Adaptive divergence in shoot gravitropism creates hybrid sterility in an Australian wildflower

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Abstract

Natural selection is a significant driver of speciation. Yet it remains largely unknown whether local adaptation can drive speciation through the evolution of hybrid sterility between 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 hybrid sterility in an Australian wildflower, *<I>Senecio lautus</I>*. 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 using a hybrid population subjected to eight rounds of recombination and three rounds of selection in the field. It revealed that shoot gravitropism responds to natural selection in the expected direction of the locally adapted population. This provided an ideal platform to test whether genetic differences in gravitropism contribute to hybrid sterility in *<I>S. lautus</I>*. Using this advanced hybrid population, we discovered that crossing individuals with extreme differences in gravitropism reduce their ability to produce seed by 21%, providing strong evidence that this adaptive trait is genetically correlated with hybrid sterility. Our results suggest that natural selection can drive the evolution of locally adaptive traits that also create hybrid sterility, thus indicating an evolutionary connection between local adaptation and the origin of new species.

Significance statement

New species originate as populations become reproductively isolated from one another. Despite recent progress in uncovering the genetic basis of reproductive isolation, it remains unclear whether intrinsic reproductive barriers, such as hybrid sterility, evolve as a by-product of local adaptation to contrasting environments or evolve through non-ecological processes, such as meiotic drive. Here, we show that differences in a plant's response to the pull of gravity have repeatedly evolved amongst coastal populations of an Australian wildflower, thus implicating a role of natural selection in their evolution. We found a strong genetic correlation between variation in this adaptive trait and hybrid sterility, suggesting that intrinsic reproductive barriers contribute to the origin of new species as populations adapt to heterogeneous environments.

Main text

Introduction

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 are therefore susceptible to changes in environmental conditions. Consequently, 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 (6) and therefore are 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 (7) (i.e., Dobzhansky-Muller genetic incompatibilities). However, given the paucity of examples that directly link loci that contribute to both local adaptation and intrinsic reproductive isolation, we remain ignorant as to whether natural selection drives speciation through the concomitant evolution of extrinsic and intrinsic barriers between populations (8).

Two notable examples genetically link local adaptation and intrinsic reproductive isolation, one via pleiotropy and the other via tight genetic linkage. Selection for pathogen resistance genes in *Arabidopsis thaliana* results in a pleiotropic effect of hybrid necrosis, which dramatically lowers reproductive success (9). In contrast, the tight genetic linkage between alleles selected for copper tolerance and alleles that cause hybrid mortality in *Mimulus guttatus* 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 various functions (e.g., stress tolerance and seed development) is a powerful example of a genetic mechanism that could often drive species divergence (11), we need more genetic and ecological studies to further understand when natural selection drives the correlated evolution of local adaptation and intrinsic reproductive isolation (12). So far, most results on the genetics of intrinsic reproductive isolation suggest that several mechanisms are driving the evolution of hybrid sterility across taxa (see Presgraves (13) for a review), such as genetic conflict (e.g., the evolution of distorter genes and their suppressors) (14), and parental conflict (e.g., endosperm failure in plants) (15). However, it remains unclear whether or not the local environment has a key role in creating intrinsic reproductive isolation (see Fishman and Sweigart (16) for a review of the mechanisms), thus limiting our understanding of the mechanisms creating genetic correlations between local adaptation and intrinsic reproductive isolation.

Here, we introduce a novel system of study where we take advantage of the repeated evolution of divergent growth habits to study the contribution of local adaptation to the evolution of intrinsic reproductive barriers in an Australian wildflower, *Senecio lautus* (G. Forst. ex Willd) (17, 18). Adjacent populations exhibit erect or prostrate growth habits dependent on whether they inhabit the sand dune or rocky headlands, respectively. Previous population genetic studies in *S. lautus* found that different sets of genes related by similar functions were repeatedly differentiated between populations with these two contrasting growth habits (19, 20). 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 many plants, auxin genes are also involved in creating variation in height (21, 22), branching (23, 24) and reproduction (e.g. pollen tube growth (25)). Given the observed divergence in height and branching, with little gene flow between erect and prostrate populations of *S. lautus* (26, 27), the evolution of auxin-related genes may explain the presence of both adaptation and reproductive isolation in *S. lautus*. Studies across the plant kingdom have established that the auxin hormone has a conserved function in governing the way in which plants orient themselves to light and gravity cues (28). For example, mutant surveys in *Arabidopsis* revealed that many auxin-related genes (with functions influencing the

biosynthesis, transport or signaling of the auxin hormone) were required for shoot gravitropism - directional growth response of the shoot against the pull of gravity (23, 28-30). With this in mind, we reasoned that if divergence in auxin-related genes contributed to the evolution of local adaptation, shoot gravitropism would be divergent between adjacent erect and prostrate populations and would differentially respond to natural selection within their local environments. Finally, if local adaptation were driving the evolution of intrinsic reproductive isolation in *S. lautus*, crosses between hybrid individuals with extreme differences in an adaptive trait would be genetically incompatible and not produce seed.

We tested these three predictions on seven coastal population pairs of *S. lautus* (Fig. 1a, Fig. S1 and Table S1), where there is strong evidence of local adaptation between adjacent Dune and Headland populations (31-34). Populations inhabiting sand dunes (Dune hereafter) are usually erect, while populations growing on adjacent rocky headlands (Headland hereafter) are usually 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, high solar radiation, low salinity, and low nutrient sand substrate, whereas Headland populations are exposed to high salinity, high nutrients, and powerful winds (32). These Dune and Headland 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 (17, 27, 31, 35).

The two ecotypes also show variable levels of intrinsic reproductive isolation, where hybrid sterility in F1 Dune-Headland hybrids is generally weak. For example, there is <15% crossing failure at Lennox Head (31, 32) and <9% at Cabarita Beach (31). In contrast, hybrid sterility was found to be strong in F2 Dune-Headland hybrids from Lennox Head and an F2 generation created from four ecotypes (58% crossing failure (32, 36)). Genetic incompatibilities appear to largely be removed in the F2 generation as F3 hybrids are fertile (32, 36). Therefore, the inability to produce seed in F2's is likely due to a small number of strong recessive negative epistatic interactions between these ecotypes (37, 38). Furthermore, demographic analyses recently revealed minimal gene flow levels between adjacent Dune and Headland populations (27), indicating that reproductive barriers (intrinsic or extrinsic) have prevented hybridization from occurring in the field. The presence of reproductive isolation between multiple locally adapted erect and prostrate populations of *S. lautus* provides an excellent opportunity to understand whether local adaptation driven by morphological and physiological traits can lead to the accumulation of intrinsic reproductive barriers.

