

Homoploid hybrid speciation in action

Richard J. Abbott,¹ Matthew J. Hegarty,² Simon J. Hiscock³ & Adrian C. Brennan¹

¹ School of Biology, University of St Andrews, St Andrews, Fife, KY16 9TH, U.K.

² Institute of Biological, Environmental & Rural Sciences, Aberystwyth University, Penglais, Aberystwyth, Ceredigion, SY23 3DA, U.K.

³ School of Biological Sciences, University of Bristol, Bristol, Somerset, BS8 1UG, U.K.

Author for correspondence: Richard J. Abbott, rja@st-andrews.ac.uk

Abstract Homoploid hybrid speciation is the origin of a hybrid species without change in chromosome number. Although currently thought to be a rare form of speciation, especially relative to the more common allopolyploid hybrid speciation, it is feasible that many examples of homoploid hybrid species will be discovered in the future now that genetic resources are readily available for testing their occurrence. In this review, we focus on the speed of homoploid hybrid speciation, the importance of ecological and spatial isolation in the process, and the nature of genetic changes that occur in a new hybrid during its origin and establishment in the wild. With reference mainly to the extensive work carried out on homoploid hybrid species of *Helianthus*, and to our own work on the very recently originated diploid hybrid species *Senecio squalidus*, we review evidence showing: (1) that new fertile homoploid hybrid species can originate very quickly, although a longer period is likely to be required before the species becomes fully stabilized both genomically and phenotypically; (2) ecological divergence of the hybrid species from its parents is key to successful establishment, and that this can occur even in the absence of post-zygotic isolation caused by chromosomal and/or genetic sterility barriers; (3) transgressive changes in phenotypic traits and gene expression are of great importance in adapting homoploid hybrid species to habitats that are ecologically and spatially divergent from those of the parents; (4) adaptive differences distinguishing a homoploid hybrid species from its parental species are likely to be maintained in the face of parental gene flow, and evolve in concert across populations representing multiple origins of the species; (5) in the absence of parental gene flow, i.e., under conditions of geographical isolation, rapid genetic divergence of the hybrid species is likely to be enhanced due to the combined effects of founder events, genetic drift and selection.

Keywords ecological divergence; gene flow; homoploid hybrid species; hybridization; speciation; transgressive gene expression

■ INTRODUCTION

Homoploid hybrid speciation is the origin of a hybrid species without change in chromosome number. It contrasts with the more frequent allopolyploid hybrid speciation in which there is whole genome duplication with diploid chromosome numbers of the two parent species being summed in the hybrid species. In his book *Plant Speciation*, Grant (1981) described two ways in which a homoploid hybrid species could originate without loss of sexual reproduction. The first of these required the hybrid species to be isolated from its parents by a chromosomal or genic sterility barrier and because of the genetic mechanisms involved was named “recombinational speciation”. The second required the hybrid to be isolated by what Grant termed an external isolating mechanism, such as habitat, ethological (behavioural) or geographical (spatial) isolation. Whereas Grant devoted one chapter of his book to describe the process of recombinational speciation, he devoted only a section of one chapter to homoploid hybrid speciation that relied on external isolating mechanisms. Thus, although it may not have been Grant’s intention, his greater focus on recombinational speciation tended to promote a perception that recombinational homoploid hybrid speciation was more important than ecological or spatial isolation between the hybrid and its parents. However, subsequent reviews of the incidence and process of homoploid hybrid speciation in plants by Rieseberg (1997) and

Gross & Rieseberg (2005) have made clear that ecological and spatial isolation are vital to the successful origin and establishment of a homoploid hybrid species and can enable the process to occur even in the absence of intrinsic isolation due to chromosomal or genic sterility barriers (Buerkle & al., 2000). Indeed, homoploid hybrid speciation is unlikely to occur unless there is at least some ecological and spatial isolation between the hybrid and its parent species (Buerkle & al., 2000).

The most extensive and detailed empirical work conducted on the process of homoploid hybrid speciation is that on three diploid hybrid species of sunflower (*Helianthus*), which originated in North America following hybridization between *H. annuus* and *H. petiolaris* (Rieseberg, 1991). The three species—*H. anomalus*, *H. deserticola* and *H. paradoxus*—are estimated to have originated between 60,000 to 200,000 years ago and are reproductively isolated from their two parents by chromosomal and ecological barriers (Rieseberg & al., 2003). Each of these hybrid species occurs in a habitat that is distinct from the two other hybrid species and from each parent species (Gross & Rieseberg, 2005). Thus, whereas the parent species, *H. annuus* and *H. petiolaris*, occur on mesic clay and drier sandy soils, respectively, the hybrid species, *H. anomalus*, *H. deserticola* and *H. paradoxus*, are restricted to active sand dune, arid desert, and desert salt marsh habitats, respectively. Another classic example of homoploid hybrid speciation often cited in the literature concerns the origin of *Iris nelsonii*. This

example is particularly intriguing in that three rather than two different diploid *Iris* species are thought to be involved as parents in its origin. Again, there is evidence that this species is reproductively isolated from its putative parents by ecological barriers (Arnold & al., 1990), and the species is thought to have originated relatively recently, possibly following a breakdown of ecological barriers between its parent species due to human activities. However, there remains a question mark over whether *Iris nelsonii* is indeed a diploid hybrid species as the genetic evidence (Arnold, 1993) is not yet conclusive on this point.

In addition to these examples, there is a growing list of other recorded homoploid hybrid species, including cases in animals (fish, insects and birds) and fungi, as well as in plants (Gross & Rieseberg, 2005; Mallet, 2007). Although homoploid hybrid speciation is still regarded as rare, particularly in comparison to allopolyploid speciation in plants, it is nonetheless an important mechanism and further examples of species originating by this process are likely to be discovered now

that genetic resources are widely available for testing their occurrence.

In this review, we shall focus on how quickly the process of homoploid hybrid speciation takes to complete and secondly on the nature of changes that occur in the hybrid during the process. Nolte & Tautz (2010) have recently emphasized the need to focus on genetic changes occurring in the initial stages of hybrid speciation, so as to obtain a more complete understanding of the process. Where relevant, we shall discuss recent work we have conducted on the diploid ($2n = 20$) hybrid species *Senecio squalidus* (Oxford ragwort; Asteraceae). This species is of special interest in that it represents a unique example of recent ecogeographic homoploid hybrid speciation facilitated by spatial isolation following human-mediated introduction of material from a hybrid zone between *S. aethnensis* and *S. chrysanthemifolius* on Mount Etna, Sicily, to the Oxford Botanic Garden in Britain (Abbott & al., 2000; Harris, 2002; James & Abbott, 2005). This new species is highly invasive and has rapidly spread through a large part of Britain and into Ireland

Table 1. Characteristics of *Senecio squalidus* and *Helianthus* hybrid species relevant to their origin and establishment.

	<i>S. squalidus</i>	<i>H. anomalus</i>	<i>H. deserticola</i>	<i>H. paradoxus</i>
Parent species	<i>S. aethnensis</i> and <i>S. chrysanthemifolius</i>	<i>H. annuus</i> and <i>H. petiolaris</i>	<i>H. annuus</i> and <i>H. petiolaris</i>	<i>H. annuus</i> and <i>H. petiolaris</i>
Age (years)	200–300	116,000–160,000	63,000–170,000	75,000–208,000
Number of origins	Once	Possibly three times	More than once	Once
Invasiveness	Highly invasive—spread throughout much of British Isles	Non-invasive—only few populations known	Non-invasive—only few populations known	Non-invasive—only few populations known
Life cycle	Annual to short-lived perennial	Annual	Annual	Annual
Mating system	Outcrossing—strong self-incompatibility	Outcrossing	Outcrossing	Outcrossing
Reproductive isolation from parent species				
Spatial	Allopatric to parents; native to Britain & Ireland ~2000 km from parents on Mount Etna, Sicily	Parapatric to parents in Utah & Arizona, U.S.A.	Parapatric and allopatric to parents in Arizona, Nevada & Utah, U.S.A.; allopatric populations are geographi- cally close to parent species	Parapatric to parents in New Mexico & Texas, U.S.A.
Ecological	Grows in temperate cli- mate—possibly intermedi- ate to that of parents	Adapted to active sand dune habitat—an extreme habitat relative to parent habitats	Adapted to arid desert habitat—an extreme habitat relative to parent habitats	Adapted to desert salt marsh habitat—an extreme habitat relative to parent habitats; flowers later than parent species
Intrinsic	Reduced fitness in F_2 hy- brids indicates some genic incompatibilities	Predominantly chromosomal	Predominantly chromosomal	Predominantly chromosomal

