First *Senecio* Research Network Meeting

University of St Andrews
20 August 2010

FIRST *SENECIO* RESEARCH NETWORK MEETING
(20 AUGUST 2010)

Dyers Brae House, Mitchell Building, School of Biology,
University of St Andrews, UK

10.00. Richard Abbott
School of Biology, University of St Andrews, UK

Welcome & Introduction

10.15. Matt Hegarty
Institute of Biological, Environmental & Rural Sciences (IBERS), University of Aberystwyth, UK

Using genomic techniques to investigate interspecific hybridisation and genetic diversity in *Senecio*

10.30. Adrian Brennan
Royal Botanic Garden, Edinburgh, UK

Quantitative and population genetics of hybrid speciation in *Senecio* (Asteraceae)

10.45. Minsung Kim
Faculty of Life Sciences, University of Manchester, UK

Development and evolution of flower head variation in Asteraceae

11.00 – 11.15. Tea/Coffee

11.15. Graham Muir
Dept Plant Sciences, University of Oxford, UK

Speciation on Mount Etna, Sicily: a multigenic analysis of adaptation in *Senecio*

11.30. Dmitry Filatov
Dept Plant Sciences, University of Oxford, UK

Genome-wide analysis of adaptation and speciation in *Senecio* and *Silene*

11.45. Tom Batstone
School of Biological Sciences, University of Bristol, UK

Genome sequencing in *Senecio*

12.00. Klaas Vrieling
Institute Biology Leiden (IBL), Leiden University, the Netherlands

The invasiveness of *Jacobaea vulgaris* (formerly *Senecio jacobaea*)

12.15. Peter Klinkhamer
Institute Biology Leiden (IBL), Leiden University, the Netherlands

Utilisation of a mapping population produced from crossing *Jacobaea vulgaris* and *J. aquatica* (formerly *Senecio jacobaea* and *S. aquaticus*)

12.30. Discussion

13.00 – 14.00. LUNCH

14.00. Daniel Ortiz - Barrientos
School of Biological Sciences, University of Queensland, Australia

Adaptive speciation in *Senecio pinnatifolius*

14.15. Hans-Peter Comes
Facbereich für Organismische Biologie, Paris-Lodron-Univrsitat Salzburg, Austria

Parallel evolution in *Senecio* sect. *Senecio*: the genetics of adaptive shifts in flowering time reconsidered

14.30. Eleanor Dormontt
School of Earth & Environmental Sciences, University of Adelaide, Australia

Founder effects, adaptation or maladaptation? Explaining reduced diversity and increased differentiation during spread of *Senecio madagascariensis* in Australia

14.45. Andrew Lowe
School of Earth & Environmental Sciences, University of Adelaide, Australia

Invasion genomics and adaptation in Australian fireweed

15.00. John Bridle
School of Biological Sciences, University of Bristol, UK

Adaptive evolution in *Senecio*: understanding the interaction between selection, recombination and gene flow

15.15 Rebecca Ross
Dept Plant Sciences, University of Oxford, UK

Transplant studies on *Senecio* in Sicily and the UK

15.25 - 15.45. Tea/Coffee

15.45 - 16.45. Discussion & Proposals

End of Meeting

ABSTRACTS

- **Matt Hegarty**

Institute of Biological, Environmental & Rural Sciences (IBERS), University of Aberystwyth, Aberystwyth, Ceredigion, SY23 3DA, UK

Using genomic techniques to investigate interspecific hybridisation and genetic diversity in *Senecio*

The genus *Senecio* is comprised of a broad range of taxa, many of which are the result of interspecific hybridisation and/or polyploidy. The genus thus represents an excellent resource for studies of both genetic diversity within natural populations, and the consequences of genome mergers/duplications. With the advent of new genomic technologies and, more recently, the initiation of efforts to produce a partial draft genome of *Senecio squalidus*, the resources are becoming available to further investigate these areas of interest. In this presentation, I will briefly discuss our previous work investigating interspecific hybridisation in *Senecio* and detail current and future plans using next-generation sequencing approaches.