Results

Divergence in gravitropic response is auxin dependent in *Senecio lautus*

To test the hypothesis that auxin-related genes drove the evolution of gravitropic differences between erect and prostrate *S. lautus* populations, we directly examined whether synthetic auxin and auxin transport inhibitors influence gravitropism differently between the ecotypes of a population pair. 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) (39, 40). Because a gravitropic response requires an auxin concentration gradient, we reasoned that removing the gradient would reduce the gravitropic angle in Dune individuals (Fig. S2). As expected, the 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, the 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 between Dune and Headland individuals from Lennox Head, gives credence to our hypothesis that different gravitropic responses between erect and prostrate individuals reflect divergence in auxin-related genes.

158 **Repeated height and gravitropism divergence across *Senecio lautus* populations**

159 To understand whether auxin-related genes might have repeatedly diverged with growth habit
160 in *S. lautus*, we tested whether plant height (a simple growth habit trait) predicts shoot
161 gravitropism in 16 *S. lautus* natural populations (Fig. 1a, Fig. S1 and Table S1). We measured
162 the change in angle of a seedling's shoot 24 hours after a 90° rotation (29, 41), where 0° is lack
163 of a gravitropic response whereas 90° describes a complete re-orientation of the shoot and a
164 large gravitropic response. We expected that short populations would have a smaller gravitropic
165 angle than their adjacent tall population after rotation, if these traits were correlated. Across
166 these populations, the average height of a population predicted its average gravitropic angle
167 (Fig. 2a; $F_{1,13}$=17.65, P=0.0010), with the average magnitude of gravitropism differing across
168 the two monophyletic clades of this system ($F_{1,13}$=32.58, P<0.0001). In addition, we found that
169 plant height in natural environments (field) strongly correlates with estimates of plant height in
170 common garden conditions (glasshouse) ($F_{1,9}$=12.41, P=0.0065), indicating that differences in
171 plant height are genetic and do not arise from plastic responses to the environment. Therefore,
172 changes in gravitropism appear to be biologically correlated with divergent growth habit traits
173 such as plant height, and this relationship has evolved independently in each clade.

174 We then investigated which of the seven adjacent Dune and Headland (and Alpine) population
175 pairs drove the observed pattern. Four population pairs showed the expected correlation, where
176 plants from the Headland population exhibited a smaller gravitropic angle and were shorter
177 than plants from their adjacent Dune population (Fig. 2b, c). The expected pattern was also
178 observed in divergent populations from the alpine region of Australia, where the exposed Alpine
179 population in the meadow was shorter and had a smaller gravitropic angle than the population
180 in the sheltered alpine gully (Fig. 2c). In the population pair at Millicent, plant height and
181 gravitropism did not differ between Dune and Headland population pairs (Fig. 2c), possibly due
182 to their similarity in environmental variables (35). At Stradbroke Island, we observed a
183 difference in height in the expected direction but not gravitropism (Fig. 2b) and at Point Labatt,
184 we observed a difference in gravitropism in the expected direction but not height (Fig. 2c),
185 indicating that height and gravitropism are not always genetically correlated and alternate
186 genes or pathways might be utilized (e.g., gibberellin controls dwarfism in some plants (42,
187 43)). Overall, these results are consistent with divergence in auxin-related genes evolving in
188 parallel in coastal and alpine ecotypes in this system.

189 **Local adaptation drives the evolution of height and gravitropism**

190 To directly assess the role of natural selection on the evolution of height and gravitropism, we
191 conducted two independent sets of field adaptation experiments. In each field experiment,
192 Dune and Headland parental and hybrid seeds were transplanted into replicated blocks at the
193 sand dune and rocky headland at Lennox Head. In all field experiments, natural selection
194 consistently favored the local Dune or Headland population over the foreign population (Fig. 3),
195 indicating that our experiment was exposed to the positive selection that drove local adaptation
196 in each environment.

197 First, we tested whether differences in height could drive differences in fitness in the rocky
198 headlands. We hypothesized that if natural selection was driving the evolution of height,
199 offspring produced by short hybrid parents would live longer than offspring produced by tall
200 hybrid parents in the rocky headland. We focused on prostrate growth because it is likely the
201 derived trait, given that the majority of *S. lautus* populations have an erect growth habit (19,
202 32). Our goal was to introgress Dune alleles associated with height onto a Headland genomic
203 background to examine their effect on fitness in the headland environment. Briefly, we crossed
204 Dune and Headland seeds from Lennox Head and then completed two rounds of backcrossing
205 followed by one round of crossing between the tallest 10% and the shortest 10% (Table S2 and
206 Fig. S3). We transplanted 558 of these seeds (from 28 families) into the rocky headland. As
207 predicted, shorter hybrid parents produced offspring that lived longer in the rocky headland
208 relative to offspring from taller hybrid parents ($F_{1,26.23}$=4.87, P=0.0362). These results suggest
209 that traits genetically correlated with plant height contributed to variation in early developmental
210 fitness in the rocky headlands and contributed directly or indirectly to the evolution of divergent
211 growth habit.

212 Next, we tested whether rapid adaptation to contrasting environments can lead to the evolution
213 of gravitropic responses in the direction of the local population. We hypothesized that if natural
214 selection was driving the evolution of shoot gravitropism, exposing an advanced recombinant
215 population to multiple rounds of viability selection in the field would create genetic covariation
216 between fitness and gravitropism. We crossed 23 Dune and 22 Headland individuals from
217 Lennox Head to test this hypothesis and then maintained three independent genetic lines for
218 eight generations (Table S3). This process disassembled allelic combinations and constructed
219 an F8 recombinant hybrid population (26) (Fig. 4a). We planted 2,403 of these F8 seeds (from
220 89 families) into both the sand dune and rocky headland (Fig. 4b and Table S4) and conducted
221 family-based truncation selection for three generations. The fittest families in each environment
222 were selected based on the highest germination and survival (top 50%; see Methods for
223 selection details). Siblings from these families were crossed (within a genetic line) to produce
224 the next generation. In the F10 generation, we tested whether families with the largest number
225 of survivors also produced offspring (F11) with the local gravitropic response under controlled
226 conditions.

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

238 We then tested whether adaptation to contrasting environments can recreate trait correlations
239 observed in nature. As we have shown, there is a strong correlation between gravitropism and
240 height across many (but not all) natural populations of *S. lautus*. We expect to lose this trait
241 correlation in creating the hybrid population if different genes control the traits. If natural
242 selection for these traits were strong, we could reconstruct the correlation after several rounds
243 of selection in the coastal environments of the Dune and Headland populations. There was no
244 genetic correlation between height and gravitropism in the control population grown only in the
245 glasshouse ($F_{1,114.3}$=0.08, P=0.7801, r^2=0.04) indicating genes contributing to these traits are
246 different and not pleiotropic. As predicted, the genetic correlation between height and
247 gravitropism was strong after three rounds of selection in the rocky headland ($F_{1,169.5}$=7.09,
248 P=0.0085, r^2=0.27) and weak after selection in the sand dune ($F_{1,151.3}$=3.20, P=0.0756,
249 r^2=0.09). Together, these results indicate that natural selection can act on standing genetic
250 variation and reconstitute genetic architectures favored in these coastal environments.