Information on *Helianthus* species was extracted from Schwarzbach & Rieseberg (2002), Welch & Rieseberg (2002), Gross & al. (2003), Gross & Rieseberg (2005), Lai & al. (2005).

since its origin (Abbott & al., 2009). *Senecio squalidus* and its parental species, *S. aethnensis* and *S. chrysanthemifolius*, are all self-incompatible (SI) (Abbott & Forbes, 1993; Hiscock, 2000), but are highly interfertile without disruption of SI function in hybrids (Chapman & al., 2005; Brennan & al., unpub.). Thus, advanced-generation synthetic hybrids can be produced by controlled crossing experiments (Hegarty & al., 2009). Consequently, it is feasible to study genetic and phenotypic changes that occur among synthetic hybrids following a cross, and to make comparisons between these hybrids across generations and between *S. squalidus* and its two parents. Because of its very recent origin, *S. squalidus* represents a fascinating case study of ecogeographic homoploid hybrid speciation ‘in action’. A comparison of similarities and differences between *S. squalidus* and *Helianthus* hybrid species that are relevant to their origins and establishment in the wild is provided in Table 1.

■ DEFINITION OF HOMOPLOID HYBRID SPECIES

Before embarking on a discussion of the process of hybrid speciation, it is important to define what homoploid hybrid species are. We follow Rieseberg (1997) in basing our definition on Mayr’s biological species concept (Mayr, 1942), which despite its shortcomings (see for example Mallet, 2008) remains the most widely accepted of the various species concepts recognized (Coyne & Orr, 2004). Thus, we define homoploid hybrid species as groups of interbreeding natural populations of homoploid hybrid origin that are reproductively isolated from other groups of interbreeding populations including those representing the parent species. Reproductive isolation between the hybrid species and other species may involve any combination of pre- and/or post-zygotic breeding barriers. It is worth noting that our definition is unaffected by the relative portions of each parent species’ genome present in a hybrid species. Thus, some hybrid species that are reproductively isolated from their parents may have originated as a result of only a few genes being introgressed from one species to another (Jiggins & al., 2008), whereas in other hybrid species reproductive isolation might, in part, be based on recombination of large portions of the genomes of each parent species (Rieseberg & al., 2003). It is often considered that a test of the biological species concept is that species will maintain their integrity in sympatry (Coyne & Orr, 2004), which for many allopatric species cannot be determined unless transplant experiments are undertaken (Abbott & al., 2008; Sobel & al., 2010). However, as Sobel & al. (2010) point out, if allopatric species are adapted to their local environments, then they are ecologically as well as geographically isolated from each other, and are expected to be mutually excluded from each other’s habitats. Thus, we propose that even hybrid lineages that originated and became established in geographical isolation from their parents, as for example was the case for *Senecio squalidus* (see above), can legitimately be regarded as homoploid hybrid species in the absence of strong postzygotic barriers, if they are ecologically as well as geographically isolated from their parents.

■ THE PROCESS OF HOMOPLOID HYBRID SPECIATION

Speed of origin. — Some of the earliest work on homoploid hybrid speciation investigated whether it was possible to generate synthetic, fertile and viable homoploid hybrids that were intersterile with both parents, thus providing a test of the recombinational model of hybrid speciation. Stebbins (1957) and Grant (1966a,b) showed that this could be achieved in the plant genera *Elymus* and *Gilia*, respectively, and because they generated such hybrids within a few generations of the original cross, it seemed that homoploid hybrid speciation could be a very rapid form of speciation in the wild. Confirmation of this hypothesis has come from what is undoubtedly the most thorough and detailed attempt to re-synthesise artificially an existing homoploid hybrid species, namely *Helianthus anomalus*, from a cross between *H. annuus* and *H. petiolaris* (Rieseberg & al., 1996). By modifying the pattern of backcrossing and intercrossing of hybrids in each generation, three different fertile hybrid lineages were produced by the F₅ generation that were partially intersterile with both parent species. Remarkably, these advanced-generation hybrids were morphologically and genetically similar to each other and to the wild form of *H. anomalus*, and contrasted with the wide morphological and genetic variation that was present among the earlier-generation hybrids.

All three of the above studies have several features in common. Plants were raised under controlled glasshouse conditions, and in each case F₁ hybrids were highly sterile. Selection was imposed either intentionally or unintentionally for the most fertile plants to act as parents of the next generation, although in no instance was there selection for plants showing intersterility with parental species. Rather, the latter evolved as a side-effect during the experiment. It was concluded that in each case the origin of intersterility between fertile advanced-generation hybrids and parent species was due to recombination of chromosomal rearrangements and/or genic sterility factors that distinguished the parent species. Thus, the results of these experiments supported the hypothesis that recombinational speciation is a mechanism that can lead to the rapid origin of a new homoploid hybrid species.

Computer simulation studies of the conditions that promote homoploid hybrid speciation have also indicated that the process is rapid (McCarthy & al., 1995; Buerkle & al., 2000). Moreover, a study of the rate of speciation of *H. anomalus* based on the size of parental species chromosomal blocks in the hybrid species, suggested that *H. anomalus* originated in the wild in less than 60 generations (Ungerer & al., 1998). However, a more recent analysis of the rate of genome stabilization in all three homoploid hybrid species of *Helianthus*, suggests that the process may take much longer to complete than previously thought (Buerkle & Rieseberg, 2008). Buerkle & Rieseberg (2008) concluded from their analysis that although “segregating factors that contribute to initial ecological or intrinsic isolation may become stabilized quickly. The remainder of the genome likely becomes stabilized over a longer time interval, with recombination and drift dictating the contributions of the parental

genomes.” This seems to mark an important advance in fully understanding homoploid hybrid speciation in that in many cases it might be expected that during the initial stages of the process the entity recognised as a new hybrid species might be phenotypically and genetically highly variable, but over the course of many further generations becomes more stable in form as it becomes less variable in phenotype and genotype.

Only three homoploid hybrid species are known to have originated in the very recent past, thus enabling their origin and establishment in the wild to be assessed as an ongoing process. These are *Senecio squalidus*, which originated in Britain within the past 200 to 300 years (Harris, 2002; James & Abbott 2005), the ‘Lonicera fruit fly’ (a *Rhagoletis* hybrid species) thought to have originated in North America in the last 250 years (Schwarz & al., 2005), and the ‘invasive sculpin’ (a *Cottus* hybrid species) first reported in 1992 in riverine habitats of the Rhine drainage in the Netherlands and Germany (Nolte & al., 2005). It is notable that leaf shape in *S. squalidus* is highly variable ranging from highly dissected to serrate (Fig. 1). This variation is not as extreme as that observed between the highly dissected and entire leaf shapes of the parent species, *S. chrysanthemifolius* and *S. aethnensis*, respectively (Fig. 1), but indicates that *S. squalidus* may be at an early stage in its eventual stabilization and that over time might become less variable in form.

Ecological divergence during homoploid hybrid speciation. — The successful production by Rieseberg & al. (1996) of synthetic hybrids that resembled both morphologically and genomically the wild homoploid hybrid species, *H. anomalus*, was highly informative of the pattern and pace of structural genomic and genetic changes that can occur during recombinational hybrid speciation. It was concluded from this study that selection in the glasshouse for increased fertility in a hybrid lineage could produce a recombined genome very similar in structure to that of a wild homoploid hybrid species. However, the study did not take into account the importance of ecological divergence during homoploid hybrid speciation and the type of genetic changes that can bring this about.

