*Tansley Review*

**Senecio** as a model system for integrating studies of genotype, phenotype and fitness

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Summary

Two major developments have made it possible to use examples of ecological radiations as model systems to understand evolution and ecology. First, the integration of quantitative genetics with ecological experiments allows detailed connections to be made between genotype, phenotype and fitness in the field. Second, dramatic advances in molecular genetics have created new possibilities for integrating field and laboratory experiments with detailed genetic sequencing. Combining these approaches allows evolutionary biologists to better study the interplay between genotype, phenotype and fitness to explore a wide range of evolutionary processes. Here, we present the genus *Senecio* (Asteraceae) as an excellent system to integrate these developments, and to address fundamental questions in ecology and evolution. *Senecio* is one of the largest and most phenotypically diverse genera of flowering plants, containing species ranging from woody perennials to herbaceous annuals. These *Senecio* species exhibit many growth habits, life histories and morphologies, and occupy a multitude of environments. Common within the genus are species that have hybridised naturally, undergone polyploidisation, and colonised diverse environments, often through rapid phenotypic divergence and adaptive radiation. These diverse experimental attributes make *Senecio* an attractive model system in which to address a broad range of questions in evolution and ecology.

Keywords: adaptive radiation, Asteraceae, hybrid speciation, quantitative genetics, genomics, model system, *Senecio*, self-incompatibility
1. Model systems for experimental ecology and evolution

Recent technological advances in genomic and computational technologies have allowed genetic analyses of many non-model organisms, providing important insights into genome evolution, the structure of developmental pathways and the control of gene expression. However, to answer fundamental questions in ecology and evolutionary biology we need to develop model systems that integrate these novel genetic resources with experimentally flexible systems that are also ecologically diverse. Using ecologically divergent systems is critical for understanding how genotypes relate to phenotypes across different environments, and to test the processes driving adaptation, speciation and biogeographical patterns. Nevertheless, many model systems used in evolutionary biology are only studied in laboratory settings because despite possessing key experimental attributes (e.g. short life-cycles, self-fertility), they show limited variation in life history, mating system, or habitat. The utility of such systems for detailed lab or greenhouse experiments therefore limits their value in field experiments testing hypotheses relating to evolution and ecology, such as the interaction between phenotypic plasticity and natural selection, and how and when populations specialise to particular environments. This limitation has become increasingly relevant given the urgent need to predict how, where, and how quickly populations and ecological communities will respond to ongoing environmental change. Research needs to focus on model systems with populations and species that inhabit different environments and ecosystems, but that remain amenable to large field experiments and quantitative genetic crossing designs. Specifically, we need model systems that can connect genotype, phenotype and fitness under a range of environmental conditions. In this review, we argue that Senecio is one of very few model systems that can fill this role.

2. Senecio as an experimental model system

Senecio (Linnaeus, 1753) is a large genus of flowering plants within the tribe Senecioneae of the daisy family, Asteraceae. Containing in excess of 1,200 species distributed across the globe, Senecio radiated rapidly during the Miocene c.10.7 million years ago to become one of the largest genera of flowering plants (Kandziora et al., 2017). Species of Senecio, commonly known as ragworts and groundsels, are generally herbaceous, but also occur as vines, shrubs, succulents, epiphytes and small trees (Fig. 1). The genus is aptly named after the tendency for species to produce senescent flower heads resembling tufts of white hair (Latin ‘senex’ = ‘old man’) formed by the pappus, which facilitate airborne seed dispersal.

Many species of Senecio have useful attributes of a model system, including: small to medium-sized genomes (mean c.5 Gbp; range 1.13-51.25 Gbp; Leitch et al., 2019), a rapid sexual cycle (3-5 months from seed-to-seed), ease of crossing because of widespread self-incompatibility. Species are also often perennial or short-lived perennials so can be regenerated from cuttings to create genetically identical clones, are
genetically transformable (Kim et al., 2008), and are easily grown in glasshouses and field plots. The fact that many species show these attributes, combined with their recent radiation and occupation of a diverse array of habitats, make Senecio species more amenable than many other systems for quantitative genetic designs. Such designs allow researchers to combine genetic and phenotypic studies in both the field and under controlled conditions. The genus Mimulus shares some of these experimental characteristics (e.g., short generation times, easy to grow, clonal propagation) and has a widespread distribution across different environments (Wu et al., 2008), but Senecio has the added benefit of a worldwide distribution, and a greater diversity of species that include a range of mating systems and a multitude of growth forms (Fig. 1). Such diversity in morphology and habitat makes Senecio attractive to a similar diversity of scientists, from ecologists to phylogeneticists, molecular geneticists and quantitative geneticists. We therefore propose Senecio as an attractive model system for answering fundamental questions in ecology and evolution, joining the ranks of Antirrhinum, Arabidopsis (including Boechera and other relatives), Helianthus, Mimulus, Silene and Solanum.

What questions can we answer using Senecio?

Future work will benefit from the imminent completion of high coverage and highly contiguous annotated genomes for S. squalidus and S. lautus (B. Nevado et al. unpublished; Wilkinson et al., 2019). These will be used as references for further genomic analyses of other Senecio species, including whole genome resequencing, DNA methylation and RNA-seq approaches. These new resources will make Senecio a powerful system to study the genomic architecture of selection, adaptation and divergence across environments. Combining such genetic association techniques with quantitative genetic crossing designs is a powerful way to explore how the relationship between genotype, phenotype and fitness changes across environments. Of special importance is the ease and speed at which species of Senecio can be propagated from cuttings. This ability to generate clones allows researchers to control for genotype and transplant large numbers of clones across multiple environments, and sets Senecio apart from other model systems. Such approaches can address an array of questions in ecology, evolution and evolutionary genomics (see: Box 1).

2.1 Origins and diversity of Senecio

The presence of repeated radiations in the Americas, Australasia, and across Northern Africa into Europe highlights the ability of Senecio to colonise and rapidly adapt to multiple environments (Comes & Abbott, 2001; Roda et al., 2013; Liew et al., 2018). This broad geographical distribution is reflected in species’ occupation of contrasting environments, from harsh coastlines to rainforests, deserts, mountains and alpine areas. Associated with this diversity of habitats is an array of different phenotypes (Fig. 1) typified by the Australian Senecio lautus species complex, which provides a fascinating example where recent radiation into
more than 10 contrasting environments across much of the continent has resulted in the evolution of ecotypes with a broad diversity of plant architecture and leaf form (Fig. 2). Among the numerous recorded radiations within Senecio there is evidence of local adaptation (Walter et al., 2016), parallel evolution (Roda et al., 2013; Comes et al., 2017), polyploidy (Alexander, 1979; Chapman & Abbott, 2010), introgressive hybridization (Kim et al., 2008; Osborne et al., 2016), hybrid speciation (James & Abbott, 2005; Hegarty et al., 2012) and apparently stable hybrid zones (Brennan et al., 2009; Abbott et al., 2018).

2.2 Historical use of Senecio for research

With its wealth of morphological, ecological and reproductive diversity, Senecio has attracted studies addressing many different questions in ecology and evolution. Experimental research using Senecio began in the late 19th Century (Table 1) when Anna Bateson, sister of the geneticist, William Bateson, showed that progeny generated by crossing S. vulgaris plants were taller and produced more seed per capitulum than those generated by self-fertilization, suggesting an heterotic effect (Bateson, 1888). Because S. vulgaris has a short generation time and is self-compatible but easy to cross, Trow recognised its value for genetic research and used it to establish the genetic basis of several discontinuous and semi-continuous traits within the species in the early 20th Century (Trow, 1912, 1916). Approximately 40 years then passed, before Harland emphasised the value of Senecio in both genetic and evolutionary research by using interspecific crosses to show that Senecio cambrensis was the recent allohexaploid of S. vulgaris and S. squalidus (Harland, 1954a, b). Through this early work, Bateson, Trow and Harland laid the foundation for more extensive works that followed (Table 1).

