This is a semi-technical text based support file for one particular topic on the WildWest.ie website. These files will be linked from that relevant page so use BACK button to return. These files will be used to outline certain specialised issues that may not be a natural fit for the image based format of our Website.

WARNING: We are well out of our comfort zone here but this is an exercise in trying to relate a field naturalist's experience to complex lab-based botanical techniques. We tend to lean towards a 'characteristic' based approach to classification and S. romanzoffiana seems to have no distinct morphologic differences between its populations in Europe and N. America... or genetic ones? So apologies for mistakes made; we will accept corrections; and through this process hope to ascertain the source of this species' populations in Ireland and Scotland.

Epigenetics

We recently heard a Radio broadcast discussing Epigenetics in regard to humans, animals and plants. Its basic tenet seems to be that certain 'genes' in genetically identical organisms can be suppressed in one place (group) whereas the same organism in a different environment, climate, or whatever, can express those qualities that remain dormant in differing or more difficult situations. We have been reading up on which ways polyploidy and epigenetics can impact on orchids...

The Literature: *All documents quoted here are freely available online. Thanks to the authors...*

Paper A: Different Species.

A recent research paper freely published online by Oxford University Press (**Molecular Biology & Evolution**) studied three different species of Marsh Orchids, *Dactylorhiza majalis, D. traunsteineri* and *D. ebudensis* (the Hebridean Marsh Orchid) and states...

"Epigenetic information includes heritable signals that modulate gene expression but are not encoded in the primary nucleotide sequence."

"Using two contrasting approaches that yielded largely congruent results, epigenome scans pinpointed epiloci under divergent selection that correlate with eco-environmental variables, mainly related to water availability and temperature."

Ovidiu Paun, Richard M. Bateman, Michael F. Fay, Mikael Hedrén, Laure Civeyrel, Mark W. Chase; Stable Epigenetic Effects Impact Adaptation in Allopolyploid Orchids (Dactylorhiza: Orchidaceae), Molecular Biology and Evolution, Volume 27, Issue 11, 1 November 2010, Pages 2465–2473, https://doi.org/10.1093/molbev/msq150

It is this reference to a predilection to water that caught our attention, with our interest in *S. romanzoffiana*. However the marsh orchids studied are known to be polyploidic — having double or more chromosomes — and being very prone to remarkable variations in appearance, whereas *S. romanzoffiana* is more consistent in appearance, just variable in ecology! Most diploid orchids are consistent in their appearance but when they have duplicate (or greater) sets of chromosomes then appearance can vary significantly. The North American, *S. cernua*, is well known for having many variations in appearance and for being a nightmare to define!

This allows a concept whereby stable orchid populations can be based on a common genotype but an inheritable epigenetic effect leads to 3 differing phenotypes which justifies them now being classified as species — without these 'instructions' being encoded in the DNA sequence?

Paper B: Within the same species.

A recent **Cold Spring Harbour Symposium** reported on the... **Epigenetic Variation, Inheritance, and Selection in Plant Populations.** As part of their conclusions they stated that:

"Plant populations forced to adapt to different environmental conditions often show striking phenotypic adaptations. Until recently, phenotypic diversity within and among populations was thought to be attributable to genetic variation only. However, epigenetic variation may also contribute to plant adaptation and other evolutionary processes. For epigenetic variation to have an impact on the ecology and evolution of natural populations, epialleles that arise in populations must be inherited with sufficient stability."

Access the most recent version at doi:10.1101/sqb.2013.77.014605 Cold Spring Harb Symp Quant Biol 2012 77:
http://symposium.cshlp.org/content/77/97.full
97-104 originally published online April 25, 2013 S. Hirsch, R. Baumberger and U. Grossniklaus Populations E

Can we interpret this as indicating plants may not only have different appearances but can respond differently to their environment based not on any variation in their genes but responding to how epigenetic qualifiers affect that genotype in different situations?

Paper C: Polyploidy and Phylogeny.

Another Paper published on the web (HERE), investigates the many faceted aspects of the *Spiranthes cernua* complex and also *S. romanzoffiana*. This seems to imply that the latter can also be polyploidic — perhaps all orchids can be? They also state that... "no genetic differentiation was found between trans-Atlantic specimens of *S. romanzoffiana*."