Although it has been noted that all homoploid hybrid species appear to be ecologically divergent from their progenitors (Gross & Rieseberg, 2005), it is seldom clear whether such divergence originated at the time of origin of a species and was crucial to the process, or whether it evolved after the hybrid species formed and became established as a stable entity. Only in situations where the process is studied ‘in action’, i.e., in the wild where a new homoploid hybrid species has been discovered immediately following its origin, or in experiments where newly produced hybrid offspring of the progenitors of a homoploid hybrid species are examined, is it likely to be possible to test the hypothesis that ecological divergence arises during homoploid hybrid speciation and is an essential feature of the process. The best experimental evidence available on this currently comes from studies on sunflowers.

Two similar experiments have been conducted on the origin of ecological divergence in the homoploid hybrid species, *H. paradoxus* and *H. deserticola*. In each case, seedlings of one of the hybrid species and its two parents, *H. annuus*

and *H. petiolaris*, together with seedlings of a large family of second-generation backcrossed (BC_2) plants, derived from a cross between the two parental species, were planted in the habitat of the hybrid species. Thus in the case of *H. paradoxus* the experiment was conducted in a natural saltmarsh (Lexer & al., 2003b), while for *H. deserticola* it was conducted in an arid ‘desert floor’ habitat (Gross & al., 2004). In both studies, it was shown that the hybrid species exhibited particular traits of adaptive significance in the hybrid species’ habitat that were not exhibited by either parent species. Most importantly, it was shown that variation in these traits among BC_2 individuals overlapped that exhibited by the hybrid species, and that the strength of phenotypic selection acting on these traits would move the BC_2 population toward the hybrid species phenotype. These results are consistent with the hypothesis that transgressive segregation in the offspring of hybrids between these two particular parental species can quickly generate morphological, anatomical, life history and physiological traits that enable some offspring to occupy habitats very different from those of the parents. Thus, the parental species are excluded from these novel habitats and the hybrid species becomes established because it is ecologically and spatially isolated from its parents and, therefore, reproductively isolated from them.

In the case of *H. paradoxus*, further analysis of the BC_2 family isolated several quantitative trait loci (QTLs) affecting mineral uptake traits, while other QTLs were shown to affect survival (Lexer & al., 2003a). Some of the mineral uptake QTLs mapped to the same genomic locations of QTLs for survivorship, including a candidate salt tolerance gene that was later found to map to the location of a QTL affecting mineral uptake (Lexer & al., 2004). In this and other studies of homoploid hybrid species of *Helianthus* (Rieseberg & al., 2003), it has been shown that many of the QTLs that combine to influence a trait of adaptive significance in the hybrid species normally show effects in opposing directions. When traits exhibit this kind of genetic architecture in parent species, segregation of parental QTL alleles after hybridization produces variable and transgressive trait phenotypes among hybrid offspring with the potential for rapid evolution (Rieseberg & al., 2003). Rieseberg & al. (2003) were able to demonstrate that complementary gene action created extreme phenotypes in synthetic hybrids that matched those of wild hybrids and, most importantly, showed that the combinations of parental chromosomal segments that occurred in the ancient hybrids were the same as those required to generate synthetic hybrids of similar extreme phenotype. These studies clearly demonstrate the vital role of ecological divergence and selection as part of the initial stages of homoploid hybrid speciation.

Although the genomes of sunflower homoploid hybrid species comprise large portions of the genomes of each parent species (Rieseberg & al., 2003), it is also true that in other homoploid hybrid species ecological divergence and reproductive isolation from parent species can be dependent on only a few genes introgressed from one parent to the other (Jiggins & al., 2008). Thus, in the butterfly genus *Heliconius*, the homoploid hybrid species, *H. heurippa*, has a wing colour pattern causing assortative mating and, in turn, reproductive isolation

from its parent species, *H. melpomene* and *H. cydno* (Jiggins & al., 2008). A colour pattern very similar to that of *H. heurippa* is easily recreated in synthetic hybrids between the same parent species by combining a few genes of major effect in the hybrids that encode the discrete wing colour patterns of the parent species. The colour pattern that closely matches that of *H. heurippa* is only expressed against a *H. cydno* genetic background and was obtained after three generations of

backcrossing synthetic hybrids to this species (Mavarez & al., 2006). Jiggins & al. (2008) predict that in cases such as this where only a few introgressed genes are required to produce a new homoploid hybrid species, it will be difficult to identify the species as hybrid unless the introgressed 'speciation genes' are themselves examined directly. This is because, at other loci, the hybrid species might not differ genetically from the introgressed parent.



Fig. 1. Top panel, Leaf shape variation between *Senecio chrysanthemifolius* (first row), *S. squalidus* (second row) and *S. aethnensis* (third row). The centre-most primary stem leaf of glasshouse-grown plants was sampled soon after flowering had started. Leaf perimeter to area ratios were measured for 32 to 45 individuals per species and leaves representing the 0.05, 0.25, 0.50, 0.75, and 0.95 quantiles are presented. Bottom panel, Leaf shape variation among a sample of individuals from a large population of *S. squalidus* in Edinburgh, Scotland.

The findings of the butterfly work fit the genic view of speciation (Wu, 2001), which states that reproductive isolation between species is controlled by a moderate number of individual genes rather than the entire genome. It is likely that the butterfly example represents one extreme of a continuum in relative proportions of each parent's genome represented in a homoploid hybrid species and required for ecological divergence and reproductive isolation (Jiggins & al., 2008). The other extreme is represented by sunflower homoploid hybrid species where ecological divergence and reproductive isolation is based on a combination of many different blocks of genome derived from each parent species.

Multiple origins. — When ecological divergence is an important driver of speciation it is possible that new species might originate recurrently (Schluter, 2000, 2009), and there is evidence that this may have occurred in at least four homoploid hybrid species—*Argyranthemum sundingii* (Brochmann & al., 2000; Borgen & al., 2003), *Helianthus anomalous* (Schwarzbach & Rieseberg, 2002), *H. deserticola* (Gross & al., 2003) and *Pinus densata* (X.-R. Wang & al., 2001; Song & al., 2003), although Gross & Rieseberg (2005) point out that the evidence in support is not yet full-proof for any of these examples. If a homoploid hybrid species has originated more than once, it raises the issue of whether the differently originated lineages will evolve independently of each other or in concert thereafter. A study on *H. deserticola* by Gross & Rieseberg (2007) suggests that populations of this species representing different origins of the species have evolved mainly in concert as some loci show “putative selected sweeps across the species range with the same allele fixed in each population”. Whether concerted evolution of this type is true for all homoploid hybrid species that have originated recurrently remains to be demonstrated.

Changes in quantitative traits. — Detailed comparative quantitative genetic surveys of hybrid and parental phenotypes are informative about the particular adaptations that distinguish new hybrids from parents and guide associated searches for candidate hybrid speciation genes. The transplant studies investigating survivorship and other quantitative traits (QTs) in parental and progenitor hybrid species of *Helianthus* described above have provided much information on key transgressive hybrid traits enabling adaptation to novel habitats (Lexer & al., 2003a,b; Gross & al., 2004) and similar studies of hybrid species from other genera are required to confirm the generality of these findings. In this respect, two recent studies on *Pinus densata* are of interest. *Pinus densata* is a homoploid hybrid species (X.-R. Wang & al., 2001), which is ecologically divergent from its progenitors, *P. tabulaeformis* and *P. yunnanensis*, and occurs only at high altitude (2700–4200 m) on the Qinghai-Tibetan Plateau, whereas *P. tabulaeformis* is restricted to elevations between 100 and 2600 m in northern China, while *P. yunnanensis* is mainly found in southwest China at altitudes below 2700 m. Gao & al. (2009) and Ma & al. (2010) have demonstrated that *P. densata* exhibits several physiological traits, not shown by either parent, which are likely to confer increased drought tolerance for growth in its arid high-altitude habitat.