3. Mating system diversity and evolution in Senecio

3.1 Outcrossing and self-incompatibility

As a member of the Asteraceae, Senecio inflorescences are capitula (‘flower heads’), comprising multiple uni-ovulate flowers (florets) tightly gathered across a disc-shaped receptacle. Florets are frequently differentiated into two types dependent on their position within the capitulum (‘disc’). Centrally located disc florets are usually radially symmetrical while outermost ray florets are bilaterally symmetrical with petals fused into a single large ‘ray’ (Abbott & Schmitt, 1985; Gillies et al., 2002; Kim et al., 2008; Garcês et al., 2016), which facilitates outcrossing by attracting generalist insect pollinators (Abbott & Irwin, 1988; Andersson & Widén, 1993).

The genetic control of flower development has been studied in S. vulgaris (ssp. vulgaris), which is mostly ray-less throughout its range but has a rayed variant in the UK (S. vulgaris var. hibernicus) that originated via introgressive hybridization with S. squalidus (Abbott et al., 1992; Kim et al., 2008). The emergence of a
rayed form increased the attractiveness of *S. vulgaris* to insect pollinators (Abbott & Irwin, 1988) and increased maternal outcrossing in the newly formed radiate variant (Marshall & Abbott, 1982, 1984). This provides a rare example of a shift towards outcrossing in a selfing species (Abbott et al., 1998), in contrast to the more typical trend for selfing to evolve from outcrossing (Barrett, 2013). Floret development in this rayed variant is controlled by localised expression of, and interactions between, at least three *cycloidea* (*CYC*)-like genes (Kim et al., 2008; Garcês et al., 2016). There is evidence for selection on a duplicated cluster of these genes, collectively known as *Ray*, in the form of gene transfer between species and clinal patterns of variation across ecotones, reflecting the adaptive importance of ray florets as a modulator of outcrossing (Kim et al., 2008; Chapman & Abbott, 2010).

Outcrossing in many *Senecio* species is maintained by the presence of self-incompatibility (SI), a molecular mechanism of self-pollen recognition and rejection (Hiscock, 2000a, b) that is common among species within the Asteraceae (Ferrer & Good-Avila, 2007). SI in *Senecio* is controlled sporophytically with a single genetic locus, *S*, determining both the pollen and stigma recognition phenotypes (Hiscock, 2000a, b; Brennan et al., 2002; 2006; 2011). Interestingly, the expression of sporophytic SI (SSI) in *Senecio* appears to be affected by additional unlinked modifier loci (Hiscock, 2000a, b; Brennan et al., 2011), which raises intriguing questions about its underlying molecular mechanism. To date however, unravelling the molecular basis of SSI in *Senecio* has proved challenging (Allen et al., 2011), Those studies that have been undertaken provide clear evidence that this molecular mechanism is distinct from the well-characterised SSI system of the Brassicaceae (Tabah et al., 2004; McInnis et al., 2006; Allen et al., 2011), indicating the evolution of a different mechanism of SSI in the Asteraceae and further supporting phylogenetic analyses that have shown that SI has evolved multiple times during the radiation of flowering plants (Allen & Hiscock, 2008).

Encouraging progress towards characterising the *S*-locus in members of the Asteraceae is being made through mapping genomic regions linked to SI in sunflower (Gandhi et al., 2005; Gonthier et al., 2013), which are also proving useful in identifying syntenic regions in the *Senecio* genome (B. Nevado et al., unpublished). Nevertheless, it remains a possibility that the Asteraceae harbours more than one SI system, especially as elements of an underlying gametophytic SI system have been described in certain crosses between SSI species of *Parthenium* (Lewis, 1994) and also in *Senecio* (Hiscock, 2000b; Tabah, 2004). This important area of mating system evolution will benefit from further research as more genomic tools emerge for *Senecio*.

### 3.2 Implications of self-incompatibility for genomic structure and regulation

**Suppressed recombination around the S locus**

Since both pollen and pistil *S* determinants are required for functional SI, all *S* loci characterised to date have
a haplotypic structure containing at least two tightly linked polyallelic genes responsible for male and female SI function, respectively (Iwano & Takayama, 2012). Recombination between haplotypes is necessarily minimised through extensive structural differences between haplotypes and/or high sequence divergence (Boyes et al., 1997; Shiba et al., 2003; Goubet et al., 2012). This extensive sequence divergence is created by balancing selection that maintains many functionally distinct $S$ haplotypes to maximise population mate availability (Schierup & Vekemans, 2008; Roux et al., 2013). The availability of sequenced genomes for Senecio species will allow testing of predictions about the genomic structure of the $S$ locus in this novel SSI system thereby broadening the taxonomic breadth of studies of the evolution of SI and $S$ loci.

**Dominance in SI systems**

Sporophytic expression of male determinant $S$ alleles in pollen creates dominance interactions between $S$ alleles that optimise mate availability in populations while effectively restricting self-pollination, even when allelic diversity is relatively low (Vekemans et al., 1998; Brennan et al., 2003; Billiard et al., 2007). The presence of $S$ allele dominance interactions (both for pollen and stigma $S$ determinants) is thought to have contributed to the colonising success of self-incompatible $S$. *squalidus* in the UK and *S. inaequidens* in continental Europe despite the presence of limited functional $S$ haplotype diversity (Brennan et al., 2002; Brennan et al., 2003, 2006; Lafuma & Maurice, 2007). Moreover, evidence for differences in $S$ allele dominance interactions across different UK *S. squalidus* populations suggests that $S$ allele dominance is labile and can evolve (Brennan et al., 2006). Senecio can be used to determine whether independently evolved systems of SSI have also independently evolved this flexible mechanism of $S$ allele dominance regulation. Future research on SSI in Senecio could explore the relevance of recent discoveries in the Brassicaceae, such as the control of pollen $S$ allele dominance by $S$ allele-specific sRNAs (Durand et al., 2014) and the accumulation of inbreeding depression associated with the $S$ locus (Billiard et al., 2007; Llaurens et al., 2009), generating an important test of whether such mechanisms apply beyond a single SSI system.

**3.3 Implications of self-incompatibility for hybridization and polyploidy**

As a mechanism promoting outcrossing, SI also increases opportunities for interspecific hybridization (Brennan et al., 2013; Vallejo-Marín & Hiscock, 2016). Hybridization is recognised as an important evolutionary force that rapidly generates new genetic combinations for selection to act upon, which enhances the spread of adaptive traits across related groups (Abbott et al., 2013; Harrison & Larson, 2014). Strong balancing selection promoting $S$ haplotype diversity increases the likelihood of gene flow between taxa at the $S$ locus (Castric et al., 2008), as reflected by extensive $S$ haplotype sharing between hybridizing Senecio species (Brennan et al., 2013).
Senecio provides many examples of allopolyploid hybrid species, created when interspecific hybridization is accompanied by a doubling of a hybrid’s chromosome number (Abbott & Lowe, 2004; Kadereit et al., 2006; Hegarty et al., 2012; Pelser et al., 2012). Different ploidy levels between species need not present an insurmountable barrier to gene flow following hybridization, as shown by examples of gene transfer of the Ray locus from diploid S. squalidus to tetraploid radiate S. vulgaris (Fig. 3; Kim et al., 2008; Chapman & Abbott, 2010). Sometimes ploidy differences can contribute to genetic swamping from the dominant pollen donor as sterile progeny with unbalanced chromosome numbers are produced. This process is thought to be contributing to the invasive success of diploid S. madagascariensis in encroaching upon the habitat occupied by native tetraploid S. pinnatifolius in Australia (Prentis et al., 2007).

Hybridization between S. squalidus (SI) and self-compatible (SC) S. vulgaris within the last 100 years has resulted in the evolution of the allohexaploid hybrid species S. cambrensis (SC) on at least two occasions in north Wales and Edinburgh (Fig. 3; Abbott & Lowe, 2004; Brennan & Hiscock, 2010; Hegarty et al., 2012). The process of genome duplication itself might have led to the disruption of the SI system inherited from S. squalidus, resulting in the SC condition in S. cambrensis. SC might therefore be selected for in new polyploids because they are initially rare with limited S haplotype diversity soon after origin, which could cause problems with mate availability under obligate outcrossing. However, many polyploid self-incompatible Senecio species are known (Andersson, 2001; Lafuma & Maurice, 2007) and in resynthesized S. cambrensis allohexaploids it was found that SI reappeared among some offspring in the F2 generation (Brennan & Hiscock, 2010) suggesting complex control of SSI in neopolyploids. These examples demonstrate that Senecio provides a fascinating study system to better understand how breeding system, hybridization, and polyploidy interact in evolution to generate and maintain genetic, taxonomic and ecological diversity.