251 **The genetics underlying gravitropism in *Senecio lautus***

252 To better understand the genes underlying shoot gravitropism in *S. lautus*, we performed
253 selective genotyping on the tails of the gravitropic distribution of the F11 hybrid populations
254 (Fig. 4c; Dune survivors, Headland survivors and Control). We identified candidate gene
255 regions containing the most extreme allelic differences between individuals with the smallest
256 gravitropic angle (agravitropic tail, <20°; mean of tail = 6.46±1.10°, n=68) and the largest
257 gravitropic angle (gravitropic tail, >56°; mean of tail = 62.03±0.45°, n=77). We found 55 sites
258 (0.2% of all SNPs) across 49 genomic contigs (Data S1) with an allelic difference in the
259 99.8% quantile (0.15<p<0.22), indicating a polygenic basis for the phenotype. We discovered
260 that these candidate gene regions disproportionally contained homologous genes with
261 predicted gene ontology categories of transport and localization of molecules within and
262 between cells (Table S5). This is consistent with expectations, as redistribution of auxin is
263 required for a gravitropic response (28). Five of the 55 sites (11%) are located in gene
264 homologs with functions related to the auxin pathway, including the second (*ENODL1*; early
265 nodulin-like protein 1) (44, 45) and fourth (*ABA3*; molybdenum cofactor sulfurase) (46) most
266 differentiated SNPs between the agravitropic and gravitropic F11 tails. *Arabidopsis wat1*
267 mutants, an ortholog of *ENODL1*, are deficient in auxin production, display reduced auxin

268 basipetal transport and have downregulated expression of many auxin-related genes,
269 including those involved in response to auxin, auxin biosynthesis and transport (44, 45).
270 *ABA3*, also named *LOS5* and *SIR3*, encodes a molybdenum cofactor sulfurase essential for
271 the activity of several enzymes including for the plant hormone ABA and the auxinic
272 compound sirtinol (47, 48). Genetic loss of function of *ABA3* causes impaired auxin signaling
273 as well as reduced ABA levels (46).

274 Auxin-related genes, *ENODL1* and *ABA3*, have likely contributed to the adaptive and polygenic
275 divergence of gravitropism in *S. lautus*. In both the *ENODL1* and *ABA3* genes, natural selection
276 recreated the expected allelic frequency shift in the F11 tails towards the parent with the same
277 trait (Data S1). For instance, the alleles favored in the agravitropic F11 tail were at high
278 frequencies in the Headland natural population (*ENODL1* cytosine allele (C)=0.87 and *ABA3*
279 guanine allele (G)=0.89), with the alternate alleles favored in the gravitropic F11 tail and the
280 Dune natural population (*ENODL1* adenine allele (A)=0.69 and *ABA3* A=0.97). *ENODL1* and
281 *ABA3* were in strong linkage disequilibrium in the survivors of the gravitropism adaptation
282 experiment in the rocky headland (Fisher's exact test, n=57, P=0.0008) and sand dune
283 (Fisher's exact test, n=48, P=0.0107), but not in the control population reared in the glasshouse
284 (Fisher's exact test, n=37, P=0.4093), suggesting natural selection has likely reconstituted this
285 favorable allelic combination. Individuals with a *ENODL1* C/C and *ABA3* G/G genotype were
286 associated with a reduction in gravitropism of 25° relative to all other genotype combinations
287 (Fig. 5; $t_{34.30}$=4.86, P<0.0001), indicating that not sensing the pull of gravity, or reacting to it, is
288 a recessive trait. *ENODL1* and *ABA3* have gene homologs with functions in not only
289 gravitropism and plant height but also in salt tolerance and pollen tube reception (44, 46, 49-
290 53), suggesting that auxin-related genes could not only contribute to the adaptive evolution of
291 gravitropism but also the evolution of reproductive trait differences between Dune and
292 Headland populations that could affect seed production. Overall, our results suggest that
293 divergence in auxin-regulated molecular processes contributed to the evolution of local
294 adaptation to contrasting environments in coastal populations of *S. lautus*.

295 **The consequences of gravitropism divergence for the evolution of hybrid sterility**

296 To investigate whether divergence in auxin-related genes has consequences for reproductive
297 compatibility between Dune and Headland populations, we measured crossing success
298 between agravitropic and gravitropic F11 hybrids. Such crosses directly test whether a genetic
299 correlation exists between gravitropism and hybrid sterility, and therefore evaluating the
300 hypothesis that intrinsic reproductive isolation can evolve as a by-product of adaptation to local
301 conditions. We therefore expected to observe increased hybrid sterility between F11
302 agravitropic and gravitropic individuals, and reproductive compatibility between individuals with
303 similar gravitropism values. Hybrid sterility was defined as fewer than three seeds produced
304 per flower head with at least three mating attempts for reciprocal crosses (fertile plants produce
305 at least 30 seeds per cross per flower). We performed 28 crosses in families with an agravitropic
306 response (within agravitropic tail, Fig. 4), 37 crosses in families with a gravitropic response
307 (within gravitropic tail), and 67 crosses between these agravitropic and gravitropic families
308 (between tails). Consistent with a genetic correlation between gravitropism and intrinsic
309 reproductive isolation in *S. lautus*, we found that hybrid sterility was more common (Odd's
310 ratio=4.8x, P=0.0188) in crosses between F11 agravitropic and gravitropic plants (21%) than
311 within each of these tails (5%; LR chi-square=6.86, P=0.0088). This pattern of crossing failures
312 indicates that gravitropism alleles have linked or pleiotropic effects on hybrid sterility in *S.*
313 *lautus*.