In *Senecio*, we have begun an analysis of adaptive divergence in *S. squalidus* by investigating twenty uncorrelated

morphological, developmental and physiological quantitative traits (QTs) in this species, its parents, and hybrids from Mount Etna (Brennan & al., 2009 and in prep.). A principal component analysis (PCA) of the data collected showed that *Senecio aethnensis* and *S. chrysanthemifolius* were distinguished by the first principal component (PC1), which accounted for 23.4% of the total variance, with hybrid plants exhibiting a range of intermediate PC1 values. *Senecio squalidus* also exhibited intermediate PC1 values, although in general these were much closer to those of *S. chrysanthemifolius* than to *S. aethnensis*. However, there was also an indication that *S. squalidus* individuals were distinct from Sicilian plants due to some having larger PC2 values. As part of this study, quantitative differentiation between species (*Qst*) was also estimated for individual trait values, and for PC1 and PC2 values from the principal component analysis (Brennan & al., in prep.). A significantly positive *Qst* value for PC1 was observed between *S. aethnensis* and *S. chrysanthemifolius*, and between *S. aethnensis* and *S. squalidus*, but not between *S. chrysanthemifolius* and *S. squalidus*, confirming that *S. squalidus* is most phenotypically similar to *S. chrysanthemifolius*. Patterns of between-species *Qst* estimates for individual quantitative traits varied widely, but could be placed into one of three broad categories. Thus for 14 of the 20 traits measured *S. squalidus* was most similar to *S. chrysanthemifolius*, while for three traits it was most similar to *S. aethnensis*, and for the remaining three traits the parents were more similar to each other than to *S. squalidus*. One of these latter three traits, plant height at first flowering, was significantly shorter in *S. squalidus* compared to its parents, i.e., was transgressively expressed in the hybrid.

It remains to be established whether any of these differences recorded between *S. squalidus* and its parental species are adaptive, and transplant studies are required to determine this and, in general, how adapted *S. squalidus* is to the ‘British environment’ relative to its parents. Interestingly, an independent reciprocal transplant investigation conducted on *S. squalidus* in Britain recently found evidence for rapid local adaptation related to population latitude for drought and temperature stress tolerance and flowering time traits, thus showing that adaptation to the British environment is an ongoing process in this relatively young homoploid hybrid species (Allan & Pannell, 2009).

Effects of interspecific gene flow. — Where post-zygotic reproductive isolation is relatively weak between a homoploid hybrid species and its parents, it is likely that extensive gene flow will occur in areas of species overlap and have important consequences on the genetic structure of the hybrid species. In fact, this is suggested by a recent study by Scascitelli & al. (2010) on gene flow between a stabilized introgressant of *H. annuus* (*H. annuus* subsp. *texanus*) and its two parents, *H. annuus* and *H. debilis*. Based on a survey of simple sequence repeat (SSR) variation at 88 loci across these taxa, it was evident that considerable bidirectional introgression had occurred between these taxa, which are sympatric in parts of their range. Despite the presence of a single putative hybrid incompatibility locus exhibiting strong asymmetric introgression, patterns of differentiation across chromosomes indicated that genetic islands of differentiation were smaller than 1 cM and were not associated with chromosomal rearrangements in the hybrid.

Even if strong postzygotic breeding barriers are present between a homoploid hybrid species and its parents, it is conceivable that gene flow might have an effect on the level of genetic divergence in areas of sympatry. This is suggested by the findings of recent studies investigating gene flow between the species *H. annuus* and *H. petiolaris*, which are postzygotically isolated from each other by strong chromosomal incompatibilities, but which nonetheless have acted as the parents of three homoploid hybrid species. Yatabe & al. (2007) and Strasburg & al. (2009) found that the genomes of these two species exhibit extensive ongoing introgression even close to chromosomal breakpoints. Of further interest is the study by Gross & Rieseberg (2007), which investigated selective sweeps in *H. deserticola* across 96 expressed sequence tag-based microsatellite loci. This study identified one population that was genetically more similar to its parents *H. annuus* and *H. petiolaris* than were three other populations of *H. deserticola* investigated. It was argued that this similarity could have been caused by interspecific hybridization resulting in the transfer of new parental alleles into this particular population of *H. deserticola*. Interestingly, selective sweeps at two loci were unique to the same population, suggesting that it might have experienced adaptive introgression from its parent species. Thus, it seems that opportunities for introgression with parents could be a potent force in shaping genetic differentiation in established homoploid hybrid species, but that these hybrid species will nonetheless maintain their ecogeographic distinctiveness, perhaps at the level of just a few important defining genetic differences.

Genetic divergence in the absence of gene flow. — The homoploid hybrid species, *S. squalidus*, is unique among homoploid hybrid species in that it is known to have originated in geographic isolation from its parent species (James & Abbott,

2005). Thus, gene flow from its parents was not a complicating factor during its origin or in its subsequent establishment and evolution. Instead, founder effects occurring during the introduction of hybrid material to Britain, complemented by the effects of genetic drift and selection during the origin and establishment of the new hybrid species, are likely to have had a significant effect on its genetic structure. Two comparative genetic diversity studies of *S. squalidus*, its parents, and hybrids from the hybrid zone on Mount Etna, have shown that *S. squalidus* is of mixed ancestry between *S. chrysanthemifolius* and *S. aethnensis* (James & Abbott, 2005; Brennan & al., in prep.). In addition, these studies have found that *S. squalidus* contains less genetic diversity, both in terms of number of alleles per locus and expected heterozygosity, than either of its parent species. Additional investigations of allelic diversity at the normally highly polymorphic *S* locus that controls SI have also found just 7 alleles in *S. squalidus* relative to at least 24 alleles in each of its parental species (Brennan & al., 2006; Brennan, Harris & Hiscock, unpub. results). Because all of these studies found considerable genetic diversity among hybrid plants from the hybrid zone on Mount Etna, it is highly likely that *S. squalidus* passed through a genetic bottleneck during the introduction of hybrid material to Britain and the origin of the hybrid species from this material.

Of particular note from our previous genetic surveys was the finding that *S. squalidus* plants formed a well-defined genetically distinct cluster from its parent species and hybrids from Mount Etna. This was first made clear by a survey of variation of RAPD markers that distinguished the two parent species (James & Abbott, 2005) (Fig. 2). Thus *S. squalidus* is more highly differentiated from *S. aethnensis* and *S. chrysanthemifolius* than are plants from the hybrid zone on Mount

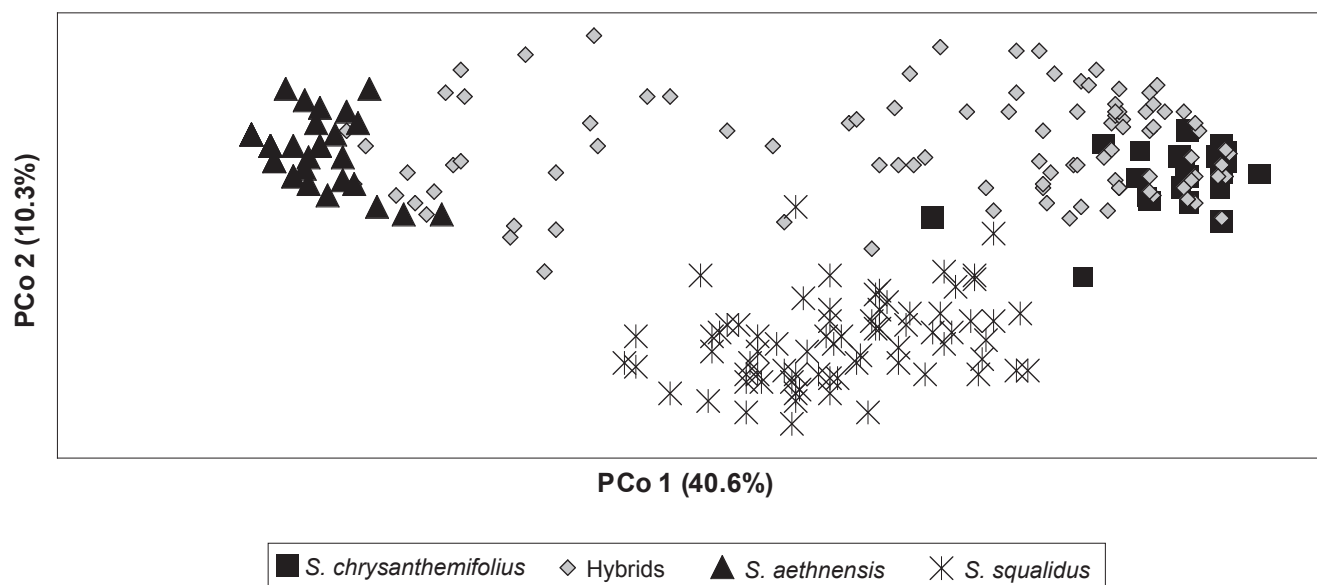


Fig. 2. Plot of individual scores against the first two axes extracted from a principal coordinate analysis of RAPD variation among individuals of *Senecio chrysanthemifolius*, *S. aethnensis* and hybrids from the hybrid zone on Mount Etna, Sicily, and of *S. squalidus* from six British populations. Individuals of *S. squalidus* form a cluster that is distinct from individuals representing its parent species and hybrids on Mount Etna. (From James & Abbott, 2005; reproduced with permission of John Wiley & Sons Inc.)