3.4 Implications of self-incompatibility for invasiveness and colonisation

According to Baker’s Law, self-compatibility is favoured in invasive populations because of enhanced reproductive assurance during colonization (Baker, 1967; Pannell & Barrett, 1998). However, many exceptions exist, and it is clear that the interactions between breeding systems and colonisation or invasiveness can be complex. In the UK, investigations of invasive S. squalidus populations have revealed individual variation in the strength of SI within and between populations; a phenomenon often referred to as pseudo-self-compatibility (PSC; Brennan et al., 2011). Despite SI being predominant in all populations of S. squalidus, a low frequency of PSC (~6-8%) sometimes higher (40% SC, 13% PSC in an Exeter sample; A. Brennan & S. Hiscock, unpublished) was detected in all UK populations sampled, and may be contributing to mating and colonizing success (Brennan et al., 2005). PSC might be of considerable adaptive importance in
confering reproductive assurance through breeding system ‘flexibility’, particularly during periods of population disturbance or establishment when the relative advantages of selfing outweigh those of outcrossing (Levin, 1996; Hiscock, 2000a, b; Stephenson et al., 2000).

PSC and SC in *S. squalidus* are heritable and appear regulated by multiple genetic loci that interact with the *S* locus to modify its expression (Hiscock, 2000b; Tabah, 2004; Brennan et al., 2011) similar to the control of unilateral interspecific incompatibility, which regulates the compatibility of crosses between closely related SI and SC species (Hancock et al., 2003). Environmental factors, such as salt concentration, also affect the expression of SI and PSC (Hiscock, 2000a, b), potentially providing a mechanism for the environment to have direct effects on recombination rates in natural populations (Stevison et al., 2017). Furthermore, it has been demonstrated experimentally that recombination can ‘resurrect’ SI to varying levels of penetrance in SC lines of synthetic *Senecio* allopolyploids generated by crossing an SC (*S. vulgaris*: 4n) with an SI species (*S. squalidus*: 2n) (Brennan & Hiscock, 2010). Therefore, rather than a qualitative trait (as it has traditionally been viewed), SI *sensu lato* could be viewed as a quantitative trait at the end of a potentially flexible continuum from SI through PSC to SC. These studies highlight the potential of *Senecio* as an informative model system for investigating evolutionary interactions between breeding systems and invasiveness.

### 4. Mechanisms and consequences of hybrid speciation

Historical and current interspecific hybridization is common in *Senecio* (e.g., Comes & Abbott, 2001; Pelser et al., 2012; Osborne et al., 2016), occurring both within and across ploidy levels, and between selfing and outcrossing species (e.g., Kadereit et al., 2006; Chapman & Abbott, 2010). Indeed, *Senecio* has been highlighted for its exceptional recent rates of hybrid speciation, particularly as opportunities for new interspecific interactions arise due to human-mediated change (Thomas, 2015). *Senecio* is notable for containing examples of both recent homoploid hybrid speciation (involving no change in chromosome number) and allopolyploid speciation (inheritance of more than two sets of chromosomes), serving as excellent models for investigating hybrid speciation (Fig. 3).

The homoploid hybrid species *S. squalidus* (Oxford ragwort) originated from material introduced to the Oxford Botanic Garden (UK) at the end of the 17th Century (Harris, 2002) derived from hybridization between two species native to Mount Etna, Sicily, *S. aethnensis* and *S. chrysanthemifolius* (Fig. 3a-b; James & Abbott, 2005). Following almost a century of cultivation in the Oxford Botanic Garden, the new homoploid hybrid species rapidly spread across Britain and into Ireland over the next 200 years (Abbott et al., 2009). *Senecio squalidus* is genetically and phenotypically divergent from its parents and their hybrids, which are restricted to Sicily (Brennan et al., 2012), with evidence of extreme divergence in gene expression (Hegarty et al., 2008, 2009). In addition to its ecogeographical isolation, recent genetic mapping studies
indicate that *S. squalidus* has inherited a combination of genetic incompatibilities from its parent species contributing further to its reproductive isolation (Brennan *et al.*, 2019). Together this genetic, phenotypic and transcriptomic divergence has likely contributed to its local adaptation to the UK (Allan & Pannell, 2009; Ross, 2010), which is the target of current transcriptomic (RNA-seq) research aimed at identifying candidate genes for local adaptation (Nevado and Hiscock, pers. comm.).

During its spread in Britain, *S. squalidus* has repeatedly hybridised with the native tetraploid, *S. vulgaris*, resulting in the origin of the introgressant *S. vulgaris* var. *hibernicus*, a new tetraploid species, *S. eboracensis* (Irwin & Abbott, 1992; Lowe & Abbott, 2003), and multiple origins of the neoallohexaploid, *S. cambrensis* (*Figs. 3-4*; Abbott & Lowe, 2004; Hegarty *et al.*, 2012). These hybridisation events were associated with marked changes in gene expression (Hegarty *et al.*, 2006; 2012; 2013) that may have had adaptive consequences by facilitating the establishment of new hybrid taxa through ecological differentiation (see also section 6.3). Multiple independent hybridisation events, as observed in *Senecio*, provide a natural experiment with which to study the consequences of hybridisation and polyploidy in populations. Species of *Senecio* therefore provide abundant systems for future detailed genomic analyses of mechanisms favouring the origin and establishment of hybrid taxa and introgression in the wild (e.g., Alexander-Webber *et al.*, 2016).

5. Adaptation and speciation in *Senecio*

5.1 The evolution of reproductive isolation during the formation of ecotypes

Ecotypes originate when adaptation to contrasting habitats leads to a build-up of extrinsic reproductive isolation between populations. Understanding how and when ecotypes become independent species remains a fundamental issue in evolutionary biology (Abbott & Comes, 2007; Lowry, 2012). Of particular importance is how ecological divergence becomes associated with intrinsic barriers to gene flow, created by genetic divergence that produce genetic incompatibilities between ecotypes.

In the *S. lautus* complex, which consists of multiple taxonomic ecotypes and species (see Roda *et al.*, 2013), a strong correlation is observed between plant morphology and habitat (*Fig. 2*; Radford *et al.*, 2004; Walter *et al.*, 2018a). Coastal populations grow tall on sand dunes (Dune ecotype), but prostrate on rocky headlands (Headland ecotype) and cliffs along the Australian coastline (*Fig. 2*; Roda *et al.*, 2013). Dune and Headland populations often occur in adjacent habitats and are self-incompatible and interfertile. Transplant experiments have revealed that neither Dune nor Headland ecotypes, nor their hybrids, can colonise each other’s habitat (Melo *et al.*, 2014; Richards & Ortiz-Barrientos, 2016; Richards *et al.*, 2016; Walter *et al.*, 2016). The probability of surviving in the alternative environment is a function of the proportion of alleles of local origin that a hybrid individual carries (Richards *et al.*, 2016). Together, these observations suggest that limited
dispersal and natural selection against migrants and hybrids keep populations distinct despite their parapatric distribution, and that selection acting in opposing directions has played a fundamental role in the formation of ecotypes in this system, likely leading to the formation of new plant species.

Barriers to gene flow that act after pollination, such as F1 hybrid inviability or sterility, are often masked by heterosis (Lowry et al., 2008). This can bias perception as to how intrinsic and extrinsic reproductive barriers evolve in a system that has diverged recently. For instance, in transplant experiments of *S. lautus*, F1 hybrids show strong hybrid vigour (Walter et al., 2016), suggesting that heterosis masks the deleterious effects of gene flow between populations locally adapted to contrasting habitats. However, a more detailed reciprocal transplant including all combinations of F1 hybrids amongst four ecotypes (Fig. 2, Woodland ecotype - dry sclerophyll woodland, and Tableland ecotype - subtropical rainforest edges), showed that field fitness depended on the chromosomal origins in a hybrid (Walter et al., 2016). For each of the four transplant environments, F1 hybrids with a chromosome native to the transplant environment (e.g., Dune in the dune environment) performed better than F1 hybrids where both chromosomes were from foreign ecotypes (e.g., F1\textsubscript{Headland}*Tableland in the dune environment). This suggests that alleles associated with heterosis were also involved in adaptation. F1 hybrids also show vigour in glasshouses when compared to parents, while F2 hybrids perform poorly. The presence of F2 hybrid breakdown suggests that intrinsic postzygotic isolation is created by alleles that reduce fitness when recombined into other genetic backgrounds (Walter et al., 2016).