A molecular framework for understanding the phylogeny of Spiranthes (orchidaceae), a cosmopolitan genus with a North American center of diversity1 lucy a. Dueck 2,4,5 , deniz aygoren 3,6 , and kenneth m. Cameron 3,4

Having never seen the North American specimens in the hand we can only go from the numerous photographs we have been shown and these very much share the same morphology, perhaps limited or exaggerated, depending on what side of the Atlantic they occur and the conditions and climate they are exposed to. Whether these factors can be entirely responsible for habitat preference (if it is a preference) and reproductive failure in Ireland is difficult to evaluate.

Paper D: Epigenetic information – unexplored source of natural variation.

In this article from Kew in 2011 the authors, again studying the phenotypicallly variable Dactylorhiza family, say...

"... we can detect patterns that indicate that some of these differences are due to changing epigenetic effects, which have been shown in several groups to be subject to environmental influence. Thus, taxa that are ecologically distinct but still appear genetically uniform may be the result of altered epigenetic controls of gene expression without any change in the underlying genetic material."

Ovidiu Paun & Mark W. Chase. Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey.*

Epigenetic information – unexplored source of natural variation
Lankesteriana International Journal on Orchidology 2011 11 (3)
Ovidiu Paun ; Mark W. Chase

This is the clearest description we have come across of variability in polypoidal orchids and it reflects recent studies we made of the 2 distinct species of Helleborines, *Epipactis phyllanthes* and *E. helleborine* which share a common genome but are morphologically clearly different. However this is a notoriously variable genus, as are the Marsh Orchids. Some Spiranthes share this variability but the previous paper indicates, in regard to *S. romanzoffiana* that no genetic differences occurs across the Atlantic nor are there (as far as we can discern) distinct differences in phenotype. So the difference in habitat, range, and reproductive success, could simply be a reflection of the effect of a marginal climate?

Paper E: Spiranthes Phylogeny. (Link is given in citation below.)

This paper contains is an incredibly detailed study of the phylogeny of the Spiranthes genus, including *S. romanzoffiana*, and does make specific suggestions as to the occurrence of that species in Europe. We, of course, support the view of wind dispersal versus being carried by animals, and have not heard species such as Great Black-backed Gulls being cited before. However we see little scope for the ever popular White-fronted Goose theory — unless there is some, as yet unknown, occurrence of *S. romanzoffiana* in Iceland where this goose rests during migration in September/October and, incidentally, where the Irish population of Whooper Swans breed. This is possibly a more likely species as their feeding pattern would be much more suitable for ingesting Spiranthes root stock or gathering seed hooks in their plumage.

However, the pattern of seed deposition as shown in our studies in L. Conn (www.WildWest.ie/spiranthesmigration.html) seems to totally rule out such random bird-carried events; it clearly shows a pattern of deposition that must be by wind/water transport and settlement.

Dueck's study suggests, in reference to this species, that...

"Vectors for this type of repeated long-distance dispersal of *Spiranthes romanzoffiana* might include the North Atlantic westerlies wind pattern and/or bird migrations. The latter may seem improbable for Orchidaceae given the nature of their dust like seeds that are not eaten by animals, but many wetland birds share habitats with *S. romanzoffiana* and could easily transmit seeds exozoochorously. Wilkinson (1997) suggested that exploratory movements of inexperienced birds play an important role in seed dispersal, perhaps even more than regular migrations. For instance, in a study of avian influenza virus transmission that tapped bird band returns, Wille et al. (2011) discovered that ca. 5% of great black-backed gull chicks banded in Newfoundland found their way across the Atlantic to Europe (including the UK) instead of taking their usual route due south to the coastal United States. Although not a standard migration route, vagrant seabirds, shorebirds, and waterfowl, often immature, are known to stray in both directions across the Atlantic (Peterson, 1980 ; Scott, 1994). Horsman (1999) even offered up a candidate bird

species as a possible carrier of *S. romanzoffiana* seed to the British Isles — Greenland's white-fronted geese. However, Spiranthes has not been reported in Greenland, forcing Horsman to theorize that the geese may have extirpated the orchid there by grazing on overwintering lateral buds. Nonetheless, it appears that at least one Spiranthes species has been able to cross transcontinental barriers via long-distance dispersal, establish itself abroad in a conducive environment, and thereby represent a model for explaining the current overseas distribution and allopatric speciation of other lineages in the genus.

A molecular framework for understanding the phylogeny of spiranthes (orchidaceae), a cosmopolitan genus with a North American center of diversity. American Journal of Botany: The latest version is at http://www.amjbot.org/cgi/doi/10.3732/ajb.1400225

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Reviewing the material and the evidence:

Why do *Spiranthes romanzoffiana* plants on both sides of the Atlantic grow differently?