314 To assess what might be driving this association between gravitropism and hybrid sterility, we
315 examined whether genetic line, F11 population, or specific individuals were correlated with
316 hybrid sterility. Crosses within (n=77) and between (n=55) the three genetic lines, regardless
317 of their gravitropic response, did not affect hybrid sterility (LR chi-square=1.10, P=0.2937). This
318 result indicates that the hybrid sterility found in the F11's is unlikely to be from genetic drift
319 leading to incompatible differences between the genetic lines. Crosses within (n=80) and
320 between (n=52) the three F11 populations (Dune survivors, Headland survivors and Control)
321 did not affect hybrid sterility in agravitropic and gravitropic individuals (LR chi-square=0.15,
322 P=0.6995), increasing the likelihood that gravitropism, and not another trait responding to
323 selection in one of these environments, causes intrinsic reproductive isolation in these

populations. Next, we determined whether specific F11 individuals (n=129) drove this association between gravitropism and hybrid sterility. Thirty-two individuals participated as one of the parents in a sterile cross; 26 of these individuals were crossed against a separate individual, and they successfully produced seeds. Thus, sterility is specific to each parental combination, consistent with the idea that hybrid sterility is polymorphic in the system, a result that echoes those found in other systems (54, 55). Finally, we found that crosses had symmetric effects on reducing hybrid fitness: only six crosses displayed differences in hybrid sterility in the reciprocal cross. Reciprocal effects on reproductive isolation are inconsistent with the contribution of maternal genotypes to survivorship in the field, suggesting that not only maternal genotypes contribute to sterility in these populations. Together, these results imply a genetic association between the adaptive divergence of gravitropism and hybrid sterility in *S. lautus*.

To investigate whether *ENODL1* and *ABA3* alleles were associated with hybrid sterility, we examined if the predicted allele counts of the F12 offspring from the F11 parents explained the proportion of failed crosses. We found that the interaction between *ENODL1* and *ABA3* did not have a significant effect on sterility (Table 2). The *ABA3* A allele, on the other hand, had positive effect on hybrid sterility, where individuals with the gravitropic favored A allele, which is dominant to the G allele, had a higher percentage of failed crosses (Table 2). This suggests that *ABA3* might genetically link adaptive evolution of shoot gravitropism with hybrid sterility in *S. lautus*. Overall, our results support the hypothesis that intrinsic reproductive isolation can evolve as a by-product of adaptation to local conditions.

Discussion

Whether local adaptation commonly drives the formation of hybrid sterility and inviability is still a topic of debate (5, 16). These intrinsic reproductive barriers are believed to evolve late in the speciation process, often after extrinsic and prezygotic barriers have formed (e.g., immigrant inviability, or assortative mating) (4), and genetic drift or natural selection has led to the accumulation of genetic incompatibilities (2). There are few studies (9, 10) on the genetics of local adaptation and intrinsic reproductive isolation within a species, with most studies having focused on the effect of non-ecological evolution e.g., genetic conflict on the evolution of Dobzhansky-Muller incompatibilities (13). Our study is a step-forward in understanding the role of polygenic adaptation in creating hybrid sterility during the early stages of speciation. Here, we provide empirical evidence to suggest that natural selection is a major driving force behind the hybrid sterility found between recently derived erect and prostrate populations of *S. lautus*. Further, we identified variation in a trait in natural populations that could only result from divergence in specific hormonal pathways, thus suggesting the joint function of many genes could underlie the evolution of divergent traits correlated with reproductive barriers. The results from our set of experiments suggest a novel and broadly applicable explanation to the elusive link between the genetics of local adaptation and the evolution of intrinsic reproductive isolation.

Our results suggest that natural selection contributed to the colonization of extreme environments in *S. lautus*, like those found on rocky headlands along the coast. Previous population genetic results in this system (19, 26, 35) and those presented here suggest that the auxin pathway could facilitate plant adaptation to new habitats through concomitant changes in multiple developmental and architectural traits. Transitions from erect to prostrate growth and the associated traits of short stature and many branches, are common in plants that colonize coastal headlands (56-59), indicating 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 auxins such as responses to mechanical cues (60) and halotropism (61-63). As the structure of hormonal pathways is generally conserved across plant species, the evolution of similar architectures in similar environments might prove to be a general mechanism to link adaptation with the incidental evolution of traits affecting species interactions such as ecological competition and reproductive success. For instance, in *Mimulus*, gibberellin (another essential plant hormone) has been recently implicated in local adaptation and associated with reproductive isolation between coastal and inland populations along the coast of California (42, 64). Like *S. lautus* Headland populations, coastal *Mimulus* populations are dwarf, salt-tolerant, and produce small amounts of gibberellin compared to inland populations (42, 65).

379 In *S. lautus*, previous results support a general link between local adaptation and intrinsic
380 reproductive isolation. Walter*, et al.* (36) found that additive variance responsible for local
381 adaptation in four ecotypes was lost in the creation of an F2 hybrid generation that displayed
382 elevated levels of hybrid sterility. A similar occurrence of hybrid sterility occurred in the F2's
383 that created the F11 generation used here (32), suggesting that genetic incompatibilities are
384 segregating in multiple populations and ecotypes of *S. lautus*. However, the other generations
385 of the hybrid population (F3-F11) are highly fertile, implying that a large proportion of the
386 incompatible alleles between Dune and Headland populations were removed in the F2
387 generation. Remarkably, we can recreate intrinsic reproductive isolation in the F11 hybrid
388 population when we sort F11 families according to the strength of their gravitropic response;
389 crosses between individuals with divergent gravitropism values have a reduced ability to
390 produce seed. A likely explanation is that incompatibility alleles segregate at low frequencies
391 in the population after the F2 generation, but we artificially increased the frequency of the
392 incompatible alleles by selecting individuals with extreme gravitropism. Altogether these results
393 provide strong evidence that adaptive traits, such as shoot gravitropism, are genetically
394 correlated with hybrid sterility in *S. lautus*.

395 The mechanisms by which gravitropism and hybrid sterility are genetically correlated could be
396 pleiotropy (e.g., Bomblies and Weigel (9)) or genetic linkage (e.g., Wright, Lloyd, Lowry,
397 Macnair and Willis (10)), or a combination of both for polygenic traits. We speculate that
398 pleiotropic effects of *ENODL1* and *ABA3* genes contributed to the correlated evolution of
399 adaptive gravitropism and hybrid sterility. For example, *ABA3*, a strong candidate to generate
400 variation in gravitropism, has been associated with pollen sterility in *Arabidopsis* (53), and it is
401 known to contribute to salt tolerance in other organisms (51). It is not difficult to speculate
402 evolutionary scenarios where hybrid sterility and gravitropism might be correlated responses to
403 selection on saline environments: as the population adapts, compensatory mutations for
404 reproduction can occur which are incompatible with genetic backgrounds from other
405 populations. For *ENODL1*, we could not obtain many genotypes to properly test its role on
406 hybrid sterility in *S. lautus*, but members of the *ENODL* family have a female-specific role in
407 pollen tube reception (52). For example, in *Arabidopsis*, *enodl* mutants fail to arrest growth and
408 rupture the entering pollen tube (52). The adaptive evolution of gravitropism through the
409 maternal genotype in our adaptation experiments, also supports the notion that maternal
410 *ENODLs* may be used to both communicate with the male pollen tube to enable reproduction
411 and participate in adaptation to local conditions via its effects on gravitropism and correlated
412 traits. The asymmetric reductions in seed set of *enodl* mutants contrasts with our symmetric
413 reciprocal crosses. This discrepancy suggests that other factors other than maternal genetic
414 effects contribute to reproductive isolation in *S. lautus*, possibly masking the effects of maternal
415 genotypes in our experimental design. Future work will test the effect of *ENODL1* and *ABA3* on
416 sterility using other genetic backgrounds and while considering novel genotypic combinations
417 once we include other candidate genes in our genetic analyses.