Although reproductive compatibility seems to be high among ecotypes of *S. lautus*, geographical patterns of reproductive isolation suggest that different populations of the same ecotype are becoming reproductively isolated (Melo et al., 2019). Such patterns provide the opportunity to study the evolution of reproductive isolation along a speciation continuum to identify generalisations for speciation in plants (Melo et al., 2014). Recent work has identified molecular links between adaptation and speciation in *S. lautus* (Melo et al., 2019), and found evidence that adaptive divergence also creates genetic incompatibilities between populations inhabiting contrasting habitats (Walter et al., 2019). Overall, current evidence suggests that natural selection plays a fundamental role in creating barriers to gene flow between *S. lautus* populations and that extrinsic barriers seem to have evolved early during divergence, while intrinsic barriers were initially weak but have accumulated. This work highlights the potential for using *Senecio* to better understand how adaptation and speciation occur across environments and geographical ranges.

5.2 Ecological speciation on Mount Etna

Genomic analyses have made it clear that in the presence of divergent selection, evolutionary divergence can occur despite populations continuing to exchange alleles (whether in sympatry or parapatry), with intrinsic reproductive isolation arising as a consequence later (Rundle & Nosil, 2005).
flow’ scenario is of interest to evolutionary biologists because the relative roles of major mechanisms of evolution (ecology, selection, genetic drift and gene flow) can be studied together to understand how phenotypes, genotypes, and alleles of diverging populations interact during speciation.

On Mount Etna, Sicily, two sister Senecio species inhabit ecologically distinct, high and low elevation environments. Senecio aethnensis (Fig. 4a) is found above ca. 2,000m above sea level (a.s.l.) on old lava flows, and is covered by snow each winter, while Senecio chrysanthemifolius (Fig.4b) occurs in disturbed habitats and along roadsides below ca. 1,000m a.s.l., and endures intense summer heat. At intermediate elevations the species form a hybrid zone (James & Abbott, 2005; Brennan et al., 2009). The parental species show divergence in leaf and capitulum shape and size, with plants in the hybrid zone exhibiting a range of intermediate phenotypes, with clines for different traits varying significantly in width, suggesting selection against at least some intermediate traits (Brennan et al., 2009). The two species are almost completely interfertile with hybrids generally fit and fertile, even in later generations. It is therefore likely that selection is responsible for maintaining the distinctness of the two parental species (Chapman et al., 2005; but see Brennan et al. [2014] and Chapman et al. [2016] for evidence of incompatibility due to hybrid breakdown).

Using transcriptome sequences from one individual from each of S. aethnensis and S. chrysanthemifolius (and S. vernalis as an outgroup), Osborne et al. (2013) found evidence that speciation occurred with gene flow (i.e. in parapatry versus allopatry). Supported by Chapman et al. (2013), both studies found similar estimates in mean divergence time between the two species of ca. 108,000 years ago (bootstrap range: 53,000 - 187,000), which coincides with the uplift of Mount Etna that likely produced a novel high altitude habitat in which S. aethnensis could have originated (Chapman et al., 2013; Osborne et al., 2013). The well characterised demographic history of the Mt Etna hybrid zone provides an intriguing system and an ideal basis for future work to study the genetic architecture of traits underlying adaptation, speciation and hybrid breakdown, especially in combination with fully annotated genomes for these species.

5.3 Introgression and clinal analysis in the formation of a hybrid zone during adaptive divergence

The hybrid zone between populations of S. aethnensis and S. chrysanthemifolius on Mount Etna has been studied by quantifying how traits (associated with reproduction, growth form and leaf shape) and neutral microsatellite markers change across a hybrid zone (Brennan et al., 2009). This analysis provides evidence for strong selection on leaf traits (relative to neutral markers), which have narrower cline widths (strong selection) than genetic markers (1.49 km compared to 3.24 km), and for selection against hybrids associated with the elevational gradient (1,515-1,795 m), which determines the position of the cline centre. There were also significant increases of covariances and variances of genotypes and ecological variation at the cline centre, suggesting a high frequency of relatively recent hybrids at the centre. A significant displacement of
the cline centre of floral traits compared to genetic markers of 0.41 km suggests that alleles associated with
variation in some traits may be moving to higher elevations. Reciprocal transplants throughout the hybrid
zone on Mt. Etna (or other Senecio hybrid zones), at different spatial scales, and in combination with high-
throughput sequencing, can provide an important way to explore the genetic and genomic architecture of
traits affecting fitness in Senecio in contrasting environments.

6. Genomics of adaptation and speciation

6.1 Genomics of adaptation and parallel evolution

The genetic basis of adaptation in plants remains largely unexplored outside model and crop systems.
Notable exceptions are wild sunflowers (Helianthus spp.), rockcress (Boechera stricta) and monkeyflowers
(Mimulus spp.), where studies have revealed the genetic architecture and molecular basis of adaptive traits,
including flowering time (Blackman et al., 2011), flowering phenology (Anderson et al., 2013),
physiological adaptations to contrasting environments (Rieseberg et al., 2003) and drought resistance (Lowry
& Willis, 2010; VanWallendael et al., 2019). In S. laetus, where multiple pairs of dune and headland
populations have evolved repeatedly and independently along the Australian coast, genomic approaches are
providing important insights into the genetic changes associated with rapid adaptive divergence.

Using single nucleotide polymorphisms (SNPs) distributed across the genome, Roda et al. (2013) showed
that different pairs of parapatric dune and headland populations had unique patterns of genetic differentiation,
suggesting independent adaptive differentiation. Although patterns of population pair differentiation were
unique at the SNP level, they were similar at the level of predicted function, notably for development and
growth via auxin. This suggests that paths towards adaptation and patterns of repeated speciation (between
ecotypes for multiple population pairs) are often different but lead to similar end results in terms of
phenotypes. Current research using S. laetus is exploring the molecular basis of auxin and gravity responses
(Wilkinson et al., 2019). Within Senecio, the parallel evolution of traits across populations occurs in many
other species (e.g., Ni accumulation in S. coronatus [Meier et al. 2018] and ecotype formation in
Mediterranean Senecio [Comes et al. 2017] ). The existence of multiple adaptive radiations in Senecio, each
involving adaptation to similar or contrasting environmental stresses highlights the potential of the genus to
understand the ecological genetics of adaptation and speciation.

6.2 Population genomics of ecological speciation

The interaction between recombination, gene flow and selection during speciation are still poorly understood
but actively researched both theoretically (e.g., Schilling et al., 2018) and empirically (e.g., Chapman et al.,
2016; Safran et al., 2016; Doellman et al., 2018; Stankowski et al., 2019). Senecio contains multiple systems
where these evolutionary dynamics can be studied. For example, despite differing profoundly in phenotypes, life history, and ecology, *S. aethnensis* and *S. chrysanthemifolius* on Mount Etna show surprisingly little genomic divergence (Chapman *et al.*, 2013, 2016; Filatov *et al.*, 2016). Osborne *et al.* (2016) demonstrated extensive contemporary gene flow between the two species, supported by a demographic analysis of the species (Chapman *et al.*, 2013), with more gene flow from *S. aethnensis* to *S. chrysanthemifolius* than in the opposite direction. Genomic comparisons between *S. aethnensis* and *S. chrysanthemifolius* revealed the clustering of highly differentiated genetic markers (identified by outlier scan; Chapman *et al.*, 2013) in a handful of small regions of the genome (Chapman *et al.*, 2016). However, their clustering appeared to be associated with low recombination regions and not islands of divergence. QTL mapping demonstrated that outlier genetic markers tended to underlie morphological QTL, suggesting a link between genomic divergence, morphological divergence and adaptation to altitude (Chapman *et al.*, 2016). In *S. laetus*, Roda *et al.* (2017) found evidence for clustering of adaptive alleles associated with variation in both architectural and reproductive traits. This suggests that pre- and post-pollination reproductive barriers can evolve concomitantly and facilitate speciation.

