant height, and that this relationship has evolved at least twice in the system.

To test whether differences in gravitropism have occurred repeatedly between locally adapted populations with differences in plant height, we 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 (22). In the population pair at Stradbroke Island, however, we observed differences in height but not in gravitropism (Fig. 2b, c), suggesting that similar plant architectures could have evolved through other developmental routes in coastal populations of *S. lautus*. We also assessed height and gravitropism in divergent populations from the alpine region of Australia, and 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-dependent genes contribute to differences in gravitropism across coastal and alpine populations in this system.

To directly test whether synthetic auxin and auxin transport inhibitors will influence gravitropism differently between erect and prostrate populations, we grew Lennox Head Dune and Headland seeds with synthetic auxin 2,4-D (2,4-Dichlorophenoxyacetic acid), and polar auxin transport inhibitor NPA (naphthylphthalamic acid) (29, 30). Because a gravitropic response requires an auxin concentration gradient, we reasoned that removing the gradient would reduce the gravitropic angle in Dune individuals (Fig. S5). As expected, addition of synthetic auxin 2,4-D reduced the gravitropic angle more in Dune individuals (from 66.34±2.27° to 19.44±7.64°) than in Headland individuals (LR chi-square χ^2_2=18.49, p<0.0001). Similarly, addition of auxin transport inhibitor NPA reduced the gravitropic angle in our experiments more in Dune individuals (from 62.57±3.62° to 20.47±13.67°) than in Headland individuals (LR chi-square, χ^2_3=21.18, p<0.0001). This difference in hormone response in Dune and Headland individuals supports our hypothesis that differences in the auxin pathway underlie the different gravitropic responses in *S. lautus*.

Together, these three results reveal that genetic differences in the auxin pathway have contributed to the repeated evolution of growth habit in this system. Additionally, our findings are consistent with the hypothesis that natural selection is the major evolutionary force responsible for the physiological and morphological diversity underpinning the differences in growth habit between Dune and Headland populations.

Adaptive evolution of auxin-dependent phenotypes in *S. lautus*

Having established that auxin-related genes have diverged between erect and prostrate forms in *S. lautus,* we conducted two independent sets of field adaptation experiments to experimentally assess the role of natural selection on the evolution of auxin-dependent phenotypes, 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 favored the local Dune or Headland population over the foreign

population (Fig. 3), indicating that experimental conditions captured local adaptation in both environments.

For the height adaptation experiments, we tested whether differences in height could drive differences in fitness in the rocky headlands. We hypothesized 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 as it is likely the derived trait because the majority of populations in the *S. lautus* species complex have an erect growth habit (8, 16). We introgressed Dune alleles associated with height onto a Headland genomic background. Briefly, we crossed Lennox Head Dune and Headland parentals and then completed two rounds of backcrossing followed by one round of crossing between tall hybrid offspring and between short hybrid offspring (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, and to the formation of a genetic correlation with plant height. We hypothesized 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 the Dune and Headland populations from Lennox Head for eight generations using recombination to disassemble allelic combinations and construct a recombinant hybrid population (24) (Fig. 4a). We planted 2,403 of these F8 seeds (from 89 families) into the sand dune and rocky headland (Fig. 4b) and conducted family-based truncation selection for three generations, planting a similar number of seeds and families in the F9 and F10 generation (Table S3). We selected the top 50% of surviving families in each environment, and subsequently crossed their full-siblings in the glasshouse to produce the next generation. In the F10 generation, we tested whether families that had the greatest number of survivors also produced offspring with the local gravitropic response under laboratory

conditions. In agreement with our hypothesis, F10 families with the greatest number of survivors in the sand dune produced offspring with a higher gravitropic angle (Table 1). We discovered that the relationship between fitness of the F10 families in the sand dune and the gravitropic response in the F11 generation in the glasshouse was dependent on fitness of the F10 dam family (Table 1), suggesting maternal genotypes contribute to the evolution of gravitropism in the sand dunes. On the other hand, all F10 families in the rocky headland produced offspring with intermediate levels of gravitropism (Table 1). Notably, in the F11 recombinant Control population that was grown and crossed in the glasshouse only, height and gravitropism were not genetically correlated ($F_{1,114.3}$=0.08, p=0.7801, r^2=0.04), but 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 suggest that natural selection can act on standing genetic variation and reconstitute genetic architectures that favor a correlation between survivorship and auxin-dependent phenotypes in the environments where the coastal populations are found.