- **Adrian Brennan**

Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh, EH3 5LR

Quantitative and population genetics of hybrid speciation in *Senecio* (Asteraceae)

Hybridization is increasingly viewed as an important force for speciation in plants. *Senecio squalidus*, is one well-known homoploid hybrid species. *Senecio squalidus* is an invasive species in the UK and contemporary records describe its introduction to Oxford Botanic Garden about 300 years ago from a natural hybrid zone on Mt. Etna, Sicily between the high and low altitude species, *S. aethnensis* and *S. chrysanthemifolius,* respectively. Our research aims to better understand the genetics of hybrid speciation by investigating quantitative and molecular genetic divergence between UK *S. squalidus* and Sicilian *Senecio* taxa. We demonstrate that selection against hybridization contributes to the maintenance of the hybrid zone on Mt. Etna. We also show that *S. squalidus* is distinct from both parent species and from hybrids on Mt. Etna confirming that *S. squalidus* has undergone evolutionary change during hybrid speciation due to a combination of genetic isolation and selection. Combined quantitative trait locus mapping and selection analyses are providing a more detailed picture of the genomic targets experiencing divergent selection between these species.

- **Minsung Kim**

University of Manchester, Manchester M13 9PT, UK

Development and evolution of flower head in Asteraceae

Changes in regulatory genes have been implicated in a range of evolutionary changes. It has been largely believed that these changes occur independently in different lineages. However, it is also possible that an interspecific hybridization can transfer traits between species.

A well-documented example of such interspecific exchange of trait is the flower head trait of *Senecio vulgaris* (Groundsel, Asteraceae) in UK. *S. vulgaris* is polymorphic in forms of flower heads (radiate and discoid). The radiate *S. vulgaris* form was introgressed from a radiate Sicilian species, *S. squalidus* when *S. squalidus* was introduced to the Oxford Botanic Garden about 300 years ago. Here, we have identified a locus (*RAY* locus) controlling radiate vs. discoid flower head forms in *S. vulgaris*, and confirmed that this locus was indeed introgressed from *S. squalidus* to *S. vulgaris*. This *RAY* locus turned out to be two clustered *CYCLOIDEA* homologs (named *RAY1* and *RAY2*). *CYC* has known as a key regulator controlling flower symmetry in *Antirrhinum*. *RAY* genes were expressed in the ray floret primordium and the over-expression of *RAY* genes was able to change flower head forms (radiate to discoid), confirming their role in determining the ray floret identity.

This result was the first identification of the gene that controls flower head development and also the first example in eukaryotes of inter-species trait transfer wholly produced from a single gene. These findings highlight how genes can flow horizontally between species (multi-cellular organisms), making evolution as a “web” of life, instead of a tree of life.

Our current project is to elucidate the underlying genetic mechanism(s) establishing the two floral symmetries within a single flower head by determining the expression patterns and the functions of *sCYC* genes, *sRAD* and *sDIV,* and the genetic/protein interaction among these genes.

Reference: Kim, M et al. (2008) Regulatory genes control a key morphological and ecological trait transferred between species. *Science* 322: 1116-1119.

- **Graham Muir**

Dept Plant Sciences, University of Oxford, South Parks Road Oxford, OX1 3RB, UK

Speciation on Mount Etna, Sicily: a multigenic analysis of adaptation in *Senecio*

The hybrid zone on Mount Etna, Sicily between two *Senecio* species is one of the best examples of a cline in plant population genetics. *Senecio aethnensis* grows at high altitudes on the volcano in an environment challenged by extremes in temperature and UV radiation, whereas *Senecio chrysanthemifolius* grows at lower altitudes towards the base of the volcano where conditions are less extreme. Hybrids exist at the interface between them and a cline in gene frequencies is observed between the two species which may be caused by diversifying selection (heterozygote disadvantage) or simply recent hybridization. We considered the role of gene flow and natural selection in the maintenance of key reproductive traits by sequencing genes differentially expressed in capitulum (inflorescence) buds and flower buds. Flowering physiology differs between the two *Senecio* species growing at high and low altitude, and aspects of key reproductive traits are likely to be adaptive responses maintaining these different evolutionary units. By studying genes which varied in expression, we were able to test whether these species are under diversifying selection to maintain species boundaries in the face of gene flow across the hybrid zone. We also used an isolation-migration framework to estimate the time of divergence of the high and low altitude *Senecio* species and other migration parameters since the divergence of the two species.