**6.3 Differential gene expression associated with hybrid speciation and adaptation.**

The extent and role of changed patterns of gene expression in local adaptation or the formation of new species via hybridisation is not well understood (Vallejo-Marin & Hiscock, 2016). However, there is considerable interest in the idea that the regions of the genome that show environmental (or trans-generational) mediation of gene expression may be disproportionally involved in local adaptation (West-Eberhard, 2005). Gene expression can also be altered dramatically during the rapid changes in gene number associated with speciation by hybridisation. To investigate how gene expression changes during homoploid and allopolyploid hybrid speciation, Hegarty *et al.* (2005) performed microarray experiments and sequenced genes that showed over or under expression in *Senecio* hybrids, relative to one or both parents. Gene expression was explored in natural and resynthesised allopolyploid hybrids between *Senecio vulgaris* and *S. squalidus* (**Fig. 3**), their primary triploid hybrid *S. x baxteri* (3n=3x=30) and their allohexaploid hybrid *S. cambrensis* (2n=6x=60) (Hegarty *et al.*, 2005; 2006). Early generation hybrids exhibited massive changes in gene expression affecting hundreds of genes, a phenomenon described as ‘transcriptome shock’ (Hegarty *et al.*, 2005; 2006). Interestingly, changes in levels of gene expression were greater in triploid *S. x baxteri* than in the allohexaploid *S. cambrensis* suggesting that genome duplication, leading to the formation of the allohexaploid, has an ‘ameliorating’ effect on hybridisation-induced ‘transcriptome shock’ (Hegarty *et al.*, 2006).
Transcriptome shock was also observed in the homoploid hybrid *S. squalidus* and in artificial ‘*S. squalidus*’ hybrids (propagated for five generations) produced by crossing its parental taxa (Hegarty *et al.*, 2009). The effect of transcriptome shock was more extreme in the artificial homoploid hybrids. In a subset of genes, transgressive expression (up or down regulation with respect to both parents) was maintained in subsequent generations, while other affected genes tended to move towards a pattern of expression consistent with wild *S. squalidus*. These transgressive genes were involved in photosynthesis, catalytic activity, lipid binding, protein biosynthesis and protein folding (Hegarty *et al.*, 2009). The finding of transgressive expression was consistent with similar microarray analysis in hybrid sunflowers (Lai *et al.*, 2006), which identified transgressive levels of gene expression in wild hybrids adapted to environmental conditions not tolerated by their parent species. Both studies suggest that new patterns of transgressive gene expression arising from hybridization could provide genetic novelty for selection to facilitate adaptive divergence.

Comparisons of gene expression and transcriptome sequences between *S. aethnensis* and *S. chrysanthemifolius* identified genes potentially involved in adaptation to high and low altitudes, and the relative roles of expression and sequence divergence in the genomic basis of adaptive divergence (Chapman *et al.*, 2013). However, in sequence comparisons, alleles that showed high interspecific divergence (ca. 200) were just a small fraction of the loci that were analysed (ca. 18,000) suggesting that species boundaries due to morphological differences and ecological adaptations might be maintained by just a small portion of the genome (Chapman *et al.*, 2013), a pattern consistent with recently diverged species as part of the ‘genic view’ of speciation (Wu, 2001). Future work should exploit the ability to clone and transplant individuals across environments. In this way, field experiments and the power of recent advances in NGS transcriptome analysis can be combined to explore the adaptive potential of changes in gene expression among *Senecio* taxa (e.g., Alexander-Webber *et al.*, 2016), alongside those associated with the generation of hybrids, as well as the loci showing plastic responses to environmental variation within species.

7. Integrating studies of genotype, phenotype and fitness

7.1 The challenge of integrating genotype, phenotype and fitness information in the field.

Quantifying genetic variation in fitness, and its relationship with quantitative traits, allows the responses of groups of traits to natural selection to be predicted. This information is central to understanding how populations and ecological communities will respond to the reductions in fitness predicted by ongoing global change, as well to explore how selection varies in strength and form among populations, species and in different ecological contexts (Shaw & Etterson, 2012). Knowledge on genetic and ecological correlations among traits will also help us to understand what drives populations to specialise, forming new species with
narrow ecological tolerances, rather than creating ecologically widespread species that show local adaptation across their range.

Quantitative genetic analyses of traits and fitness across ecological context demand large sample sizes and large experiments that integrate genetic, phenotypic and fitness information, ideally across a range of natural environments. To estimate genetic variation in fitness, selection should be measured on individuals of known relatedness (pedigrees or crossing designs) by quantifying phenotypic traits and fitness under field conditions, and estimating the additive genetic covariance between traits and fitness (Morrissey et al., 2012; Pujol et al., 2018; Shaw, 2019). The combination of appealing experimental attributes (short generation times, recent genetic developments and the ease to which they can be propagated) and the range of ecologies in which the fitness of phenotypes can be assessed, mean that such integrative experiments are more tractable (and powerful) in *Senecio* than in many other model systems, especially when incorporated with five powerful experimental approaches outlined below.

1) **Full-sibling families**: Controlled crosses can be carried out easily in most *Senecio* species. In most cases they can also produce sufficient seed/genetic resources to conduct the large-scale field experiments with sufficient power to quantify evolutionary parameters (e.g., Walter et al., 2019). Crossing designs containing both full and half-sibling families (i.e., individuals sharing only a father) allow partitioning of the phenotypic information to estimate the additive genetic covariation between traits, or between traits and fitness (Lynch & Walsh, 1998). The benefit of using families within a breeding design is two-fold. Firstly, transplanting multiple individuals (siblings) from the same family in different environments allows them to be tested in different environments to identify how fitness varies across environments. Secondly, with sufficient seed, the same families can be propagated in separate glasshouse and field experiments. In this scenario, we can connect the mean of each family across separate experiments, making it possible to estimate the mean phenotype in the glasshouse, and the mean fitness in the field. Connecting phenotype with fitness using family means is logistically simpler than measuring phenotypes in the field where selection may have removed variation, and plasticity may masks genotypic effects. In addition, because phenotypes are measured under controlled conditions, the number of phenotypes that can be measured can be maximised, and each trait measured more precisely by minimising environmental variation. Traits that are difficult to measure in the field, such as physiological traits, can then be included more easily in largescale experiments. Fieldwork can then focus on quantifying different and more comprehensive measures of fitness to characterise how these performance measures change with ecological, geographic or temporal variation.

2) **Clones**: Ideally, the phenotype and fitness of a given individual within a pedigreed population (or crossing design) should be assessed across different environments, controlling for genetic variation. However, this is
only possible where genotypes can be replicated (i.e. cloned) and transplanted into multiple environments, an approach that assesses the reaction of the same genotype to environmental variation. Such an approach can be implemented in very few other model systems (e.g., *Mimulus*). However, many *Senecio* species produce roots readily from cuttings, allowing the use of clones of known genotypes in field and molecular experiments, at sufficiently high replication to allow useful estimates of genetic variation in fitness to be made under field conditions, as well as exploration of the genomic bases of such variation.

3) Artificial hybridization: Detecting ongoing natural selection, and genetic variation in fitness, is difficult if populations are already close to their optimal phenotype and stabilizing selection is strong, or genetic variation low (Walsh & Lynch, 2018). The benefit of using *Senecio* as a model system to overcome such difficulties is two-fold. Firstly, many *Senecio* species show evidence of recent rapid adaptive radiation, providing the opportunity to characterise the strength and direction of selection during the early stages of adaptive diversification using transplant experiments (Walter *et al.*, 2016; Richards *et al.*, 2019). Secondly, *Senecio* species often display high levels of intraspecific variation, often with a well-defined structure among populations (i.e., ecotypes). In many cases, species also produce viable hybrids (unlike highly divergent taxa), allowing their use in multi-generation artificial hybridisation experiments (e.g., Richards *et al.*, 2019; Walter *et al.*, 2019; Wilkinson *et al.*, 2019). These crosses can be used to recreate genetic and phenotypic variation that is currently absent in natural populations and may reflect earlier stages of divergence among sister species. If transplanted into the field, the fitness of these alleles and genotypes can be used to explore the ecological consequences of such variation, as has also been conducted with great success to study hybrid speciation in *Helianthus* sunflowers (Llexer *et al.*, 2003a; Llexer *et al.*, 2003b).