418 Given that gravitropism has a polygenic basis and auxin related-genes have many pleiotropic
419 effects on growth and development, and reproduction, our results suggest many genes likely
420 drove local adaptation and only a subset of them contributes to hybrid sterility as they become
421 part of a Dobzhansky-Muller incompatibility. This systems' view of the evolutionary process,
422 where pleiotropic effects are pervasive, could provide fertile ground for the origin of adaptations
423 and new species. Future genetic studies focusing on isolating sets of loci causing hybrid sterility
424 in *S. lautus* will reveal the molecular mechanisms by which intrinsic reproductive barriers evolve
425 together with local adaptation and will allow testing of these novel predictions on the origin of
426 new species. Overall, our study showcases a powerful strategy to explore the genetic basis of
427 local adaptation and speciation in natural systems. We postulate that the evolution of hormonal
428 pathways in plants provides a simple mechanism for the rapid modification of co-regulated traits
429 that facilitate the colonization of new habitats and the correlated evolution of hybrid sterility.

430 **Materials and Methods**

431 **Synthetic auxin and auxin transport inhibitor experiments**

432 We tested if auxins govern gravitropism in *S. lautus* by evaluating gravitropic responses in
433 seedlings treated with chemicals affecting auxin signaling. Specifically, we used 2,4-

434 Dichlorophenoxyacetic acid (2,4-D), a carrier-dependent synthetic auxin (39, 66), and
435 Naphthylphthalamic acid (NPA), an efflux inhibitor (40). Gravitropism was measured *in vitro* on
436 agar plates on approximately 40 maternal families from Lennox Head sand dune and rocky
437 headland. Seeds from an ecotype were combined in a falcon tube and were first sterilized with
438 a quick rinse in 70% EtOH, followed by four 10-minute inversions in a sterilizing solution of 6%
439 sodium hypochlorite and 1% Tween 20. Seeds were then rinsed three times with distilled water
440 and vertically orientated on Murashiga and Skoog (MS) agar plates containing 0.15% MS,
441 0.05% 2-(*N*-morpholino)-ethanesulfonic acid (MES), 0.15% sucrose, 1% agar and 2,4-D and
442 NPA at concentrations of either 0 μM, 0.5 μM, 5 μM, and 50μM. For the chemical
443 concentrations, we used the following stock solutions: 2,4-D: 1mM in Ethanol and NPA: 10mM
444 in dimethyl sulfoxide (DMSO). We created 1ml dilutions of stock solutions (in ethanol or DMSO),
445 which were dissolved in 500 ml of media.

446 We placed eight seeds on each MS plate and incubated the plates at 21°C in a dark growth
447 cabinet to avoid any light effects. After seven days, all plates were rotated clockwise by 90°
448 and a photograph of each plate was taken 24 hours after rotation. All photographs were
449 imported into ImageJ (67) to determine gravitropism by measuring the angle to which the stem
450 reorientated to the horizontal (30, 68, 69). Seedlings were excluded from analyses if they were
451 shorter than 5mm, contacted the plate's edge, or germinated after rotation. We were left with a
452 total of 188 seedlings to quantify the gravitropic response. The 50μM concentration of 2,4-D
453 treatment was excluded, as less than six seeds germinated under this high concentration (Data
454 S4). For each chemical, we used a mixed linear model using the lmer function of lme4 package
455 in R v3.1.3 (70): $y_{ijkl} = A_k + E_i + C_j + E_i \times C_j + e_{l(ijk)}$, where agar plate ($A_k$) is the MS plate that the
456 seeds were grown on, ecotype (E_i) is Dune or Headland and concentration (C_j) are the 4
457 different concentrations of the chemical. Agar plate was fitted as a random effect, while ecotype,
458 concentration and their interaction ($E_i \times C_j$) were fixed effects, and $e_{l(ijk)}$ is the residual error.
459 Gravitropism (y_{ijkl}) was compared using a Type II Wald chi-square test.

460 **Phenotyping of natural populations**

461 *Height measurements*

462 We measured plant height in all 16 populations in the glasshouse and 12 of the populations in
463 their native field environment (Data S2 and Data S3). Height (vegetative) was measured as the
464 vertical distance from the soil to the plant's highest point that has vegetative leaves (flowers
465 and stems are not included). In the field, we measured height in 32 individuals evenly across
466 the range of each population. In the controlled conditions of the glasshouse, we sampled an
467 average of 14 individuals per population after plants reached maturity.

468 For both the glasshouse and field measurements, we used a linear model to determine whether
469 Dune populations were taller than their adjacent Headland pair: $y_{ijk} = P_i + E_{j(i)} + e_{k(ij)}$, where pair
470 (P_i) is an adjacent Dune and Headland population at the same locality and ecotype ($E_{j(i)}$) is
471 Dune or Headland and is nested within pair. All factors are fixed effects and $e_{k(ij)}$ is the residual
472 error. Population height (y_{ijk}) for each pair was compared using a one-tailed t-test (Table S6).
473 The Alpine populations were also included with the prediction that the sheltered Alpine
474 population (A03) would be taller than the exposed Alpine population (A07). All statistical results
475 reported here were produced in JMP 13 (SAS 2015).

476 *Gravitropism measurements*

477 Gravitropism was measured *in vitro* on agar plates in a dark growth cabinet using seeds from
478 all 16 natural populations. For each population, 2-4 seeds per family were grown for ~40
479 maternal families (1,278 seeds in total). Plates were incubated, rotated, photographed, and
480 gravitropism was measured in ImageJ (67), as outlined above. Overall, there was a 63.8%
481 germination success, but seeds were excluded with the criteria above. This left a total of 736
482 seedlings across all 16 populations (57.6% of the total number of seeds planted; Data S2). To
483 test the hypothesis that Dune populations would have a stronger gravitropic response in their
484 stem than their adjacent Headland pair, we used a mixed linear model: $y_{ijkl} = P_i + E_{j(i)} + A_k +$
485 $e_{l(ijk)}$, where pair (P_i) is an adjacent Dune and Headland population at the same locality, ecotype
486 ($E_{j(i)}$) 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 (Table S6). 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).