In preparing the webpage (to which this review is an appendage) we placed Irish and North American sites and habitats side by side. We also studied the distribution and reproductive patterns of both populations. We have not studied the erudite DNA research as, if our thesis of a transatlantic connection is proved right, then they may share a common heritage? We are aware of a comparison between North American populations and Irish populations. However, the Canadian population may be more relevant especially the East Newfoundland population identified in our research. There is also much that morphology can teach us as well as… 'good old fieldwork.'

Three effects need to be considered in studying behaviour of differing taxa within a group of similar orchids. How do we determine the significance of perceived differences. Can they be…

- **Genetically based**.
- Not genetically different but clearly having either different appearance (phenotype) or habit, which seems to be controlled by **some epigenetic effect**.
- Neither genetically different or subject to any epigenetic control or release of a normal gene expression. They are purely reacting to arriving in a different environment, **being marginalised**, and just trying to cope.

Genetically based difference.

We tend to be intuitively sceptical of rare plant genome sequencing. It is not affordable to sequence in great detail non-commercial plant crops, so small parts of a vast genome are surveyed. These small sections can prove identical and a quick conclusion would be to state they are the same species even though our eyes tell us they are recognisably and inheritably different. Often we can reconcile the specialities of the geneticist and the naturalist but the simple explanation we were offered recently may provide one clue… "*There will be differences in other parts of the genome we have not surveyed!*" To use genetics to prove the 'race' of a rare plant may be impossible as it depends on a wide variety of other tests done and having other proven specimens to compare your sample with. There simply is not that amount of data available for many rare plants. For many rare orchids there may be only a handful of sequences done, some of these are from herbarium collections many years old, others could have been misidentified, in the past, as the family is notably taxonomically variable. But if a 'phenogroup' are consistent in appearance and are able to replicate themselves for years then they are clearly some distinct taxon.

In North America, *Spiranthes romanzoffiana* is one of numerous taxa of Spiranthes to occur. In Ireland, it is one of two. Its 'cousin' *S. spiralis,* whilst of a common pattern, is obviously a quite different species. Also its distribution is different — occurring in Europe and absent from North America. This immediately implies that there may be a reason for *S. romanzoffiana* spanning the two continents and no such reason for *S. spiralis,* though some literature we are reading seems to imply that it, too, may have originated in North America and has just died out there for some reason?

However, the two populations of *S. romanzoffiana* are phenotypically and genetically (we believe) the same. It seems very likely that the specimens found in Ireland and Scotland may have come from North America — and may still be coming from there. Former claims of genetic differentiation between the Scottish and Irish stocks no longer seem to be accepted. Little work has been done comparing the Irish and US populations genetically and any indicators seen may not be

significant. These stocks, also, may not be in communication as the samples analysed were from New York — a long way from Newfoundland — the most likely point for any seed being shared with the west of Ireland.

We would tend to assume that because of their adaptation to wind transport, and the survival mechanisms put in place on Spiranthes seeds, that it is more than probable that some viable seed will travel the Atlantic. The plants it produces are remarkably similar but the way it grows, the ecology, and its inability to reproduce in Ireland, speaks of a plant that is at the margins of its range and may be enduring some controlling mechanism, be it epigenetic or environmental, that naturally limits its range.

If *S. romanzoffiana* had been isolated in Ireland for centuries, or millennia, we would expect to see a much greater phenotype alteration from what would then be its ancient North American cousins. Contact with keen botanists in Canada and the US have shown us plants remarkably similar to the ones we know and love in Leitrim, Mayo and Galway. There is no significant variation in the background genetic structure but the differing environment these migrants from North America find themselves in could be sufficiently severe as to trigger some epigenetic effect which controls or limits the plants ability to produce seed.

An Epigenetic effect.

The study on Marsh Orchids (Paper A above) claims a correlation between epiloci (points on the genome where multiple chromosomes interact) that they have found in the specimens studied, and the environmental conditions in which those species live, mainly regarding water and temperature.

Assuming that the 'switching mechanism' is an epigenetic effect this would easily explain how these 3 marsh orchids have very different appearances, have very specific ecological niches and why one, the Hebridean Marsh Orchid has a very limited range.