Etna. An analysis of allele sharing in a survey of 13 SSRs, six allozymes and three indels (102 alleles in total, Brennan & al., in prep.) showed that while hybrid *Senecio* on Mount Etna contained more unique alleles than *S. squalidus* relative to parental *Senecio* species (11 versus 5), many more alleles are shared between the hybrid plants and both parents than between *S. squalidus* and its parents (62 versus 35). Furthermore, allele frequency differences were found to be significantly smaller when comparisons were made between Mount Etna hybrid *Senecio* and parental *Senecio*, than between *S. squalidus* and parental *Senecio* (Brennan & al., in prep.). Thus, both allele sharing and allele frequency differences in *S. squalidus* compared to parents and Sicilian hybrids contributed to the overall genetic differentiation observed for *S. squalidus*. Together, these results suggest that genetic differentiation during homoploid hybrid speciation is enhanced and hastened by physical barriers to ongoing introgression. Further investigations to quantify and distinguish the influences of founder effects and selection on genetic diversity during this speciation event using computer simulation and high-throughput gene-space sequencing methods are currently being conducted in our laboratories. In addition, transplant experiments that compare the relative fitness of *S. squalidus* and its parent species in Britain are required to assess the adaptive significance of genetic changes identified in this new hybrid species. Such experiments will show whether geographical isolation caused by adaptation of *S. squalidus* to the British environment was of major importance in the origin of the species (see Sobel & al., 2010).

Changes in gene expression. — The combination of two divergent genomes within a single hybrid nucleus can result in rapid genomic change in homoploid hybrid species caused

by recombination (Rieseberg & al., 1995), and activation and proliferation of transposable elements (Ungerer & al., 2006; Kawakami & al., 2010). In addition, it can lead to the generation of novel patterns of gene expression, which could give rise to transgressive phenotypes that are of adaptive significance. Gene expression which is not merely a combination of that observed in the parental species is commonly referred to as “non-additive”, and has been observed extensively in allopolyploid hybrids such as *Arabidopsis suecica* (J. Wang & al., 2006), and *Senecio cambrensis* (Hegarty & al., 2006, 2008). Non-additive gene expression can arise due to factors such as novel interactions between gene regulators, changes in the epigenetic regulation of gene expression and variation in gene copy number (reviewed in Hegarty & Hiscock, 2008). Many of these factors are also likely to play a role in mediating gene expression in homoploid hybrids. Indeed, a recent microarray expression study in homoploid hybrid sunflowers (Lai & al., 2006) demonstrated non-additive gene expression in a wild homoploid hybrid adapted to a habitat which neither parent can tolerate. In this study, gene expression was compared between the hybrid *Helianthus deserticola* and its parental species *H. annuus* and *H. petiolaris*. Lai & al. (2006) identified 154 genes differentially expressed between the hybrid and *H. annuus* and 174 between the hybrid and *H. petiolaris*. Whilst this study did not attempt to assess deviation from the parental midpoint value (MPV) as is now commonplace for studies of non-additive gene expression, 58 genes were identified as showing ‘transgressive’ expression—that is, expression levels which are not merely non-additive but which lie significantly outside the expression range of either parent. This ‘transgressive’ group was shown to over-represent transport-related genes (i.e.,

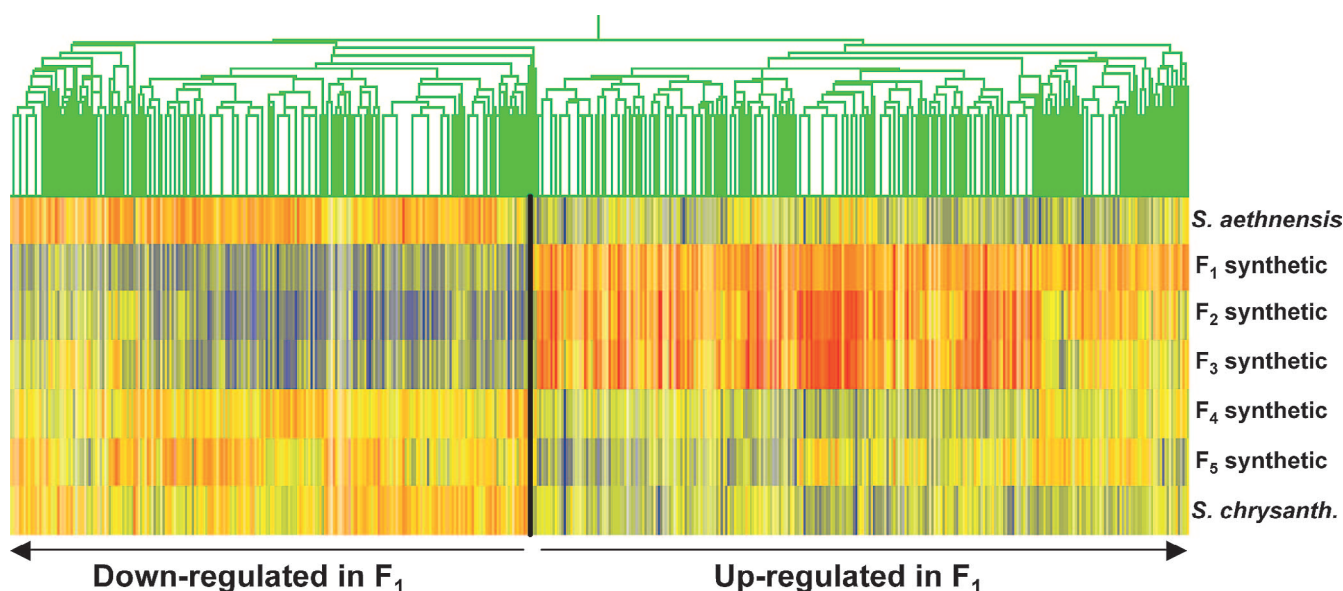


Fig. 3. Non-additive patterns of gene expression in early generation resynthesized *Senecio squalidus*. Hierarchical clustering (based on similarity of expression pattern) of 986 cDNA clones showing non-additive gene expression in the F_1 synthetic hybrid relative to the parental midpoint. Red coloration indicates up-regulation whilst blue denotes down-regulation (relative to a derived baseline for each clone). A majority of clones affected in the F_1 hybrid (690 of the 986 clones) display similar expression patterns in both the F_2 and F_3 generations, but return to a more additive expression pattern in the F_4 and F_5 hybrids. (From Hegarty & al., 2009; reproduced with permission of John Wiley & Sons Inc.)

protein transporters of potassium, sodium and chloride ions), which could explain the phenotypic divergence that enables *H. deserticola* to survive in an arid environment that neither of its parents can tolerate. Non-additive gene expression in hybrid plants, therefore, may represent a source of phenotypic novelty upon which selection may act.