Our results imply that natural selection of hormonal pathways can aid in the rapid evolution of multiple integrated phenotypes when encountering a new selection pressure.

The genes underlying gravitropism in *S. lautus*

Having shown that natural selection drove the rapid evolution of gravitropism in *S. lautus* under experimental conditions in the field, we identified genes underlying adaptation by detecting extreme allelic differences between the F11 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 performed selective genotyping on the tails of the gravitropic distribution in the F11 recombinant populations (Fig. 4c; Dune and Headland survivors, and Control individuals) and created a gravitropism candidate gene set with allelic differences in the 99.8% quantile (0.15<*p*<0.22). We identified 55 sites (0.2% of all SNPs) across 49 genomic scaffolds in this quantile (Data S2) and discovered that these genes disproportionally represented gene ontology categories of transport and localization of molecules across and between cells (Table S4).

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) and fourth (*ABA3*; molybdenum cofactor sulfurase) most differentiated SNPs between the agravitropic and gravitropic F11 tails. In both the *ENODL1* and *ABA3* gene, the allele frequency of the F11 tails shifted in the direction of the most common allele in the native population with the same phenotype (Data S2). For instance, the alleles favored in the agravitropic F11 tail were at high frequencies in the Headland population (*ENODL1* C=0.87 and *ABA3* G=0.89), with the alternate alleles being favored in the gravitropic F11 tail and the Dune population (*ENODL1* 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 drove the evolution of this 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; 45% of the difference in gravitropism between these F11 tails). Together, these results suggest that a small gravitropic angle is a recessive phenotype, and that natural selection has targeted few genetic regions of large effect. *ENODL1* and *ABA3* have functions in gravitropism, plant height, salt tolerance and pollen tube growth across different plant species (31-37), and therefore are strong candidates to not only explain the adaptive evolution of gravitropism, but other phenotypic differences between Dune and Headland populations. Moreover, *ENODL1* was also associated with field survivorship in a previous BSA study in this system (24) and with height in one of the BSA populations we used here for genetic mapping of convergent traits.

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 on the evolution of intrinsic reproductive isolation

We are now in a unique position to address whether adaptation and speciation have a common molecular basis. Specifically, we can test whether divergent gravitropic forms are reproductively isolated. To assess this problem, we investigated the effects of gravitropism on reproductive success

in the F11 hybrids. 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. We found that crosses failed more often between F11 agravitropic and gravitropic plants (21%) than crosses within each of these tails (5%; Fisher's exact test, n=132, p=0.0079). Of the 11 failed crosses with known genotypes, only one cross had the *ENODL1* C/C genotype, none had the *ABA3* G/G genotype, and none were double homozygous for the two genes, suggesting that evolution of a small gravitropic angle possibly relied on the recessive × recessive interaction. These results imply a genetic association between adaptation and intrinsic reproductive isolation among populations that occupy contrasting environments.

Discussion

Here we have discovered that divergence in auxin-related genes contribute to the repeated evolution of growth habit in *S. lautus*, suggesting that evolution in hormone signaling could facilitate colonization of new habitats through concomitant changes in multiple developmental and architectural traits. Consistent with our results, transitions from erect to prostrate growth and the associated phenotypes of height, main stem angle and number of branches, are common in plants that colonize coastal headlands (38-41). This indicates that there are strong selective agents common to headland environments. For example, powerful winds or salty substrates could impose early selective pressure in traits controlled by auxins such as responses to mechanical cues (42) and halotropism (43-45). Further, we discovered that divergence in growth habit was genetically correlated with intrinsic reproductive isolation in *S. lautus*, implying that hormone signaling divergence might lead to pleiotropic or indirect evolution of genetic incompatibilities that reduce hybrid fitness or pollen-pistil interactions (e.g., differences in pollen tube growth (46)) that prevent fertilization. Together, these results suggest that divergence in plant hormonal pathways that affect multiple traits during plant development and reproduction could be a powerful mechanism to link the genetic, ecological and physiological basis of adaptation and speciation in plants.