- **Dmitry Filatov**

Dept Plant Sciences, University of Oxford, South Parks Road Oxford, OX1 3RB, UK

Genome-wide analysis of adaptation and speciation in *Senecio* and *Silene*

Together with Richard Abbott and Simon Hiscock we recently started a collaborative project devoted to evolutionary genomics of adaptation and speciation in *Senecio*. We will combine high-throughput genomic and population genetic analyses to identify protein coding and regulatory regions across the *Senecio* genome and to analyze evolutionary dynamics of mutations in these regions. In particular, we plan to obtain the sequence of the non-repetitive fraction of the genome of *S. squalidus* and will use it as a partial reference genome in identification of coding regions and functional elements. These regions will be compared between closely related *Senecio* species and among individuals within species to identify regions that may have been involved in adaptation of *Senecio* to contrasting environments. We will use population genetic analyses of DNA polymorphism and divergence across the *Senecio* genome to test modern theories of adaptation and speciation. To illustrate the power of this approach I will present our (fairly preliminary) data for another project in my lab that aims to conduct genome-wide DNA polymorphism analysis in White Campion (*Silene latifolia*) using high-throughput sequencing of cDNA.

- **Tom Batstone**

School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG

Genome sequencing in *Senecio*

We have recently begun a project utilising the power of next generation sequencing technologies to study the basis of adaptation and species divergence in *Senecio squalidus*. Work in Bristol will focus on producing a partial reference genome of *S. squalidus* which will be used as the basis for subsequent small RNA profiling and ChIP-seq experiments. The genome of *S. squalidus* is estimated to be ~1700Mbp, but a large proportion of this is expected to consist of various repetitive elements. We are employing an approach based on methyl-filtration, using a methyl binding domain protein, to eliminate the heavily methylated repetitive fraction of the genome, followed by 454 sequencing. Pilot 454 sequencing data comparing both methyl-filtered and native genomic DNA indicates that this approach successfully reduces the proportion of repeat sequence. Although this method will not yield a full genome sequence of *S. squalidus*, we hope to produce a usable representation of coding and regulatory sequences as the basis for further studies. We are also investigating the use of illumina sequencing technology to complement the 454 data and improve both depth and overall coverage, including use of paired end libraries that have been normalized using a combination of Cot filtration and double strand specific nuclease treatment. Ultimately we intend to supplement the present strategy by further illumina sequencing with the aim of producing a complete genome sequence.

- **Klaas Vrieling**

Plant Ecology and Phytochemistry, Institute Biology Leiden (IBL), Leiden University, Sylvius Laboratory, Sylviusweg 72, 2333BE, P.O.Box 9505, 2300RA, Leiden, the Netherlands

The invasiveness of *Jacobaea vulgaris* (formerly *Senecio jacobaea*)

Common ragwort is a notorious pest weed in Australia, New Zealand and North America and it is less so in its native area in Europe. We extended the EICA hypothesis (Blossey and Nötzold 1995) by incorporating the specialist-generalist dilemma (Van der Meijden 1996) to explain evolutionary shifts in allocation and hence the success of invasive species. We tested the prediction of the Shifting Defence Hypothesis (Doorduin and Vrieling 2010 in press) by comparing ragwort from invasive and native areas in common garden and laboratory experiments. As predicted by the Shifting Defence hypthesis invasive plants decreased their quantitative defences, increased their qualitative defences, became more susceptible to specialist herbivores and less susceptible to generalist herbivores and finally increased there reproductive output with more than 35% (Joshi and Vrieling, 2005). This suggests that a fast evolutionary change in allocation in combination with the absence of specialist herbivores gave invasive ragworts an increased competitive ability in their new range and hence shaped a pest species. Molecular research showed that multiple introductions occurred excluding the possibility of preadaptation (Doorduin et al 2010, Doorduin et al. in prep). Future research will focus on physiological and genetic mechanisms involved in the reallocation.

❖ **Peter Klinkhamer**
Plant Ecology and Phytochemistry, Institute Biology Leiden (IBL), Leiden University, Sylvius Laboratory, Sylviusweg 72, 2333BE, P.O.Box 9505, 2300RA, Leiden, the Netherlands

Utilisation of a mapping population produced from crossing *Jacobaea vulgaris* and *J. aquatica* (formerly *Sencio jacobaea* and *S. aquaticus*)

❖ **Daniel Ortiz Barrientos**
School of Biological Sciences, University of Queensland, St. Lucia, QLD 4072, Australia