The work of Lai & al. (2006) used a microarray platform comprised solely of cDNA clones from tissues subjected to a variety of environmental stresses (e.g., salt or drought stress). Because hybridization may theoretically affect the expression of genes that are not involved in stress responses (i.e., ‘housekeeping’ genes), these results do not give a genome-wide picture of changes to gene expression associated with homoploid hybrid speciation. In addition, because Lai & al. (2006) focussed on an established hybrid species, their results do not provide information on the timing of gene-expression change after hybrid formation. To further explore gene expression changes associated with homoploid hybrid speciation, we used custom cDNA microarrays to compare gene expression in wild U.K. *Senecio squalidus* to its progenitors, *S. aethnensis* and *S. chrysanthemifolius*. We also recreated the hybrid under glasshouse conditions and produced F_1 – F_5 generations of hybrids via randomised mating and compared these to their parental plants in order to determine the timing of transcriptional changes associated with homoploid hybridization (Hegarty & al., 2009).

Our analysis of wild *S. squalidus* identified 311 array features (4.9% of features on the array) which displayed gene expression significantly different to the average of the parental expression values. Of these, 80.7% showed upregulation relative to the parental midpoint. We then filtered the data further to identify genes with expression levels outside the range of either parent (‘transgressive’ expression) and found that 65% of genes displaying non-additive expression also showed transgressive

expression patterns. Amongst these genes were a number involved in defence and stress responses, which are also typically affected in allopolyploid hybrid systems. More interestingly, we observed significant upregulation of two genes, encoding glutathione S-transferase and ATP-sulfurylase, which are known to be upregulated as a consequence of sulphur deficiency. As soils in Britain generally contain much lower levels of sulphur than the volcanic soil of Mount Etna where *S. squalidus* arose, upregulation of these genes may represent the effects of adaptive divergence since the species’ introduction to Britain.

Our survey of gene expression in synthetic *S. squalidus* hybrids was also informative. We identified 690 array features (10.89% of the array) displaying non-additive expression across the first three generations of synthetic hybrids, typically displaying the greatest deviation from the parental midpoint in the F_2 generation. However, all of these non-additive features displayed a return to additivity in the F_4 and F_5 generations (Fig. 3), which was correlated with an observed increase in seed germination after reductions in the F_2 and F_3 generations (Fig. 4). It might be, therefore, that some of the extreme expression patterns observed in the F_2 – F_3 generations were eliminated due to selection against those patterns at the level of seed viability. Indeed, the majority of genes displaying non-additive and ‘transgressive’ expression in the F_4 – F_5 generations are different to those seen in the F_2 – F_3 generations. Mitochondrial and photosynthetic genes were most heavily affected in the synthetic hybrids, including several genes known to be involved in adaptation to varying UV light conditions. These genes also displayed significant differences in expression between the parental taxa, suggesting a role in local adaptation to UV light conditions at the two parental altitude ranges on Mount Etna. In the majority of cases, these genes did not show significant variation from the parental midpoint in wild *S. squalidus*. This suggests that early transgressive effects in these genes have been ameliorated over evolutionary time. Our findings show that extreme, transgressive changes to gene expression are generated rapidly in early hybrid generations and provide hybrids with genetic novelty which may influence their successful evolution into new species. However, selective pressures are a necessary component in determining whether these novel genetic traits are maintained or eliminated over time. Future experimentation in *Senecio* will attempt to determine whether hybrids are indeed better adapted to the environmental conditions present in the hybrid range, such as soil sulphur content and UV light exposure and, if so, whether these traits may have been fixed by selection in the wild British population.

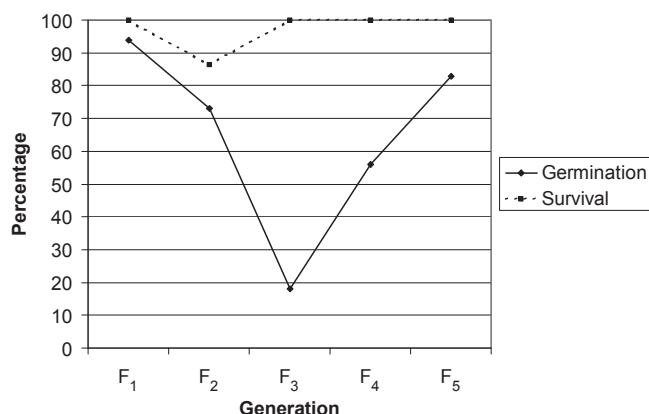


Fig. 4. Seed germination and survival rates for resynthesized *Senecio squalidus* hybrid lines. Percentage seed germination after three weeks and percentage of germinating seedlings that survived to six weeks in the first five generations of resynthesized *S. squalidus* hybrids. Survival in the F_2 generation was reduced due to the occurrence of albino individuals which could not photosynthesize effectively. (From Hegarty & al., 2009; reproduced with permission of John Wiley & Sons Inc.)

■ CONCLUSIONS AND FUTURE RESEARCH

Most of our current understanding of homoploid hybrid speciation is derived from the comprehensive research conducted on three homoploid hybrid species of sunflower, *Helianthus anomalus*, *H. deserticola* and *H. paradoxus*, each of which originated following hybridization between *H. annuus* and *H. petiolaris*. This work, conducted by Rieseberg and co-workers, has shown that new fertile homoploid hybrid species

that are reproductively isolated from their parents can originate rapidly. However, a longer period is likely to be required before the new species exhibits stabilized and distinctive phenotypic and genomic variation relative to that which it exhibits soon after its hybrid origin. Theory shows that ecological and spatial divergence of the hybrid species from its parents is key to successful establishment, and that this can occur even in the absence of post-zygotic isolation caused by chromosomal and/or genetic sterility barriers. While for many traits a hybrid species might exhibit intermediate phenotypes to its parents, often it either combines a distinctive mix of parental-like traits or exhibits transgressive traits that enable it to occupy a novel and perhaps more 'extreme' habitat relative to those of its parents. Studies of *Helianthus* homoploid hybrid species have shown that the generation of transgressive traits has been of great importance in adapting these species to habitats that are ecologically and spatially divergent from those of the parents.

Our work on the newly originated homoploid hybrid species, *Senecio squalidus*, is valuable in determining how this species has diverged from its two parent species and their hybrids that occur on Mount Etna, Sicily. The origin of *S. squalidus* differs from that of all other known homoploid hybrid species in that human actions played a direct role in geographically isolating hybrids by introducing them into Britain from Sicily. A small population of introduced hybrid material was then cultivated for many years allowing genetic change to take place and for a successful invasive hybrid species to evolve, in the absence of gene flow from the two parent species. Phenotypically, *S. squalidus* is similar to one of its parents, *S. chrysanthemifolius*, for most traits recorded, but for some traits it is more similar to the other parent, *S. aethnensis*, while for some others it exhibits a transgressive phenotype. In fact, our studies of changes in gene expression in *S. squalidus* relative to its parents, suggest that many changes are transgressive and some of these could have been important in adapting the new species to conditions in the U.K. Moreover, it is apparent from our studies of early generation synthetic hybrids that increased variation in gene expression is correlated with loss of fitness, but that gene expression is stabilized and fitness restored very quickly due presumably to the effects of selection.

A notable finding from our studies on *S. squalidus* is that this new species is already genetically divergent from the wide range of hybrid genotypes that are found in the hybrid zone between *S. aethnensis* and *S. chrysanthemifolius* on Mount Etna (James & Abbott, 2005; Brennan & al., in prep.). This divergence is due to a combination of reduced genetic diversity and greater allele frequency differences in *S. squalidus* compared with both parent species and Sicilian hybrid material.