4) Molecular methods for relating genotype to phenotype: The transcriptome is a critical intermediary between genetic and phenotypic variation. Appropriately designed experiments can answer fundamental questions about the genomic architecture underlying phenotypic responses to the environment. *Senecio* can be used in experiments that propagate large numbers of clones of a single genotype, which are then transplanted across environmental conditions to produce powerful analyses of differential gene expression. Changes in levels of gene expression across the genome can then quantify the effects of the environment, tissue-type, sex, or developmental stage. RNA-seq has been used to study differential expression between divergent species on Mt Etna (Hegarty *et al.*, 2008; Chapman *et al.*, 2013), between polyploids and their diploid progenitors (Alexander-Webber *et al.*, 2016), and also intraspecific variation of specific traits, such as nickel hyper accumulation in *S. coronatus* (Meier *et al.*, 2018). Differential expression analyses tend to yield lists of tens to hundreds of genes that differ in the number of transcripts produced between conditions. However, functional annotation of genes provides a means of deriving meaningful results from such lists. For example, Meier and colleagues found significant enrichment for transporter proteins, and qRT-PCR
confirmed elevated expression of two known nickel transporters (IRT1 and IREG2), which were associated with intra-specific variation in nickel accumulation.

5) Genetic transformation and gene editing: More explicit tests of function are possible in Senecio using Agrobacterium-mediated transformation (Kim et al., 2008; Garcês et al., 2016). By combining gene expression and functional analyses with phylogenetic analyses, Chen et al. (2018) showed that ray florets in S. vulgaris are regulated by three CYC2-like paralogs. The phylogenetic history of this gene family across Senecio, and Asteraceae more broadly, can show how gene duplication and introgression can give rise to greater floral complexity. Finally, the development of the CRISPR/Cas9 gene editing technology for non-model organisms (Shan et al., 2018) will allow fast targeted mutagenesis in Senecio to test the function of loci identified from comparative genomic studies and field experiments. Such technologies will provide an excellent opportunity to relate gene function to evolutionary ecology and the gain and loss of key traits across such a diverse plant genus.

7.2 Exploring the evolution of environmental sensitivity

The ability of a single genotype to produce different phenotypes in response to environmental variation (phenotypic plasticity) allows complex organisms to survive in a variable world. Across environments where this plasticity is adaptive, it reduces the exposure of alleles to selection (Ghalambor et al., 2007), acting against the formation of clines (local adaptation) at many loci. Such responses of genotypes to environmental variation in time and space determines the distribution of populations, their range limits and how they will respond to future environmental disturbances (Chevin et al., 2010). The relatively recent radiation of Senecio (10.7My), their occupation of contrasting environments, and their ability to hybridise makes Senecio a powerful system to study how environmental sensitivity evolves in different environmental contexts.

Local adaptation in many plant systems (Hereford, 2009), including numerous Senecio species (e.g., Walter et al., 2016), confirms that plasticity within a given species can only be adaptive across the limited range of environments that typically define their ecological niche (DeWitt et al., 1998). Studies have also identifiedheritable variation in plasticity for several important traits (Nussey et al., 2005), suggesting that genetic variation for plasticity persists that may permit future evolution of different forms of environmental sensitivity. However, such studies are rare in plants, and require the development of model systems that can integrate the approaches described above (especially clonal propagation).

In other systems, reciprocal transplants of clones, seeds, or seedlings within and outside existing habitats has been used to explore genetic variation in phenological traits at range margins (e.g., Sheth & Angert, 2016). Ongoing Senecio research on Mount Etna is complementing these studies by testing how adaptation to
contrasting environments affects their capacity for phenotypic plasticity when exposed to environmental variation (G. Walter et al., unpublished). Therefore, *Senecio* can be used to understand how local adaptation (i.e. specialisation) may reduce the forms and magnitude of plasticity possible within a species, with concomitant consequences for fitness and resilience to future environmental change.

### 7.3 Understanding trade-offs in life history and among environments

Understanding life history trade-offs is crucial for understanding how organisms acquire and utilise resources throughout their lifetime, and why they form species with narrow niches over longer timescales (Futuyma & Moreno, 1988; Agrawal et al., 2010). Field transplants of ecotypes of *S. lautus* have shown developmental mismatches with the environment that results in mortality during seedling establishment. Such mortality reflects fitness trade-offs between contrasting environments and life stages, suggesting specialisation for environment-specific resource allocation strategies (Walter et al., 2018b). How variation in resource allocation across environments is mediated by biotic challenges (e.g., immunity or resistance to fungi) remains a key issue in understanding ecological range margins.

### 7.4 Genetic constraints to adaptation

Trait correlations bias the distribution of genetic variation towards certain multivariate phenotypes, at the expense of other phenotypes. If genetic variance is orientated away from the direction of natural selection, there will be little genetic variance for fitness, and the rate of adaptation will be slowed (Lande, 1979; Walsh & Blows, 2009). For much of the 20th century, genetic correlations were thought to be stable, making it difficult to see how rapid evolution in novel directions could occur (Arnold et al., 2008). However, recent comparative evidence has shown that genetic correlations can evolve rapidly (Doroszuk et al., 2008; Eroukhmanoff & Svensson, 2011), and experiments with *Senecio* suggest that changes in genetic correlations could overcome constraints to adaptation, allowing rapid ecological diversification (Walter et al., 2018a). By integrating the approaches outlined above, experiments can capitalise on the tractability of *Senecio* to identify how the environment affects genetic variation in traits and fitness, identify the conditions that alter genetic correlations, and assess how and when the evolution of trait correlations leads to rapid evolutionary responses to novel environments.

### 8. Conspectus

*Senecio* is a powerful, but as yet underutilised model system that has a unique potential to answer many fundamental questions in ecology and evolutionary biology. Most recently, four studies using Australian species of *Senecio* in field and glasshouse experiments combined artificial hybridization with molecular and quantitative genetics to identify mechanisms underlying adaptive divergence leading to speciation (Roda et
In Europe, the development of genetic and genomic resources for \textit{S. squalidus} and its close relatives has facilitated comparative genomics of species adapted to contrasting environments, and the identification of candidate genes for local adaptation (Hegarty\textit{et al.}, 2008; Chapman\textit{et al.}, 2013; B. Nevado\textit{et al.} unpublished). Meanwhile, detailed genomic and ecological analyses of closely related but ecologically divergent species in Sicily have revealed how selection acts across different genomic and ecological backgrounds (Brennan\textit{et al.}, 2009), the role of gene flow and geological processes in promoting adaptive divergence (Chapman\textit{et al.}, 2013; Osborne\textit{et al.}, 2016), and the way that gene expression variation may be involved in these processes. Alongside these studies, focused ecological analyses can use \textit{Senecio} to better understand the biotic and abiotic factors that limit species distributions across taxa and biogeography. While ongoing next-generation sequencing can further develop \textit{Senecio} as a system for studying genome evolution within a phylogenetic context. Quantitative genetic research can develop \textit{Senecio} by taking clones of individuals from breeding designs, and transplanting them across environments to identify genetic variation that can promote adaptive evolutionary responses to environmental change. Combining transcriptomic analyses with such experiments can then identify the genetic basis of plasticity. Such experiments using the \textit{Senecio} model will ultimately yield an integrative ‘triangle’ linking genotype, phenotype and fitness.

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Supporting information

Note S1: Photo credits for Fig. 1 and Fig. 4.

Note S2: Details of the methods to produce Fig. 2.