Adaptive speciation in *Senecio pinnatifolius*

Natural selection should create species. We expect that it can favour traits that incidentally lead to the evolution of reproductive isolation. The early stages of such divergence, however, remain a mystery. Many species carry populations inhabiting contrasting environments, and correlations between abiotic variables, like temperature or salinity, and morphology often arise in the form of ecotypes. These correlations may be coupled with the evolution of reproductive isolation, thus making the origin of ecotypes fertile grounds to study the early stages of speciation. Here, I describe the development of a new plant model system to investigate the origin of new species. *Senecio pinnatifolius*, or the variable groundsel, is native to Australia and inhabits both montane and costal habitats. Many of its forms appeared to have evolved several times in response to divergent natural selection, and some display variable levels of both intrinsic and extrinsic reproductive isolation. Next-generation sequencing estimates of divergence between parapatric populations of this species suggest that there is extensive gene flow between ecotypes, yet they remain distinct. Several models of evolution of ecotypes, and their implications for our understanding of speciation will be discussed. The ease of doing genetic, ecology and genomics in this genus, suggest that *Senecio* can become a model genus system for the study of both adaptation and speciation.

❖ **Hans-Peter Comes**
Facbereich für Organismische Biologie, Paris-Lodron-Univrsitat Salzburg, 5020 Salzburg, Austria

Parallel evolution in *Senecio* sect. *Senecio*: the genetics of adaptive shifts in flowering time reconsidered

Studying the genes that shape ecologically important traits may bring new insights to our understanding of the genetics of adaptation and speciation. Flowering time is one such major trait in plants because it determines the environmental conditions of reproduction with respect to climate, herbivory, pathogen pressures, and the presence of pollinators. The genetic architecture of this trait has been extensively studied in model organisms and crops but far less is known about the genetic changes and evolutionary consequences underlying variation in flowering time in natural systems. Particular phenotypic traits such as flowering time often evolve repeatedly when independent natural populations are exposed to similar ecological conditions.

Annual, winter-annual, and short-lived perennial species of *Senecio* sect. *Senecio* (Asteraceae), with their centre of diversity in the Mediterranean, should provide an ideal system for studying the molecular mechanisms that underlie flowering time in nature. The specific purpose of this talk is to draw attention again to this possibility and, in particular, to the fact that the number of molecular and developmental pathways from early to late flowering types (or vice versa) is probably limited in this group because flowering time has been shown to follow an oligo- rather than polygenic model of inheritance in some species (*S. vulgaris*, *S. vernalis*). Moreover, previous QTL studies in *S. vulgaris* have indicated that the genomic region controlling flowering time (termed '*SOD*') occurs in the same linkage group as the 'ray floret locus' controlling capitulum type (presence vs. absence of ray florets).

Next-generation sequencing technologies, the development of *Senecio* genomic recources, and knowledge gained from recent work that has isolated and characterized two tightly linked regulatory genes controlling capitulum type (Kim *et al*., 2008), should make it possible to identify, sequence and characterize the *SOD* gene(s). Successful research in this direction might offer promising perspectives for (i) revealing phylogenetic trends of flowering time evolution during the Quaternary radiation of this group; (ii) comparing the genetic basis of similar flowering time traits that have evolved in different locations and species (i.e. testing for evolutionary parallelism at the molecular level); and (iii) studying the phylgeographic patterns of *SOD* allelic variation, including signatures of recurrent positive selection, at different spatial scales. Together, such (and similar) approaches in *Senecio* should bring new insights to our understanding of the genetics of parallel plant adaptation and (incipient) speciation.

Reference: Kim, M et al. (2008). Regulatory genes control a key morphological and ecological trait transferred between species. *Science*, 322: 1116–1119.

- **Eleanor Dormontt**

Australian Centre for Evolutionary Biology & Biodiversity (ACEBB), School of Earth & Environmental Sciences, DP418, The University of Adelaide, SA 5005

Founder effects, adaptation or maladaptation? Explaining reduced diversity and increased differentiation during spread of *Senecio madagascariensis* in Australia