The roles of hormone signaling in plant adaptation and intrinsic reproductive isolation have been explored independently but only in rare cases have the two been linked. The hormonal pathways most commonly involved in plant adaptation are the abscisic acid pathway, which is involved in stress response (47), the jasmonate signaling pathway that contributes to the growth-defense conflict (48), and the auxin pathway that responds to salt tolerance (43-45) and mechanical stress responses (42). On the other hand, hormonal pathways involved in intrinsic reproductive isolation include the cytokinin signaling pathway in hybrid sterility (49), and the auxin pathway in pollen tube growth (46). In more recent work in *Arabidopsis* (50) and *Mimulus* (51), variation in genes involved in the gibberellin pathway were associated with variation in both morphological differentiation and local adaptation to different environments. The consequences of such adaptation for the evolution of intrinsic reproductive isolation remain unclear, but at least in *Mimulus*, many of the gibberellin genes associated with local adaptation reside within a chromosomal inversion strongly associated with flowering time differences between coastal and inland populations (51, 52). Our study takes us a step further by directly linking natural selection and divergence in hormone signaling with intrinsic reproductive isolation in a highly replicated system of evolution, thus paving the way to uncover the molecular basis of repeated evolution and its consequences on the evolution of intrinsic reproductive isolation.

Overall, our study illustrates a powerful strategy to explore the genetic basis of adaptation in natural systems that takes us away from a gene-centric paradigm and moves us towards identifying sets of genes related by function. We conjecture that the evolution of hormonal pathways in plants provides a simple mechanism for the rapid formation of integrated phenotypes that facilitate colonization of new habitats, and to the incidental evolution of intrinsic reproductive isolation as a consequence.

Materials and Methods

Reference genome

Two Lennox Head Headland individuals were used for the creation of the 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 for roots to grow and then transferred to 12 hours light/day in a constant temperature room at 25°C for seven days for shoots to grow. Seedlings were then transferred into pots with standard potting mix and grown in the glasshouse. Siblings from the seeds produced were grown and crossed by rubbing flower heads together to produce a family of individuals capable of selfing (rare in *S. lautus*). One generation of selfing was completed to increase homozygosity. We extracted DNA from leaf tissue using the CTAB protocol (53) with column cleaning and overnight incubation. All sequencing was performed by Beijing Genomics Institute (BGI).

A draft genome of *S. lautus* was de novo assembled using second-generation short reads and AllPaths-LG 6.1.2 by utilizing a series of eight read libraries (Table S2). The reads were trimmed to remove residual adapter sequence 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 (54) 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 genome was de novo assembled using next-generation Pacbio long reads and CANU 1.6 (55). A total of 11,481,638 reads (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. All sequencing was performed by BGI.

Genetic mapping of convergent traits

Creation of BSA 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 (6, 8, 22, 56). 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 (57), we crossed multiple individuals from pairs of adjacent populations to create F1s (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 auxins: 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 rising from these. Trait distributions and population statistics are provided in Data S3.

Genotyping of BSA populations

Genomic DNA of these pools described above was extracted following a modified version of the CTAB protocol (53). Young leaves were collected and stored at -80°C and ground using a Tissuelyser (Qiagen, Chatsworth, CA). DNA was quantified with the Picogreen reagent (Invitrogen, Carlsbad, CA) and pools of recombinant individuals were created as described in ref. (22). For each recombinant population we pooled DNA from plants from the 10% tails of the distribution 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 (58) were created and sequenced at Floragenex (University of Oregon High Throughput Sequencing

Facility) following the methodology described in ref. (22) but increasing sequencing depth and coverage (50x depth and 2M reads / sample).