Note S3: References cited in Table 1.
Tables, Boxes and figures
Table 1. Use of Senecio in genetic, evolutionary, systematic and ecological research. Within each section of the table, studies are listed in approximate historical sequence. Full references of citations are listed in Note S3. Research on taxa formerly within Senecio but now placed within the genus Jacobaea is not cited in the table.

| Topic                                | Taxa                              | References                                                                 |
|--------------------------------------|-----------------------------------|---------------------------------------------------------------------------|
| **Genetics, Cytology, Development**  |                                   |                                                                           |
| Heterosis                            | S. vulgaris                       | Bateson (1888)                                                            |
| Genetics of traits                   | S. vulgaris                       | Trow (1912, 1916ab); Skinner (1952); Basford (1961ab); Comes (1998); Brennann et al. (2016) |
|                                      | S. lautus                         | Roda et al. (2017)                                                        |
|                                      | S. squalidus                      | Ingram & Taylor (1982); Hiscock (2000a); Hiscock et al. (2003); Tabah et al. (2004); McInnis et al. (2005) |
| Genome size variation                | Diverse Senecio                   | Ornduff et al. (1963); Lawrence (1980, 1985); Lafuma & Maurice (2007); Lopez et al. (2013) |
| Maternal effects                     | S. vulgaris                       | Aarssen & Burton (1990)                                                   |
| Heteroplasmy                         | S. vulgaris                       | Frey (1998); Frey et al. (2005)                                          |
| **Evolution**                        |                                   |                                                                           |
| Hybridization & introgression        | S. vulgaris x S. squalidus        | Harland (1954a); Ingram (1977, 1978); Ingram et al. (1980); Marshall & Abbott (1980); Abbott et al. (1992); Kim et al. (2008); Lowe & Abbott (2015) |
|                                      | S. vulgaris x S. viscosus         | Gibbs (1971)                                                              |
|                                      | S. viscosus x S. squalidus        | Crisp & Jones (1978)                                                     |
|                                      | S. vulgaris x S. squalidus        | Comes & Kadereit (1990); Comes (1994a)                                   |
|                                      | S. pinnatifolius x S. madagascariensis | Prentis et al. (2007)                                           |
| **Hybrid speciation – Allopolyploidy**| S. cambrensis                     | Harland (1954b); Rosser (1955); Weir & Ingram (1980); Ingram & Noltie (1984, 1986, 1987, 1995); Ashton & Abbott (1992); Lowe & Abbott (1996); Abbott & Forbes (2002); Hegarty et al. (2006, 2008, 2011, 2012) |
|                                      | S. mohavensis                     | Coleman et al. (2001); Kadereit et al. (2006); Alexander-Webber et al. (2016) |
|                                      | S. hoggariensis                   | Kadereit et al. (2006)                                                    |
| Hybrid speciation – Homoploidy       | S. eboracensis                    | Irwin & Abbott (1992); Lowe & Abbott (2000, 2003, 2004)                  |
|                                      | S. squalidus                      | James & Abbott (2005); Brennan et al. (2012, 2019); Hegarty et al. (2009) |
| Hybrid zone structure & maintenance | S. aethnensis x S. chysanthemifolius | James & Abbott (2005); Chapman et al. (2005); Brennan et al. (2009); Ross et al. (2012) |
|                                      | S. vernalis x S. glaucus          | Abbott et al. (2018)                                                      |
|                                      | S. hercynicus x S. ovatus         | Raudnitscka et al. (2007); Bog et al. (2017a)                             |
| **Floral polymorphism**              | S. vulgaris                       | Richards (1975); Oxford & Andrews (1977); Marshall & Abbott (1982, 1984ab, 1987); Abbott (1985, 1986); Ross & Abbott (1987); Abbott & Irwin (1988); Oxford et al. (1996); Abbott et al. (1998); Kim et al. (2008); Garces et al. (2016) |
| **Breeding/Mating system variation** | Diverse Senecio                   | Gibbs et al. (1975)                                                       |
|                                      | S. vulgaris                       | Hull (1974); Campbell & Abbott (1976); Marshall & Abbott (1982, 1984ab, 1987); Ross & Abbott (1987); Warren (1988); Damgaard & Abbott (1995); Irwin et al. (2016); Love et al. (2016) |
|                                      | S. vernalis                       | Comes (1994b)                                                             |
|                                      | S. squalidus                      | Abbott & Forbes (1993); Hiscock (2000a); Hiscock et al. (2003); Brennan et al. (2002, 2003a); 2005, 2006); Tabah et al. (2004); McInnis et al. (2006); Allen et al. (2010, 2011) |
|                                      | S. inaequidens                    | Lafuma & Maurice (2007)                                                   |
| Ecotypic divergence & speciation     | S. vulgaris                       | Brennan & Hiscock (2010)                                                  |
|                                      | S. cambrensis                     | Crawford (1966b); Abbott (1976a); Briggs (1976, 1978); Ren & Abbott (1991); Theaker & Briggs (1993); Müller-Schärer & Fischer M (2001) |
|                                      | S. sylvaticus                     | Kumlzer (1969)                                                            |
|                                      | S. viscosus                       | Akeroyd et al. (1978)                                                     |
|                                      | S. lautus                         | Radford & Cousens (2004); Melo et al. (2014); Richards et al. (2016, 2019); |
| Systematics | Biosystematics | Molecular systematics | Phylogeography & speciation | Phylogeny | Ecology | Physiological variation | Life history | Chemical defence by pyrrolidine alkaloids | Disease susceptibility to: |
|-------------|---------------|----------------------|----------------------------|-----------|---------|------------------------|-------------|------------------------------------------|--------------------------|
| S. squalidus | S. vulgaris | Richards & Ortiz-Barrientos (2016); Walter et al. (2016, 2018ab, 2019) | S. vulgaris | Kent (1956, 1960); Harris (2002); Abbott et al. (2009); Allan & Pannell (2009) | Senecio species adapted to dry and wet habitats | Amlin et al. (1968); Hartmann et al. (1989); Frischknecht et al. (2001); Cheng et al. (2017) | S. sylvaticus, S. viscous | S. vulgaris, S. vernalis | S. vulgaris |
| S. aethnensis – S. chrysanthemeinvolius | S. vulgaris | Allan & Pannell (2009) | S. vulgaris | Harvest & Ogden (1970); Popay & Roberts (1970ab); Kadereit (1984c); Abbott (1986); Fenner (1986ab); Ren & Abbott (1991); Leiss & Müller-Schärer (2001) |  |  | S. vulgaris | S. vernalis | Hartmann & Zimmer (1986) |
| S. laetus complex | S. laetus | Chapman et al. (2013); Muir et al. (2013); Osborne et al. (2013); Brennan et al. (2014, 2016) | Mediterranean Senecio | Walter et al. (2018b) |  |  | S. laetus | S. laetus | Castells et al. (2014) |
| Herbicide resistance | S. vulgaris | Roda et al. (2013ab, 2017) |  |  |  |  |  | S. nemorensis complex | Bog et al. (2017b) |
| Parallel divergence & speciation | Mediterranean Senecio | Comes et al. (2017) |  |  |  |  |  | Diverse Senecio | Langel et al. (2011) |
| | | |  |  |  |  |  | | - Erysiphe fischeri |
| | | |  |  |  |  |  | | - Puccinia lagenophora |
| | | |  |  |  |  |  | | - Puccinia expansa |
| | | |  |  |  |  |  | | Plant-pollinator interactions |
| | | |  |  |  |  |  | | Interspecific competition |
| | | |  |  |  |  |  | | Long distance dispersal |
| | | |  |  |  |  |  | | | S. flavus, S. mohavensis |
| | | |  |  |  |  |  | | | S. inaequidens | Pelser et al. (2012) |
| | | |  |  |  |  |  | | | | - Erysiphe fischeri |
| | | |  |  |  |  |  | | | - Puccinia lagenophora |
| | | |  |  |  |  |  | | | - Puccinia expansa |
| | | |  |  |  |  |  | | | Plant-pollinator interactions |
| | | |  |  |  |  |  | | | Interspecific competition |
| | | |  |  |  |  |  | | | Long distance dispersal |
| | | |  |  |  |  |  | | | | - Erysiphe fischeri |
| | | |  |  |  |  |  | | | - Puccinia lagenophora |
| | | |  |  |  |  |  | | | - Puccinia expansa |
| | | |  |  |  |  |  | | | Plant-pollinator interactions |
| | | |  |  |  |  |  | | | Interspecific competition |
| | | |  |  |  |  |  | | | Long distance dispersal |
Box 1 – Future research using Senecio as a model system

Below we detail research directions we consider likely to be particularly productive using Senecio as a model system.