As regards the distribution of *S. romanzoffiana* in the Irish province, the correlation with water is outstanding — so outstanding that it may imply more specific geographical factors rather than an evolutionary adjustment to a cold and wet climate. (Go back to website for examples of this.) There is also the worrying implication that the history of the species is short in Europe and we probably must accept the lessons of their discovery. They were not in Ireland or Scotland for many years before they were found! That would leave a period of less than 200 years for an epigenetic effect to be widely applied to the species whereas the literature talks about these changes happening in distant ancient times through a natural variant or 'sport' whose qualities are then inherited through the generations, in parallel with their unaltered genetic sequence.

We are not clear how precise the correlation with water, described in Paper A, is. Nor have we seen such detailed mapping of specimens for Scotland as we have prepared in our research. There are startling new colonies being reported from there and there could be many similar observations to ours from L. Conn that might show even more clearly the true reason for the distribution patterns of our water-loving Spiranthes?

Marginalised environmentally.

This brings us to our last option. Irish *S. romanzoffiana* are not genetically different to North American specimens nor have they any clear morphology that distinguishes them from their cousins (siblings?) but they are simply responding to an environment more severe than the one they are used to — or they have arrived here via a unique mechanism that places them in neat rows along the shores of our lakes rather than the species selecting that habitat!

In a way we are slow to embrace the obvious conclusion, partly the need for the extra evidence we have garnered this year, and partly to avoid prejudgement or jumping to a conclusion. With research this Summer and international contacts this Autumn/Fall, two facts have become clear:

1. There is no clear physical or appearance difference between the specimens, bar height and lushness.
2. Whereas they have a huge range in North America, from east to west, and from 0 – 3,300 m. altitude, they do occur abundantly in Newfoundland and that environment is remarkably similar to Ireland's, with cold and wet Autumns. But the species does produce seed there!
3. But, in Newfoundland, there is no association with lake shores and specimens aren't routinely flooded.

Specimens in Newfoundland seem robust and abundant in places in the Summer. Perhaps there is a local climate effect or a soil characteristic that suits them better. Also, in Ireland over the past several years, we have observed that *S. romanzoffiana* seems to thrive in its early stages up to the time it is in full flower, and we can have some fine specimens close to 40 cm. high with very significant inflorescences. However, very soon after the spike is in full flower, the plants seem diminished and insects and bees are less abundant. This seems to have marked the onset of an early Autumn that has been very consistent over the past several years. The plants seem suddenly to lose vigour?

Perhaps this 'Summer cut-off' is less of a feature in Newfoundland and that, combined with no flooding of the Spiranthes colonies, provides two essential factors that contribute it to being able to produce abundant seed there whereas it has proved nigh impossible to find matured seeds on Irish specimens.

Of course, if this is the case, this would totally rule out any genetic or epigenetic factor as a cause for the difference in distribution or reproduction observed in the west of Ireland — it is a purely a consequence of the environment. The plant is trying repeatedly to colonise a new territory within reach of its seeds but has an environment that is in some way hostile… so the plants keep coming and going in new sites from seeds, and surviving for a number of years in old sites

The evidence of an environmental base to the observed pattern of Spiranthes distribution in Ireland does inevitably come back to the fact that there are examples of hundreds of specimens arranged neatly around flat depressions near water or like sentries along a straight shore with a consistent slope. Where one can argue about the possibility of some species of bird carrying the seed, or root, to Ireland there is no bird that is so organised as to tidily plant the seed at a consistent height above, or at the water level, at time of arrival!

End…

It has been very useful to us to have struggled through issues we found so confusing but, now, a bit clearer. We apologise for a fairly simplistic use of language in investigating complex botanical scenarios but, if the language is not simple, it may disguise what is a rather exciting truth. We have no absolute proof but it seems that 'our' *S. romanzoffiana* are not different either in genetics or epigenetic effects but their distribution is merely reflecting the way that they have arrived in this country (by seeds gently lapping onto the shores of Autumn lakes) and, then, the way they respond to a cold wet Autumn some years later as the mature plant roots start to produce shoots and emerge. This suggests that our present high Autumn water levels may not suit the plant and they require lower water levels so the seed can be deposited on a sand/muddy shore with little plant cover. This could be another factor in explaining the apparent periodicity of their population explosions? Wet Summer and Autumn weather may not hinder vegetative reproduction (see website) but is not conducive to seed production and the development of colonies away from the shore or in adjoining hilly or rocky land where they would gain protection from floodwaters but might be overgrown by lush hill or bog vegetation.

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