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 SNPs, determine allelic frequencies and calculate genetic differentiation between pairs of pools, RADseq data was processed using the PoPoolation2 pipeline (59). Illumina reads were mapped to the PacBio genome draft of *S. lautus* using BWA 0.7.15 (60) with default parameters. We then used SAMtools 1.5 (61) to create an mpileup file for each pair of pools (Phred quality score of 20). Finally we used the Popoolation2 pipeline 1201 (59) to extract variable positions (minimum count of 2, minimum coverage of 15, and maximum coverage of 800), estimate allelic frequency differences (62) between pools and calculate Fisher Exact tests (FET) of allelic differentiation (63-65). We thus obtained an average number of SNPs per comparison of 39,161 ± 7,244 SNPs located in 10,826 ± 1,405 genomic scaffolds. We called candidate SNPs for each comparison 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 scaffolds containing at least one candidate SNP were considered candidates for the comparison (Table S1 and Data S5).

To determine if candidates from BSA of the three traits show footprints of natural selection we re-analyzed previous pool-seq samples of natural populations (8, 22) and survivors from reciprocal transplants(24) (Table S3). We used the pipeline described above to identify candidate SNPs and contigs for pairwise comparisons between adjacent populations (8, 22) or survivors from transplants to different environments(24). 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 scaffolds were defined as described previously for both types of pairwise comparisons.

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 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 flats in a controlled temperature room at 25°C with a 12-hour light/day cycle. We sampled the shoot apical meristem (SAM) along with the youngest 3 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 (66). Total RNA was quantified using a NanoDrop (Thermo Fisher Scientific, Waltham, USA) and quality was checked using a bioanalyzer (Agilent technologies, USA) at the Australian Genome Research Facility. Only samples with RNA integrity number (67) > 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 HiSeqTM 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 (68) to align pair-end reads and Samtools 1.5 (61) to obtain a sorted BAM files. Stringtie 1.3.4d (69) was used to

assemble mapped reads of each population to transcripts, to merge the transcripts of the four populations into a non-redundant set of transcripts contained in the four populations, and to quantify transcript abundances in each of the four populations. Finally, we used the Ballgown (70) 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 BSA using databases from *Arabidopsis thaliana*, which is the plant model where most studies of auxin signaling 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 scaffolds showing associations to adaptive traits in BSA we also searched for genes not detected in the transcriptome by doing a BLATx of the scaffolds against TAIR protein database. We then retrieved the function of significant hits using the DAVID server (71, 72) (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 was grown for ~40 maternal families (1,278 seeds in total). The seeds from a population were combined in a falcon tube and were first sterilized with a quick rinse in 70% EtOH, followed by four 10 minute inversions in a sterilizing 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 agar plates containing 0.15% MS, 0.05% 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 (73) to determine gravitropism by measuring the angle to which the stem reorientated to the horizontal (74-76).

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 S11). 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 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 signaling. Specifically we used 2,4-Dichlorophenoxyacetic acid (2,4-D), a carrier-dependent synthetic auxin (29, 77) and Naphthylphthalamic acid (NPA) an efflux inhibitor (30). We collected seeds from Lennox Head sand dune and rocky headland and grew them on MS 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 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 (73), as outlined above.

Approximately 40 seeds from different maternal families were planted for each population and chemical concentration. Seeds were excluded from analyses following same procedure above, leaving a total of 188 seedlings to calculate gravitropism. Treatments where few seeds germinated were excluded (Data S12). For each chemical we used a mixed linear model using the lmer function of lme4 package in R v3.1.3(78): $y_{ijkl} = A_k + E_i + C_j + E_i \times C_j + e_{l(ijk)}$, 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 and placing 1-2mm under the ground within a grid cell (Fig. 3b) that was randomly assigned