It is expected that future research on homoploid hybrid speciation will uncover additional examples of homoploid hybrid species in diverse plant, animal and fungal groups in which interspecific hybridization occurs. The discovery of new examples of homoploid hybrid species will provide new opportunities for investigating the different ways in which they originate, particularly when these species represent early stages in origination. We anticipate that resolving the mechanisms underlying the reproductive isolation and adaptive novelties of homoploid

hybrid species will continue to be a major focus of research with considerable effort aimed at isolating and characterizing the genes and genomic regions involved in this. In our own research on the origin and evolution of *Senecio squalidus*, we are currently undertaking a comparison of genetic linkage and QTL maps of *S. aethnensis*, *S. chrysanthemifolius* and *S. squalidus* to further investigate genomic relationships between the new hybrid species and its parents, and have also embarked on a genomic analysis of adaptation in the three species by means of genomic screens of the entire gene-space (the non-repetitive gene-rich fraction of the genome) of *S. squalidus* to identify genes associated with adaptation and divergence of this species in Britain. The recent advances in DNA sequencing technologies (454 and Illumina) now permit unprecedented access to genomic data allowing us to begin to determine the relative importance of genomic changes to coding versus regulatory regions of genes in adaptation of the three *Senecio* species to contrasting environments. We will further interrogate the *Senecio* genome to analyse genetic and epigenetic changes at candidate loci potentially involved in generating 'transgressive' expression patterns associated with hybrid speciation and potential adaptation of *S. squalidus* to low sulphur and UV light conditions that were identified from our recent transcriptomic studies (Hegarty & al., 2009). In this way, we intend to obtain over the next few years a detailed understanding of the genetic and adaptive changes responsible for the origin, establishment and rapid spread of what is the most recently originated homoploid hybrid plant species currently known in the wild.

■ ACKNOWLEDGEMENTS

We thank Tod Stuessy for inviting us to contribute this paper, and also two anonymous reviewers for their helpful comments on an earlier version of the manuscript. Our research on homoploid hybrid speciation in *Senecio* has been funded by the Natural Environment Research Council (NERC) through grants NER/T/S/2001/00995 (to SJH/RJA), NE/D014166/1 (to RJA), and NE/D005353/1 and NE/G018448/1 (to SJH).

■ LITERATURE CITED

- Abbott, R.J., Brennan, A.C., James, J.K., Forbes, D.F., Hegarty, M.J. & Hiscock, S.J. 2009. Recent hybrid origin and invasion in the British Isles by a self-incompatible species, Oxford ragwort (*Senecio squalidus* L., Asteraceae). *Biol. Invasions* 11: 1145–1158.
- Abbott, R.J. & Forbes, D.G. 1993. Outcrossing rate and self-incompatibility in the colonizing species *Senecio squalidus* L. *Heredity* 71: 155–159.
- Abbott, R.J., James, J.K., Irwin, J.A. & Comes H.P. 2000. Hybrid origin of the Oxford ragwort, *Senecio squalidus* L. *Watsonia* 23: 123–138.
- Abbott, R.J., Ritchie, M.G. & Hollingsworth, P.M. 2008. Introduction. Speciation in plants and animals: Pattern and process. *Philos. Trans., Ser. B* 363: 2965–2969.
- Allan, R. & Pannell, J.R. 2009. Rapid divergence in physiological and life-history traits between northern and southern populations of the British introduced neo-species, *Senecio squalidus* L. *Oikos* 118: 1053–1061.

- Arnold, M.L. 1993. *Iris nelsonii* (Iridaceae): Origin and genetic composition of a homoploid hybrid species. *Amer. J. Bot.* 80: 577–583.
- Arnold, M.L., Hamrick, J.L. & Bennett, B.D. 1990. Allozyme variation in Louisiana irises: A test for introgression and hybrid speciation. *Heredity* 65: 297–306.
- Borgen, L., Leitch, I. & Santos-Guerra, A. 2003. Genome organization in diploid hybrid species of *Argyranthemum* (Asteraceae) in the Canary Islands. *Bot. J. Linn. Soc.* 141: 491–501.
- Brennan, A.C., Bridle, J.R., Wang, A.-L., Hiscock, S.J. & Abbott, R.J. 2009. Adaptation and selection in the *Senecio* (Asteraceae) hybrid zone on Mount Etna, Sicily. *New Phytol.* 183: 702–717.
- Brennan, A.C., Harris, S.A. & Hiscock, S.J. 2006. The population genetics of sporophytic self-incompatibility in *Senecio squalidus* (Asteraceae): The number, frequency, and dominance interactions of *S* alleles across its British range. *Evolution* 60: 213–224.
- Brochmann, C., Borgen, L. & Stabbetorp, O.E. 2000. Multiple diploid hybrid speciation of the Canary Island endemic *Argyranthemum sundingii* (Asteraceae). *Pl. Syst. Evol.* 220: 77–92.
- Buerkle, C.A., Morris, R.J., Asmussen, M.A. & Rieseberg, L.H. 2000. The likelihood of homoploid hybrid speciation. *Heredity* 84: 441–451.
- Buerkle, C.A. & Rieseberg, L.H. 2008. The rate of genome stabilization in homoploid hybrid species. *Evolution* 62: 266–275.
- Chapman, M.A., Forbes D.G. & Abbott, R.J. 2005. Pollen competition among two species of *Senecio* (Asteraceae) that form a hybrid zone on Mt Etna, Sicily. *Amer. J. Bot.* 92: 730–735.
- Coyne, J.A. & Orr, H.A. 2004. *Speciation*. Sunderland, Massachusetts: Sinauer.
- Gao, D.-H., Gao, Q., Xu, H.-Y., Ma, F., Zhao, C.-M. & Liu, J.-Q. 2009. Physiological responses to gradual drought stress in the diploid hybrid *Pinus densata* and its two parental species. *Trees* 23: 717–728.
- Grant, V. 1966a. Selection for vigor and fertility in a species cross in *Gilia*. *Genetics* 53: 757–775.
- Grant, V. 1966b. The origin of a new species of *Gilia* in a hybridization experiment. *Genetics* 54: 1189–1199.
- Grant, V. 1981. *Plant speciation*, 2nd ed. New York: Columbia Univ. Press.
- Gross, B.L., Kane, N.C., Lexer, C., Ludwig, F., Rosenthal, D.M., Donovan, L.A. & Rieseberg, L.H. 2004. Reconstructing the origin of *Helianthus deserticola*: Survival and selection on the desert floor. *Amer. Naturalist* 164: 145–156.
- Gross, B.L. & Rieseberg, L.H. 2005. The ecological genetics of homoploid hybrid speciation. *J. Heredity* 96: 241–252.
- Gross, B.L. & Rieseberg, L.H. 2007. Selective sweeps in the homoploid hybrid species *Helianthus deserticola*: Evolution in concert across populations and across origins. *Molec. Ecol.* 16: 5246–5258.
- Gross, B.L., Schwarzbach, A.E. & Rieseberg, L.H. 2003. Origin(s) of the diploid hybrid species *Helianthus deserticola* (Asteraceae). *Amer. J. Bot.* 90: 1708–1719.
- Harris, S.A. 2002. Introduction of Oxford ragwort, *Senecio squalidus* L. (Asteraceae), to the United Kingdom. *Watsonia* 24: 31–43.
- Hegarty, M.J., Barker, G.L., Brennan, A.C., Edwards, K.J., Abbott, R.J. & Hiscock, S.J. 2008. Changes to gene expression associated with hybrid speciation in plants: Further insights from transcriptomic studies in *Senecio*. *Phil. Trans., Ser. B* 363: 3055–3069.
- Hegarty, M.J., Barker, G.L., Brennan, A.C., Edwards, K.J., Abbott, R.J. & Hiscock, S.J. 2009. Extreme changes to gene expression associated with homoploid hybrid speciation. *Molec. Ecol.* 18: 877–889.
- Hegarty, M.J., Barker, G.L., Wilson, I.D., Abbott, R.J., Edwards, K.J. & Hiscock, S.J. 2006. Transcriptome shock after interspecific hybridization in *Senecio* is ameliorated by genome duplication. *Curr. Biol.* 16: 1652–1659.
- Hegarty M.J., Hiscock, S.J. 2008. Genomic clues to the evolutionary success of polyploid plants. *Curr. Biol.* 18: 435–444.
- Hiscock, S.J. 2000. Genetic control of self-incompatibility in *Senecio squalidus* L. (Asteraceae): A successful colonizing species. *Heredity* 85: 10–19.
- James, J.K. & Abbott, R.J. 2005. Recent, allopatric, homoploid hybrid speciation: The origin of *Senecio squalidus* (Asteraceae), in the British Isles from a hybrid zone on Mount Etna, Sicily. *Evolution* 59: 2533–2547.
- Jiggins, C.D., Salazar, C., Linares, M. & Mavárez, J. 2008. Hybrid trait speciation and *Heliconius* butterflies. *Philos. Trans., Ser. B* 363: 3047–3054.
- Kawakami, T., Strakosh, S.C., Zhen, Y. & Ungerer, M.C. 2010. Different scales of *Tyl/copia*-like retrotransposon proliferation in the genomes of three diploid hybrid sunflower species. *Heredity* 104: 341–350.
- Lai, Z., Gross, B.L., Zou, Y., Andrews, J. & Rieseberg, L.H. 2006. Microarray analysis reveals differential gene expression in hybrid sunflower species. *Molec. Ecol.* 15: 1213–1227.
- Lai, Z., Nakazato, T., Salmaso, M., Burke, J.M., Tang, S., Knapp, S.J. & Rieseberg, L.H. 2005. Extensive chromosomal repatterning and the evolution of sterility barriers in hybrid sunflower species. *Genetics* 171: 291–303.
- Lexer, C., Lai, Z. & Rieseberg, L.H. 2004. Candidate gene polymorphisms associated with salt tolerance in wild sunflower hybrids: Implications for the origin of *Helianthus paradoxus*, a diploid hybrid species. *New Phytol.* 161: 225–233.
- Lexer, C., Welch, M.E., Murphy, J.L. & Rieseberg, L.H. 2003a. Natural selection for salt tolerance quantitative trait loci (QTLs) in wild sunflower hybrids: Implications for the origin of *Helianthus paradoxus*, a diploid hybrid species. *Molec. Ecol.* 12: 1225–1235.
- Lexer, C., Welch, M.E., Raymond, O. & Rieseberg, L.H. 2003b. The origin of ecological divergence in *Helianthus paradoxus* (Asteraceae): Selection on transgressive characters in a novel hybrid habitat. *Evolution* 57: 1989–2000.
- Ma, F., Zhao, C.-M., Milne, R., Ji, M.-F., Chen, L.-T. & Liu, J.-Q. 2010. Enhanced drought-tolerance in the homoploid hybrid species *Pinus densata*: Implication for its habitat divergence from two progenitors. *New Phytol.* 185: 204–216.
- Mallet, J. 2007. Hybrid speciation. *Nature* 446: 279–283.
- Mallet, J. 2008. Hybridization, ecological races and the nature of species: Empirical evidence for the ease of speciation. *Philos. Trans., Ser. B* 363: 2971–2986.
- Mavárez, J., Salazar, C., Bermingham, E., Salcedo, C., Jiggins, C.D. & Linares, M. 2006. Speciation by hybridization in *Heliconius* butterflies. *Nature* 441: 868–871.
- Mayr, E. 1942. *Systematics and the origin of species*. New York: Columbia Univ. Press.
- McCarthy, E.M., Asmussen, M.A. & Anderson, W.W. 1995. A theoretical assessment of recombinational speciation. *Heredity* 74: 502–509.
- Nolte, A.W., Freyhof, J., Stemshorn, K.C. & Tautz, D. 2005. An invasive lineage of sculpins, *Cottus* sp. (Pisces, Teleostei) in the Rhine with new habitat adaptations has originated from hybridization. *Proc. Roy. Soc. London, Ser. B, Biol. Sci.* 272: 2379–2387.
- Nolte, A.W. & Tautz, D. 2010. Understanding the onset of hybrid speciation. *Trends Genet.* 26: 54–58.
- Rieseberg, L.H. 1991. Homoploid reticulate evolution in *Helianthus* (Asteraceae): Evidence from ribosomal genes. *Amer. J. Bot.* 78: 1218–1237.
- Rieseberg, L.H. 1997. Hybrid origins of plant species. *Annual Rev. Ecol. Syst.* 28: 359–389.
- Rieseberg, L.H., Raymond, O., Rosenthal, D.M., Lai, Z., Livingstone, K., Nakazato, T., Murphy, J.L., Schwarzbach A.E., Donovan, L.A. & Lexer, C. 2003. Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* 301: 1211–1216.
- Rieseberg, L.H., Sinervo, B., Linder, C.R., Ungerer, M.C. & Arias D.M. 1996. Role of gene interaction in hybrid speciation: Evidence from ancient and experimental hybrids. *Science* 272: 741–745.