Field experiments

All field experiments were conducted at the sand dune and rocky headland at Lennox Head (NSW) in the exact location where native *S. lautus* grow. We tracked each individual by gluing each seed to a toothpick with non-drip superglue and placing them 1-2mm under the ground within a grid cell (Fig. 4) that was randomly assigned (for details see Walter*, et al.* (32)). 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 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. S3). 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 among one another (n=16, tallest 10% of the population), and the shortest individuals among one another (n=18, shortest 10% of the population). See Table S2 for the number of individuals and families contributing 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, totaling 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 S5).

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) as individuals with the same parents, and block (B_k) as 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 (71, 72) as described in Roda, Walter, Nipper and Ortiz-Barrientos (26). 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 S3 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 modeling (73, 74) implemented with the 'Aster' package in R (70). 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 approach 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 three rounds of selection, where survival success was measured at day 138 in the F9 generation, and at day 232 in the F10 generation. See Table S4 for the number of seeds and families planted per genetic line and environment for the three transplant experiments.

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 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 self-fertilization (rare in *S. lautus*). One generation of selfing was completed to increase homozygosity. We extracted DNA from the leaf tissue of one individual using a modified CTAB protocol (75).

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 utilized 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 ~843 MB long and consisted of 96,372 scaffolds with an N50 of 21 KB. Although 843 MB is much shorter than the expected haploid size of 1.38 GB (76) of the whole 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.

F11 gravitropism measurements

In the F11 generation described above, we measured gravitropism as the angle of the stem after a 90° rotation of a seedling. This included 39 Dune survivor families, 37 Headland survivor families and 25 inbred control families with 12 individuals per family (1,212 seeds in total). These families were germinated in three separate batches ~seven 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 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 (67) to measure gravitropism as described above. Data S6 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 offspring and high fitness Headland families produce agravitropic offspring. 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 the 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 is the F10 family fitness values for the individuals that were crossed to

593 create the F11 offspring where gravitropism was measured. All factors were included as fixed
594 effects, and $e_{n(ijklm)}$ was the residual error. Genetic line C was removed from analyses as it has
595 little variation in fitness values, which means it did not converge. Shapiro-Wilk W test shows
596 the residuals from the model are normally distributed for both the sand dune (W=0.98,
597 P=0.3879) and rocky headland (W=0.98, P=0.2776). The linear model was performed in JMP
598 13 (SAS 2015).

599 *Genetic association between height and gravitropism*

600 We tested the genetic association between height and gravitropism after segregation in an
601 advanced recombinant population. We implemented a mixed linear model for the three F11
602 populations (Dune survivors, Headland survivors, and a Control population) that accounts for
603 family variation: $y_{ijk} = H_i + F_j + e_{k(ij)}$, where gravitropism ($y_{ijk}$) is the angle of the growth response
604 12 hours after a 90° rotation, height (H_i) is the vertical distance from the soil to the top of the
605 vegetative leaves, measured after maturity in the glasshouse and family (F_j) is a random effect
606 that consists of individuals that have the same dam and sire. The mixed linear model was
607 performed in JMP 13 (SAS 2015).

608 *Genotyping of F11 gravitropism tails*

609 To isolate gravitropism candidate genes, we genotyped 77 gravitropic (>56°) and 68
610 agravitropic (<20°) F11 individuals (Data S6). We extracted DNA from leaf tissue using a
611 modified CTAB protocol (75) and quantified the DNA using the PicoGreen reagent (Invitrogen,
612 Carlsbad, CA). To determine which parent the alleles were derived from, we included 39 Dune
613 parentals (D01) and 41 Headland parentals (H01). Leaves from the Lennox Head Dune and
614 Headland natural populations were collected directly from the field and the same DNA
615 extraction protocol was followed. Each F11 individual was duplicated in independent wells and
616 libraries of Restriction-site Associated DNA (RAD) tags were created at Floragenex following
617 Baird*, et al.* (77), but using the PstI restriction enzyme. We sequenced 380 samples on four
618 lanes of an Illumina HiSeq 4000 with 91bp single-end reads at Floragenex. A total of 1.39 billion
619 reads with a mean of 3.62 million reads per sample were produced. Reads were aligned to the
620 Illumina reference genome using Bowtie 1.1.1 (78) and a FASTQ quality score of above 20.
621 We then used SAMtools 0.1.16 (79) to create an mpileup file of all samples with a minimum
622 Phred quality score of 10, minimum sequencing depth per sample of 6x and minimum per cent
623 of population genotyped of 75%. This approach produced 26.8K variable positions (224K
624 variants before filtering) with a minimum distance between variants of 50bp. The gravitropism
625 candidate gene set consisted of SNPs in the 99.9% quantile of the distribution of differentiated
626 SNPs between the gravitropism tails. The region of the scaffold containing the SNP was
627 annotated using the BLASTx NCBI database (80).

628 *Senecio lautus gravitropism candidate genes*

629 We tested for overrepresentation of gene function and linkage disequilibrium between
630 gravitropism loci in the gravitropism candidate gene set. A statistical overrepresentation test
631 was performed in PANTHER (http://pantherdb.org/) using the TAIR identification for 32 unique
632 gravitropism candidate genes matched to a reference list of 27,502 *Arabidopsis thaliana* genes.
633 To calculate linkage disequilibrium between loci, a likelihood-ratio chi-square test was
634 performed in JMP 13 (SAS 2015) with each F11 population independently (Dune survivors,
635 Headland survivors and Control; Data S7).

636 **Intrinsic reproductive isolation**

637 *Gravitropism and hybrid sterility correlation*

638 We tested whether hybrid sterility was associated with gravitropism by randomly crossing within
639 and between the F11 gravitropic and agravitropic groups in a controlled temperature room and
640 recording successful and failed crosses. To maximize sample size, all three replicate genetic
641 lines (A, B, and C) were used across all three environments (Dune survivors, Headland
642 survivors, and Control). A total of 138 crosses were completed (Data S8), 71 crosses between
643 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 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. Here, hybrid sterility could be caused by failure to produce fertilized ovules (prezygotic), or by fertilized ovules failing to develop into viable seeds (postzygotic). 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 two within the tails).

If gravitropism contributed to divergence in *S. lautus*, 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: $y_{ijkl} = T_i + G_j + P_k + e_{l(ijk)}$, where tails ($T_i$) is 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.