(for details see ref. (79) and ref. (20)). 50% shade cloth was initially suspended over all plots to replicate the shade given by surrounding vegetation. Seeds were watered twice a day to keep the soil moist and replicate ideal germination conditions to maximize 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, to isolate the height alleles on a common genomic background, we introgressed the tall trait onto a headland genomic background by backcrossing the tallest F1 to a Headland parental. This created a BC1F1 generation, where the tallest individuals (n=11, tallest 20% of the population) were again backcrossed to the Headland parental, creating a BC2F1 generation. We then grew and crossed the tallest BC2F1 individuals (n=18, tallest 10% of the population) among one another and the shortest individuals (n=19, shortest 10% of the population) among one another. These BC2F2 seeds were planted into the rocky headland at Lennox Head on the 2nd 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 843 BC2F2 seeds. The entire field experiment had a total of 4,708 seeds (108 families) but the other genotypes were not relevant to this study. 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 (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 30 Lennox Head Dune and Headland individuals as described earlier. We replicated the construction of the F8 using three independent replicate crossing lines (A, B and C), all derived from the same base population. F8 seeds were planted into the sand dune and rocky headlands as described above and the 50% highest surviving families within an environment (and genetic line) were identified, and full-sibling seeds from these families were grown in the glasshouse. To produce the subsequent generations, we used an among-family based selection scheme (80) by conducting crosses among the best surviving families for both the sand dune and rocky headland separately. In crossing the surviving families, we also doubled the crosses per family, ensuring we maintained ~100 full-sibling families for each selection generation planted into the natural environments. Selection was conducted on the F8, F9 and F10 generations.

We selected the top 50% of surviving families in the F8, F9 and F10 field selection experiment using Aster modelling (81, 82) implemented with the 'Aster' package in R (78). 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, day 138 in the F9 generation, and day 232 in the F10 generation. Finally, to produce the F11 generation, we crossed the F10 generation such that survivors were designated as sires and dams. We then crossed two sires each to two dams in a full-sib, half-sib crossing design.

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 (39 Dune families, 37 Headland families and 25 inbred control families with 12 individuals per family (1,212 seeds in total were germinated). The 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 for roots to grow and then transferred to light for 4 days 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 hours light/day 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 (73) 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: $y_{ijklmn} = B_i + V_j + R_{k(i)} + D_{l(ik)} + S_{m(ik)} + e_{n(ijklm)}$, where time-block ($B_i$) is the three time points in which the F11 seeds were grown (~seven days apart); viability (V_j) is the number of days until the death of F11 plants in controlled conditions; and replicate, which consists of the three independent genetic replicates, is nested within time-block ($R_{k(i)}$). Dam fitness was nested in replicate and time-block ($D_{l(ik)}$) and sire fitness was also nested in replicate and time-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. Replicate 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 ($y_{ij}$) 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 the CTAB protocol (53) with column cleaning and overnight incubation 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 ref. (58) 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 (83) and a FASTQ quality score of above 20. 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 (84).

Senecio lautus gravitropism candidate genes

In the gravitropism candidate gene set we tested for overrepresentation of gene function and linkage disequilibrium between gravitropism loci. 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 group independently (Control population, Dune survivors and Headland survivors; Data S15).

Intrinsic reproductive isolation

We tested whether intrinsic reproductive isolation was associated with the gravitropism phenotype by randomly crossing within and between the F11 gravitropic and agravitropic groups in a controlled temperature room and recording successful and failed crosses. To maximize sample size, all three replicate genetic lines (A, B and C) were used across all three populations (Dune survivors, Headland

survivors and Control). A total of 132 crosses were completed (Data S16), 67 crosses between the tails and 65 within the tails (agravitropic tail = 28 and gravitropic tail 37). 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. A failed cross was considered when, in the presence of pollen, less than three seeds were produced per flower head from both plants with three mating attempts. We performed a two-tailed fisher's exact test in JMP 13 (SAS. 2015) to determine whether there was a significant association between cross type (within vs between gravitropism tails) and failed crosses.

Next, we tested whether intrinsic reproductive isolation was associated with ENDOL1 and *ABA3* by calculating the percentage of failed crosses with each allelic combination in the F11 population (Data S17 and Data S18).

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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 BSA mapping populations and performed BSA analyses. FR and

MJW performed physiological and molecular biology experiments. GMW reared and phenotyped the natural populations in the glasshouse and 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. RN and JW called the genotypes of the F11 population. SA assembled the current draft genome of *S. lautus*. 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.