- Rieseberg, L.H., Van Fossen, C. & Desrochers, A.M. 1995. Hybrid speciation accompanied by genomic reorganization in wild sunflowers. *Nature* 375: 313–316.
- Scascitelli, M., Whitney, K.D., Randell, R.A., King, M., Buerkle, C.A. & Rieseberg, L.H. 2010. Genome scan of hybridizing sunflowers from Texas (*Helianthus annuus* and *H. debilis*) reveals asymmetric patterns of introgression and small islands of genomic differentiation. *Molec. Ecol.* 19: 521–541.
- Schluter, D. 2000. *The ecology of adaptive radiation*. Oxford: Oxford Univ. Press.
- Schluter, D. 2009. Evidence for ecological speciation and its alternative. *Science* 323: 737–741.
- Schwarz, D., Matta, B.M., Shakir-Botteri, N.L. & McPherson, B.A. 2005. Host shift to an invasive plant triggers rapid animal hybrid speciation. *Nature* 436: 546–549.
- Schwarzbach, A.E. & Rieseberg, L.H. 2002. Likely multiple origins of a diploid hybrid sunflower species. *Molec. Ecol.* 11: 1703–1715.
- Sobel, J.M., Chen, G.F., Watt, L.R. & Schamske, D.W. 2010. The biology of speciation. *Evolution* 64: 295–315.
- Song, B.-H., Wang, X.-Q., Wang, X.-R., Ding, K.-Y. & Hong, D.-Y. 2003. Cytoplasmic composition in *Pinus densata* and population establishment of the diploid hybrid pine. *Molec. Ecol.* 12: 2995–3001.
- Stebbins, G.L. 1957. The hybrid origin of microspecies in the *Elymus glaucus* complex. *Cytologia*, Suppl. Vol.: 336–340.
- Strasburg, J., Scotti-Saintagne, C., Scotti, I., Lai, Z. & Rieseberg, L.H. 2009. Genomic patterns of adaptive divergence between chromosomally differentiated sunflower species. *Molec. Biol. Evol.* 26: 1341–1355.
- Ungerer, M.C., Baird, S.J.E., Pan, J. & Rieseberg, L.H. 1998. Rapid hybrid speciation in wild sunflowers. *Proc. Natl. Acad. Sci. U.S.A.* 95: 11757–11762.
- Ungerer, M.C., Strakosh, S.C. & Zhen, Y. 2006. Genome expansion in hybrid sunflower species is associated with retrotransposon proliferation. *Curr. Biol.* 16: R872–R873.
- Wang, J., Tian, L., Lee, H.-S., Wei, N.E., Jiang, H., Watson, B., Madlung, A., Osborn, T.C., Doerge, R.W., Comai, L. & Chen, J. 2006. Genomewide nonadditive gene regulation in *Arabidopsis* allotetraploids. *Genetics* 172: 507–517.
- Wang, X.-R., Szmidt, A.E. & Savolainen, O. 2001. Genetic composition and diploid hybrid speciation of a high mountain pine, *Pinus densata*, native to the Tibetan Plateau. *Genetics* 159: 337–346.
- Welch, M.E. & Rieseberg, L.H. 2002. Patterns of genetic variation suggest a single, ancient origin for the diploid hybrid species *Helianthus paradoxus*. *Evolution* 56: 2126–2137.
- Wu, C.-I. 2001. The genic view of the process of speciation. *J. Evol. Biol.* 14: 851–865.
- Yatabe, Y., Kane, N.C., Scotti-Saintagne, C. & Rieseberg, L.H. 2007. Rampant gene exchange across a strong reproductive barrier between the annual sunflowers, *Helianthus annuus* and *H. petiolaris*. *Genetics* 175: 1883–1893.