ENODL1 and ABA3 association with hybrid sterility

We implemented a linear model to test the hypothesis that *ENODL1* and *ABA3* alleles are correlated with hybrid sterility. From the F11 individuals that were crossed, we extracted their genotypes and predicted the genotypes of their offspring (F12). Genotypes that were ambiguous due to heterozygous parents were removed, which reduced the sample size to 61 crosses in which 11 were failed crosses (Data S9). We tested the effect of *ENODL1* and *ABA3* alleles and their interaction on the percentage of failed crosses: $y_{ijk} = E_i + A_j + EA_{ij} + e_{k(ij)}$, where *ENODL1* ($E_i$) is the allele counts for the *ENODL1* C allele in the F12's; *ABA3* (A_j) is the allele counts for the *ABA3* G allele in the F12's; and *ENODL1* x *ABA3* (EA_{ij}) is their interaction. All factors were included as fixed effects, and $e_{k(ij)}$ was the residual error. Shapiro-Wilk W test shows the residuals from the model are normally distributed (W=0.94, P=0.6314). The linear model was performed in JMP 15 (SAS 2015).

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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 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. 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* genome. CB guided the physiological experiments. MJW and DO wrote the paper with input from all authors. DO secured the funds and is mentor and supervisor for the research program.

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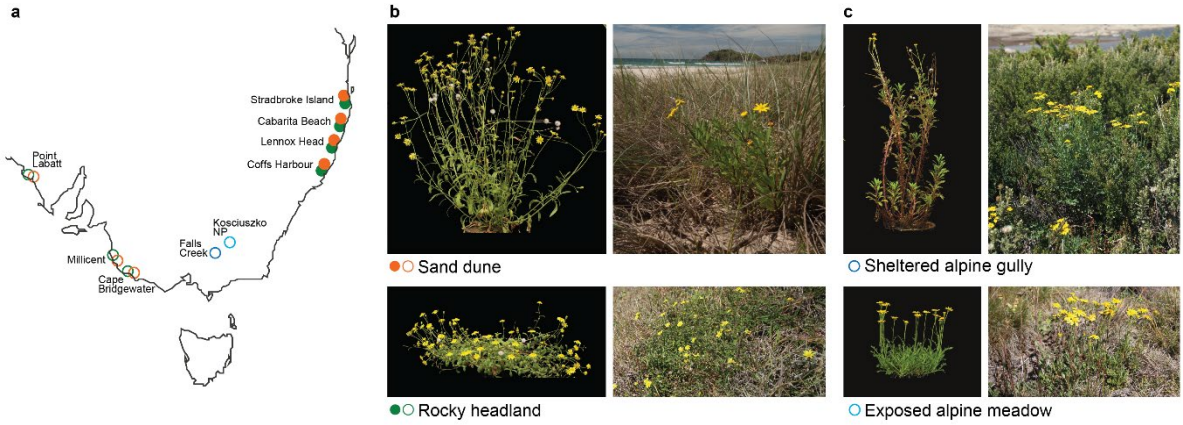
898 **Figures and Tables**



899

900 **Fig. 1. Sample locations and growth habit differences between adjacent *Senecio lautus***
901 **populations. a**, Map of Australia showing locations of the 16 populations used in this study. The
902 seven coastal localities have a Dune (orange) and Headland (green) population occurring adjacent to
903 each other. The populations are split into two monophyletic clades (35), the eastern clade (closed
904 circles) and the south-eastern clade (open circles). **b**, *Senecio lautus* native to the sand dunes have
905 an erect growth habit and *S. lautus* native to the rocky headlands have a prostrate growth habit. **c**,
906 Alpine populations of *S. lautus* include a sheltered alpine gully and an exposed alpine meadow,
907 containing individuals with an erect and prostrate growth habit, respectively.

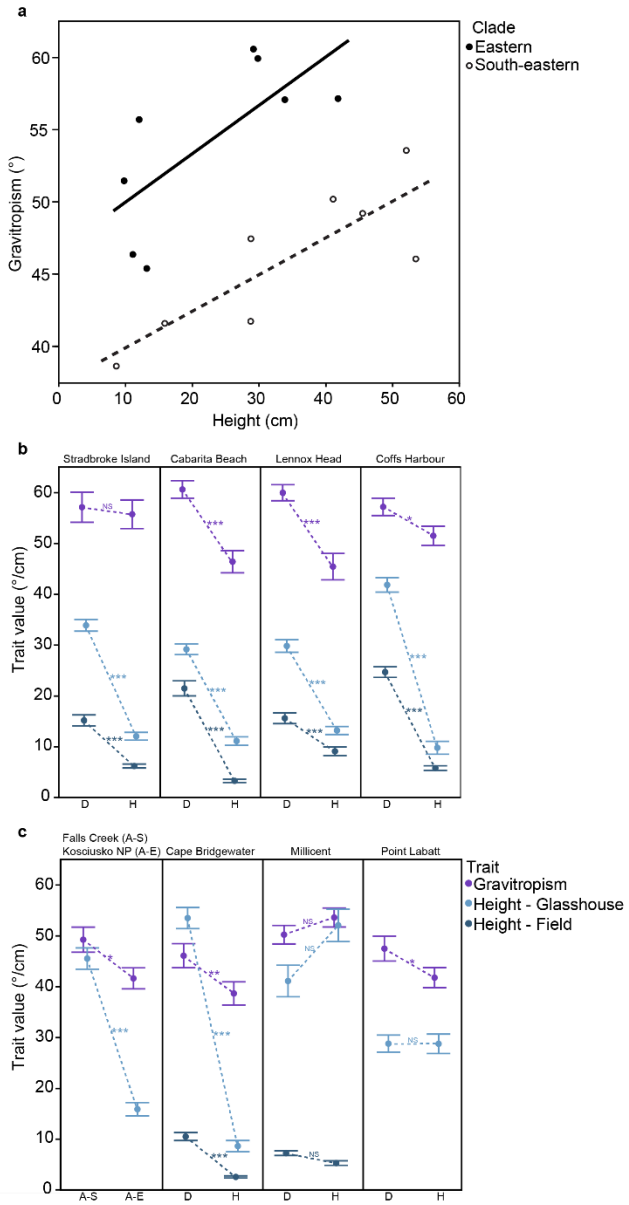


Fig. 2. Gravitropism and height variation across 16 *Senecio lautus* populations. a, The correlation between gravitropism and height across *S. lautus* populations split into their monophyletic clades – see Fig. 1 for details. Each point in the graph represents a population mean where height was measured in the glasshouse and gravitropism was measured 24 hours after a 90° rotation. **b**,**c**, Divergence in gravitropism (°), height (cm) 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 ± SE; one tailed Student's t-test (Table S6), *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, NS not significant.