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Figures and Tables

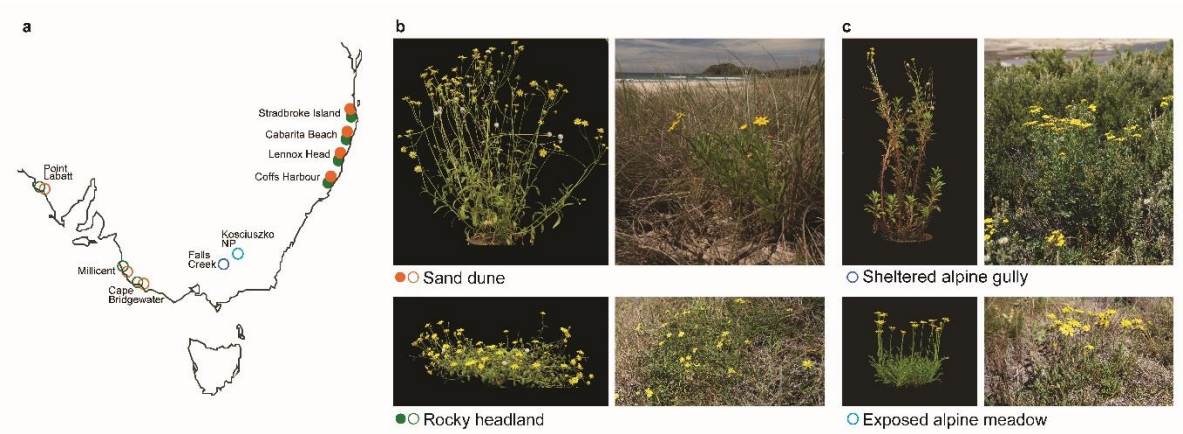

Fig. 1. Sample locations and growth habit differences between adjacent *S. lautus* populations. **a**, Map of Australia showing locations of the 16 populations used in this study. The seven coastal localities have a Dune (orange) and Headland (green) population occurring adjacent to each other. The populations are split into two monophyletic clades (22), 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.