1. Responses to climate change. Due to its widespread distribution and occupation of numerous habitats, Senecio allows the response of populations and communities to climate change to be tested across a range of biotic and abiotic interactions. Such tests could include: (a) Responses in ecologically similar but phylogenetically different Senecio (e.g., across Mediterranean or Alpine habitats in Europe, Africa, and Australasia). (b) Comparisons of ecologically different but phylogenetically similar species: e.g. Alpine species can be compared to closely related taxa at lower elevations, as on Mt Etna. (c) Field transplants across elevational gradients to simulate future climate scenarios. These transplants can also assess how transitions in plant, mycorrhizal, microbial and insect communities across elevational gradients interact with changes in the fitness and phenotypes of different Senecio species.

2. Factors determining patterns of adaptive radiation. Adaptive radiation appears rapid and common in Senecio. A major challenge is to identify the factors that have driven adaptive diversification, leading to its broad distribution across environments and geography. Potential approaches could be: (a) Bringing phylogenetic and morphological data together to test whether innovation in key traits created species radiations in the Asteraceae. This could include traits associated with long-distance dispersal (i.e., the pappus), variation in leaf morphology, or the production of toxic alkaloids that deter herbivores. (b) Studying the genetics and evolution of barriers to gene flow in Senecio using examples of ecotypic divergence, recent hybrid speciation and stable hybrid zones. (c) Using the many self-incompatible and self-compatible species of Senecio to study the evolution of diverse mating systems, especially within a broad phylogenetic context. (d) Using the repeated examples of morphological convergence and divergence and trait gains and losses in Senecio to test for genomic convergence in the evolution of complex traits within a single plant genus; for example, the evolution of growth habit in ecotypes of S. laetus could be expanded by studying how dramatic variation in growth habit has evolved in varied, but closely related, Senecio species.

3. Ecological and evolutionary importance of phenotypic plasticity. Many species of Senecio show considerable variation in phenotypic plasticity in response to environmental heterogeneity (e.g. in S. vulgaris) and can be used in experiments to determine the relative importance of phenotypic plasticity versus adaptation in responding to such heterogeneity. The utility of Senecio in glasshouse and field experiments, especially when combined with quantitative genetic breeding designs, makes it possible to combine large experimental studies with emerging genetic approaches: (a) Using genetic transformation based on existing Agrobacterium-mediated methods or the development of CRISPR/Cas9 gene editing, it will be possible to conduct functional analyses of genes associated with specific traits, as well as characterise the function of loci identified through field and -omics studies. (b) Transplanting a breeding design across environmental variation allows us to study changes in relative fitness, which can then be related to transcriptomic data to identify how differential gene expression contributes to fitness variation. Quantitative PCR could then be used to study the specific regions of the genome underlying differential expression, and the role of small RNAs (siRNA and miRNA) in determining patterns of differential expression to identify the regulatory basis of local adaptation and phenotypic plasticity.

4. Developmental genetics of trait adaptations. The diverse array of traits and habitats of Senecio species provide a rich resource for understanding the developmental and genetic pathways of many traits, for example, leaf shape variation. Studying the ecological variation underlying already identified pathways could complement work on Arabidopsis and relatives.

5. Life history evolution. With a diverse array of life history strategies in closely related species, Senecio can be used to study the ecological genetics of developmental plasticity, and the evolution of life history and resource allocation trade-offs, particularly where some species have overlapping generations, and others do not. For example, Senecio species with diverse growth habits could be used to study transgenerational effects on adaptive phenotypes, and how the nature of such transgenerational effects is shaped by environmental predictability and variability.

6. The ecology and evolution of invasive species. Notable examples of invasive species within Senecio are prime models for detailed analysis, especially where they are invading habitat already dominated by other Senecio: (a) In Australia, S. madagascariensis is colonising ecosystems occupied by a closely related native Senecio species. (b) In the UK, the recently-formed hybrid species S. squalidus has colonised much of the British Isles in the last 200 years, providing an important model system to study how invasive species form, spread and adapt rapidly to their new geographic range. (c) South African Senecio inaequidens has spread rapidly across Europe, the Americas and Australia, and shows differences in chromosome number across its range, allowing detailed investigation of how ploidy level correlates with invasiveness.

7. Ecological and evolutionary importance of hybridisation. Senecio contains many species that have been formed by recent hybridisation, with associated effects on chromosome number, genome size, and gene regulation. These include multiple examples of homoploid hybrid and allopolyploid species, and provide excellent opportunities to test how introgression changes the architecture of genetic variation across geography, and promotes hybrid speciation.

8. How biotic and abiotic factors shape the distribution of widespread versus more specialised species. In S. laetus, populations of ecotypes often exhibit very narrow distributions despite a wide distribution of their habitat, allowing studies to investigate what determines the distribution of populations, and their fundamental versus realised niches. For example, to test how abiotic stress tolerances and biotic interactions together determine range limits, and whether this changes across habitats.
Figure legends

Fig. 1: With a worldwide distribution, species of *Senecio* show dramatic diversity in morphology and the habitats they occupy. Images are protected by copyright; this information is provided in parentheses and Note S1. 1. *S. wootonii* (CC BY-NC-SA 3.0 W. Anderson), 2. *S. pseudoarnica* (CC BY-NC 2.0 K. McFarland), 3. *S. soldanella* (CC BY-NC 3.0 M.E. Harte), 4. *S. flaccidus* (CC BY C.K. Kelly), 5. *S. claussenii* (CC BY N. Taylor & D. Zappi), 6. *S. petasitis* (© J. Moura), 7. *S. moqueguensis* (CC BY D.B. Montesinos-Tubée), 8. *S. candidans* (CC BY S.J. Tonge), 9. *S. barbertonicus* (CC BY-SA O. Leillinger), 10. *S. haworthii* (CC BY D. Avery), 11. *S. rowleyanus* (© Surreal Succulents, UK), 12. *S. nemorensis* (CC BY-NC C.T. Lin), 13. *S. halleri* (© I. Rey), 14. *S. leucanthemifolius* (CC BY-SA Petr Brož), 15. *S. graciliflorus* (CC BY Prashant Awale), 16. *S. cannabifolius* (CC BY Wikimedia), 17. *S. scandens* (CC BY-NC S. Duan), 18. *S. gregorii* (© P. Campbell).

Fig. 2: Australian *S. lautus* complex contains 12 ecotypes that have diverged rapidly from each other. (a) Ecotypes vary dramatically in leaf morphology and growth form, reflecting the diversity of habitats they occupy. (b-c) Ecotypes are distributed widely across Australia. Clades (represented by uppercase letters) group by geography, rather than ecology. (d) Morphological divergence of the ecotypes. Points and confidence intervals represent the mean and one standard error for each population measured. Colours represent ecotypes from panel c. See Note S2 for details of the data analysis.

Fig. 3: Hybrid origins of new *Senecio* taxa in the British Isles. Dates of first records are in red. For *S. vulgaris*, the non-radiate (nr) and radiate (r) forms are presented. Figure reproduced from Abbott and Rieseberg (2012) with permission.

Fig. 4: European *Senecio* species that have been studied extensively. *Senecio aethnensis* (a) and *S. chrysanthemifolius* (b) occur on Mount Etna (Sicily, Italy) at different altitudes. Hybrids between these two species were cultivated at the Oxford Botanic Garden (UK), from which they escaped in the late 18th century and spread across the UK, becoming a new species, *S. squalidus* (c). Hybridisation between *S. squalidus* and *S. vulgaris* (d) created the neoallohexaploid, *S. cambrensis* (e) and the tetraploid *S. eboracensis* (Fig. 3). Image of *S. vulgaris* © Botanical Society of Britain & Ireland, *S. cambrensis* © Frau-Doktor.