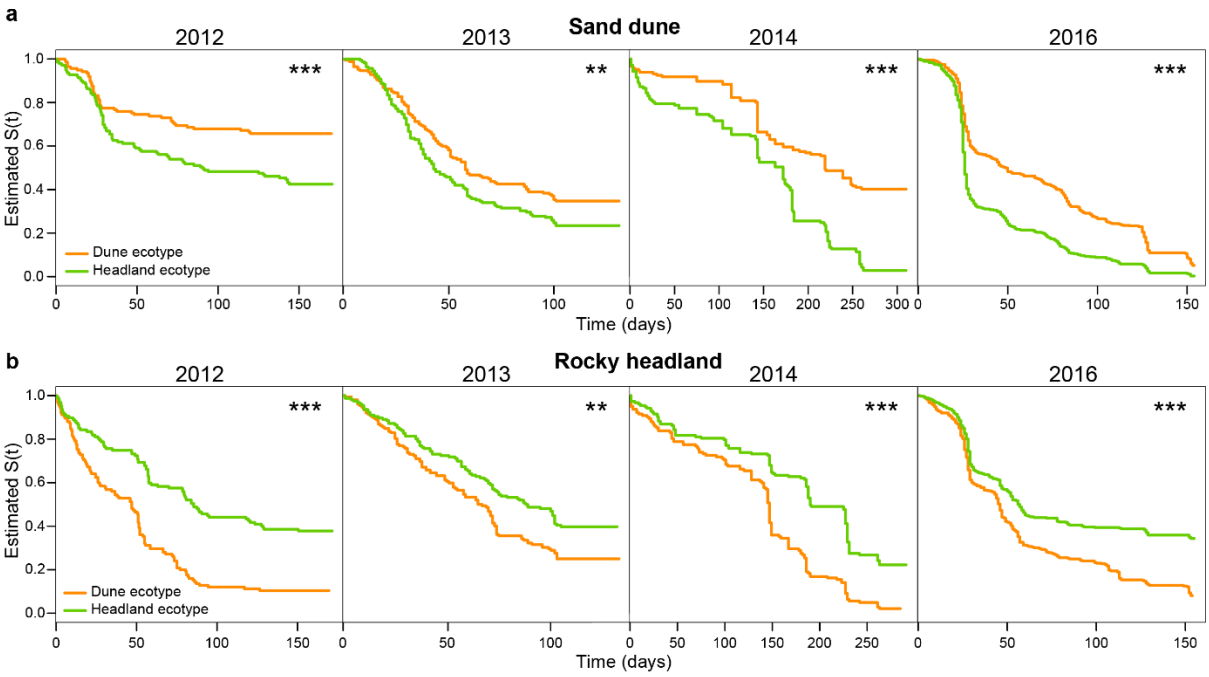


Fig. 3. Parental survival curves in the height (2016) and gravitropism (2012-2014) adaptation experiments at the sand dune and rocky headland at Lennox Head. a,**b**, Survival (Estimated S(t)) over the length of the field experiments is shown for the Lennox Head Dune population (orange) and the Lennox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

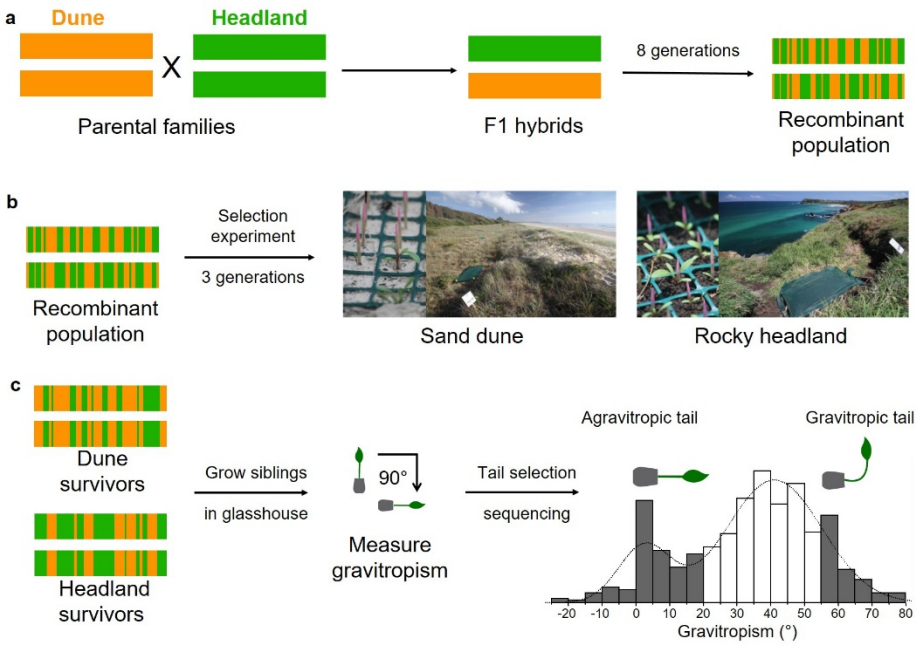


Fig. 4. The creation of the recombinant hybrid generation, the design of the gravitropism adaptation experiments and sequencing of the tails of the gravitropic distribution. a, 23 parental Dune and 22 parental Headland individuals from Lennox Head were crossed randomly and with equal contribution for 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.

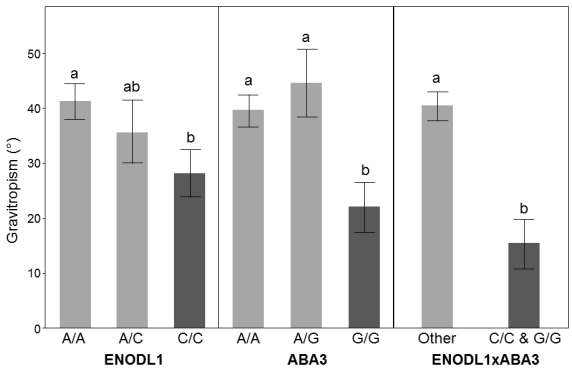


Fig. 5. Association between *ENODL1* and *ABA3* alleles and gravitropism in *Senecio lautus.* The average gravitropism angle is shown for each allelic combination, independently and when the agravitropic alleles (dark grey) are combined. Different letters denote significant differences between genotypes at a significance level of α = 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.

955 **Table 2. General linear model for the effect of *ENODL1* and *ABA3* alleles on hybrid**
956 **sterility.** The genotypes of the F12's were predicted based on F11 parental genotypes with
957 ambiguous genotypes removed. *ENODL1* is the allele counts for the *ENODL1* C allele in the
958 F12's, while *ABA3* is the allele counts for the *ABA3* G allele. *ENODL1* x *ABA3* is the effect of
959 all observed allelic combinations between these two gravitropism candidate loci and the
960 percentage of failed crosses.
961

Source	DF	SS	F-Ratio	P-value
ENODL1	1	6.51	0.078	0.791
ABA3	1	925.52	11.076	**0.021**
ENODL1* x *ABA3	1	9.77	0.117	0.746

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