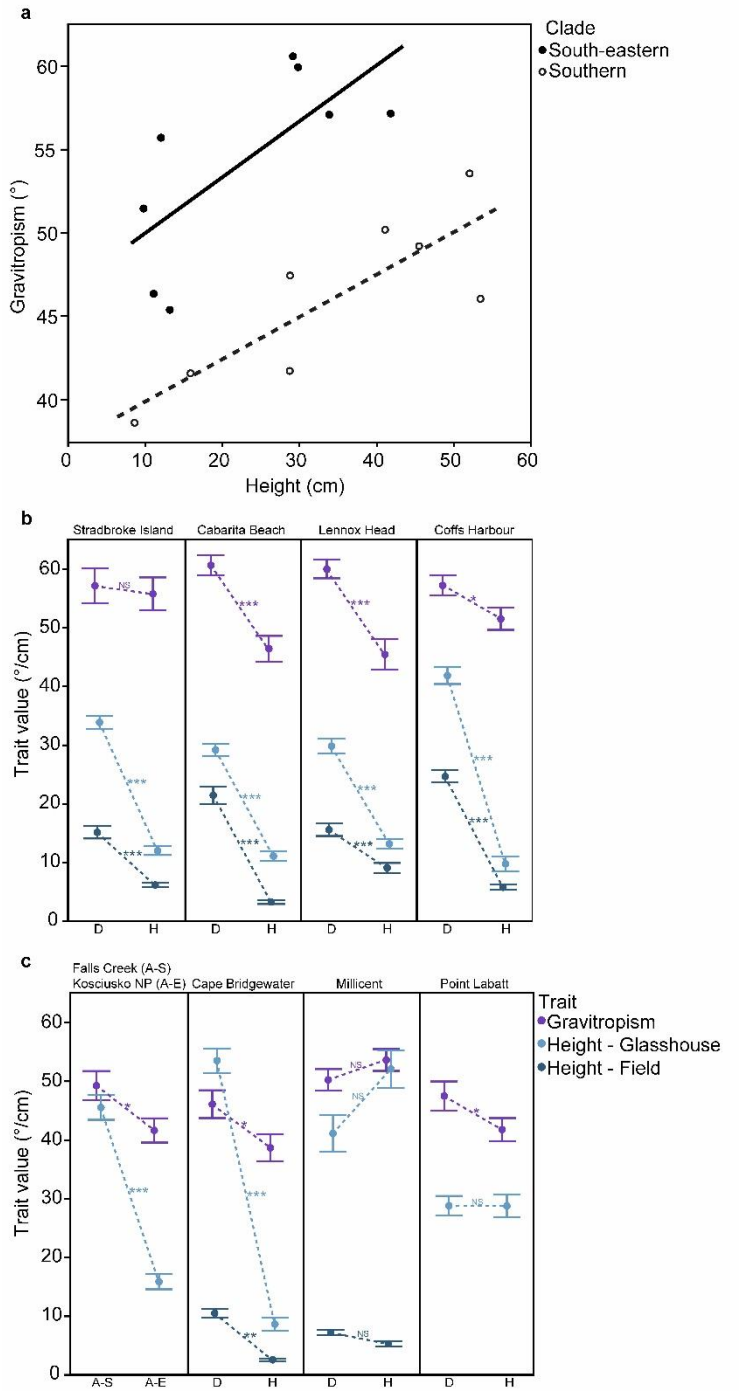

Fig. 2. Gravitropism and height variation across 16 *S. lautus* populations. a, The correlation between gravitropism and height across *S. lautus* populations split into their monophyletic clades – see Fig. 1 for details. Each point in the graph represents a population mean where height was measured 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**, South-eastern clade and **c**, southern 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, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, NS not significant.

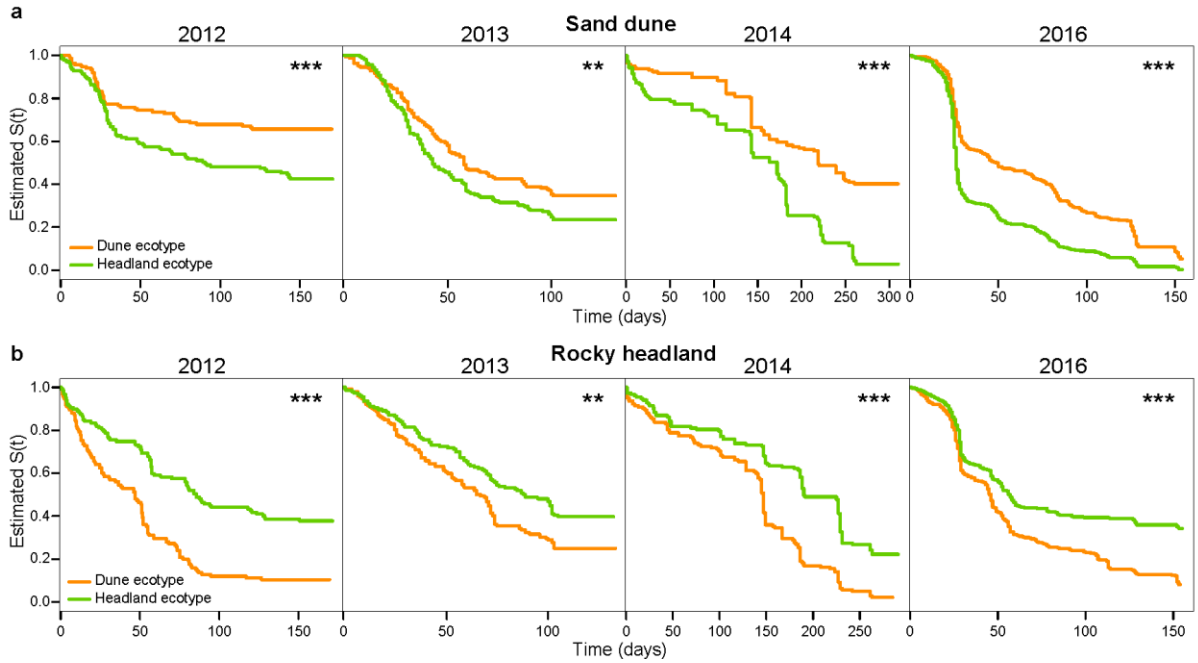

Fig. 3. Parental survival curves in the height (2016) and gravitropism (2012-2014) adaptation experiments at the sand dune and rocky headland at Lennox Head. a,**b**, Survival over the length of the field experiments is shown for the Lennox Head Dune population (orange) and the Lennox Head Headland population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