Using nine published microsatellite loci, 11 South African (native) and 20 Australian (invasive) populations of *Senecio madagascariensis* were screened to characterise the distribution of genetic diversity across both ranges. Within Australia, populations around the invasion origin harboured diversity comparable to that found in the native range, suggesting that initial introduction(s) circa 1918 contained a representative sample of native diversity. Population level diversity was negatively correlated with distance from putative point of introduction and average pairwise F_{ST} greater between populations from more recently colonized areas than those around the invasion origin. These results are consistent with several hypotheses; multiple, possibly sequential, founder effects during spread within Australia; selection of adapted genotypes during spread leaving a suite of maladapted populations around the origin; or subsequent introductions of less diverse material from the native range. Global F_{ST} and Nei,s total gene diversity (H_T) were not significantly different between the native and invasive ranges but within population diversity (H_S) was significantly smaller in the invasive range. AMOVA comparisons revealed more diversity within populations in the native range and between populations in the invasive range. Taken together these results illustrate that although overall diversity statistics can be similar between native and invasive ranges, the distribution of that diversity can differ markedly.

- **Andrew Lowe**

Australian Centre for Evolutionary Biology & Biodiversity (ACEBB), School of Earth & Environmental Sciences, DP418, The University of Adelaide, SA 5005

Invasion genomics and adaptation in Australian fireweed

- **John Bridle**

School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG

Adaptive evolution in *Senecio*: understanding the interaction between selection, recombination and gene flow

- **Rebecca Ross**

Dept Plant Sciences, University of Oxford, South Parks Road Oxford, OX1 3RB, UK

Transplant studies on *Senecio* in Sicily and the UK

NOT ATTENDING

- **Simon Hiscock**

School of Biological Sciences, University of Bristol, Woodland Road, Bristol,
BS8 1UG, UK (Simon.Hiscock@bristol.ac.uk)

Obtaining a reference *Senecio* genome

I hope that the meeting will galvanize some sort of 'world-wide' *Senecio* consortium with some common global aims. For me the most important 'tool' we need is a reference genome for a 'type'/'model' *Senecio*, namely *S. squalidus*. We can then all work from that in future genomics research on other species - diploids and allopolyploids. I have therefore asked Tom Batstone whi is attending the meeting to request that members of the consortium attempt to pool some resources (money) into helping to achieve this goal. The reason for this is NERCs recalcitrance on letting us use money allocated to 454 sequencing into more data effective and cost effective Illumina runs. Tom will explain that with a combination of 454 and Illumina we can get the whole genome - not simply the gene space (as proposed with 454) and this will be of immense value to the whole community. Once we have a good diploid *Senecio* genome sequence we can build all the synteny studies and genome architecture and architecture change in hybrids and allopolyploids around it in future projects. The Compositae Genome Project people in the US have deliberately left the *Senecio* section to us in their mammoth sequencing project so we need to bear this in mind.

- **Dietrich Ober**

Biochenical and molecular Evolution Section, Intitute of Botany and Botanical Garden, Christian Albrechts Universitat zu Kiel, Kiel, Germany (dober@bot.uni-kiel.de)

Evolution of biosynthetic pathways for secondary metabolite in *Senecio*

The research of our group focuses on a better understanding of those evolutionary processes that result in the establishment of new biosynthetic pathways as the prerequisite for the enormous chemical and physiological diversity within plant secondary metabolism. Currently, we are using the pyrrolizidine alkaloid (PA) biosynthesis as a model system. PAs are toxic compounds that are constitutively produced as defence against herbivores. We intend to identify the evolutionary origin of the specific enzyme systems, the regulatory elements and the evolutionary forces that shaped the biosynthetic pathway. Based on our analyses of the first specific enzyme of this pathway we have hints that this pathway evolved several times independently during angiosperm evolution, twice within the Asteraceae.

Senecio plants are a well established model system for our molecular and biochemical analyses. Presently we use our knowledge about the tissue and cell specificity of PA biosynthesis to isolate specific cells of *Senecio* roots of which we believe that they might be involved in PA biosynthesis. We hope to enrich those cDNAs that encode proteins involved in PA biosynthesis. The long-term aim of our research is a comparison of the biosynthetic enzymes and regulatory factors that are involved in PA biosynthesis from different plant lineages, for which we were able to show that at least the first specific enzyme (homospermidine synthase) is of independent origin. As tools we are establishing cDNA libraries of PA-synthesizing cells by direct and by subtractive methods.

Without doubt, our researtch would benefit significantyl of the knowledge of the genome of a certain *Senecio* species. Many techniques presently used for identification of new genes require genome databases, especially if only shorter sequence fragments are identified. For many of our evolutionary questions it is also essential to know definitely how many homologues sequences exist in a certain genome. This can only be answered by the complete genome data.

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