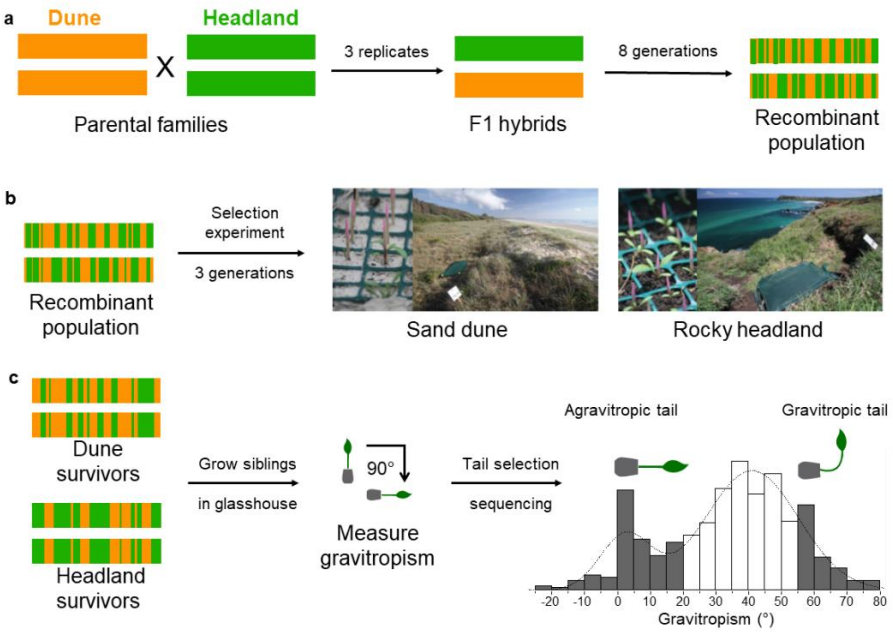

Fig. 4. The creation of the recombinant hybrid generation, the design of the gravitropism adaptation experiments and sequencing of the tails of the gravitropic distribution. a, A total of 30 parental Dune and Headland individuals from Lennox Head were crossed randomly and with equal contribution for 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°. Agravitropic plants have small gravitropic angles, while gravitropic plants have large gravitropic angles as they reorient their growth and grow upright. Individuals in the tails of the gravitropism distribution were sequenced on four lanes of the Illumina HiSeq 4000 platform.

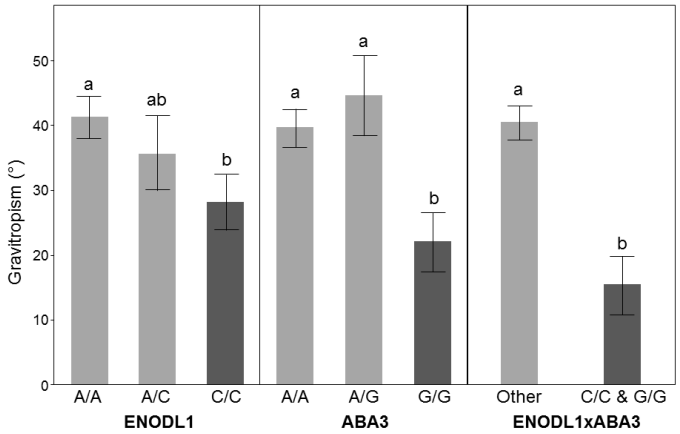

Fig. 5. Association between *ENODL1* and *ABA3* alleles and gravitropism in *S. lautus.* The average gravitropism angle is shown for each allelic combination, independently and when the agravitropic alleles (dark grey) are combined. Different letters denote significant differences between genotypes ($t>1.98$,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

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 replicates.

	Dune				Headland			
Source	DF	SS	F-Ratio	Prob > F	DF	SS	F-Ratio	Prob > F
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 replicate	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.