



REVIEW ARTICLE

Ecotypes and the controversy over stages in the formation of new species

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Recent interest in the role of ecology in species formation has led to renewed discussion of the stages in the process of speciation. Although attempts to classify the stages in the process of species formation date back at least as far as Alfred Russel Wallace, one of the most intense debates on the subject occurred among botanists during the mid-20th Century. The present review outlines the progression of the historical debate about stages in the evolution of species, which was instigated by the genealogical classification scheme of Göte Turesson in the 1920s, championed in the mid-century by Jens Clausen, and then brought under harsh scrutiny by many in the 1960s and 1970s. At the heart of the controversy is the question of whether speciation occurs rapidly on a local scale or gradually through the formation of geographically widespread ecotypes that evolve as precursors to species. A corollary to this debate is the question of whether speciation is reversible and, if so, how does it become irreversible? A third wave of interest in stages in the process of speciation is currently underway, thus making a modern historical narrative of the debate important. Both contemporary and past evolutionary biologists have argued that viewing speciation as being composed of stages can free researchers from concerns over species definitions and focus attention on the mechanisms involved in the process. How speciation becomes irreversible and whether ecogeographically isolated ecotypes are integral to this process remain as important unresolved issues. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **106**, 241–257.

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'In the evolution of plant species there is a long series of stages of increasing evolutionary distinctness, starting with the local populations, continuing through ecological races, ecospecies, and groups of species of higher and higher order' (Clausen, 1951: 177).

INTRODUCTION

The process by which one species diverges into two distinct phylogenetic lineages has long fascinated biologists. While most biologists would agree that

speciation occurs across a continuum over time, there has been much disagreement about the nature of the process, the role of reproductive isolating barriers in speciation, and the point at which the process is completed. To resolve these issues, biologists must study speciation at various points along the continuum of the process. Repeatedly, those who have taken up this challenge have found that dividing speciation into stages is a useful framework for better understanding the entire process (Wallace, 1865; Turesson, 1922a; Clausen, 1951; Grant, 1981; Wu, 2001; Nosil, Harmon & Seehausen, 2009).

Understanding the stages that taxa pass through en route to becoming distinct species has piqued the

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interest of evolutionary biologists at least three times during the last 150 years. During each of these periods, research on the role of natural selection in diversification led to the inevitable question of whether there are discernable stages to the process of speciation. We are currently in a third wave of interest in the stages of speciation (Wu, 2001; Mallet *et al.*, 2007; Hendry *et al.*, 2009; Nosil *et al.*, 2009; Smadja & Butlin, 2011), triggered primarily by contemporary focus on the role of ecology and natural selection in the process (Schluter, 2001, 2009; Coyne & Orr, 2004; Rundle & Nosil, 2005). However, there is a rich history of debate over stages in the formation of species that often is overlooked in the modern discussion. In particular, the great experimental work and writings of the plant biosystematists (e.g. Göte Turesson, Jens Clausen, and Verne Grant) are relevant.

Consensus on plant speciation has never been reached at any point in history and has often been contentious (Turesson, 1922a, b; Faegri, 1937; Clausen, 1951; Heywood, 1959; Ehrlich & Raven, 1969; Langlet, 1971; Quinn, 1978; Levin, 1993; Baum, 2009; Schemske, 2010; Sobel *et al.*, 2010). Beyond the definition of species itself, the process of plant speciation was controversial during the 20th Century because of the question of whether widespread distinguishable ecotypes exist within species, and if they are precursors to new species.

Much of the controversy came down to the question of whether ecotypes are fundamentally different from clines (Linhart & Grant, 1996; Briggs & Walters, 1997). This controversy is unexpected given that a cline refers to the change in a single trait or allele frequency across geographical space (Fig. 1). By contrast, ecotypes are groups of populations, which are distinguished by a composite of variation in many traits and allele frequencies across loci over space. Because ecotypes are formed by multiple trait adaptations to many environmental variables that covary in space, they are best represented by the principle components of all trait variation (Fig. 1C). Unfortunately, as shown in the present review, ecotypes have been viewed by many of their detractors as misguided attempts to force variation within species into distinct and non-evolving static groups. However, supporters of the ecotype concept (Turesson, Clausen, and many others, including myself) do not view ecotypes as static, but rather see them as a reflection of the nonrandom partitioning of genetic variation along the continuum of species formation. Just as sunlight can appear as a dim crack in the sky before clouds part, the coarse boundaries of ecotypes may appear as a separation of principle component clusters before speciation. However, just as clouds can fuse back together, most ecotypes

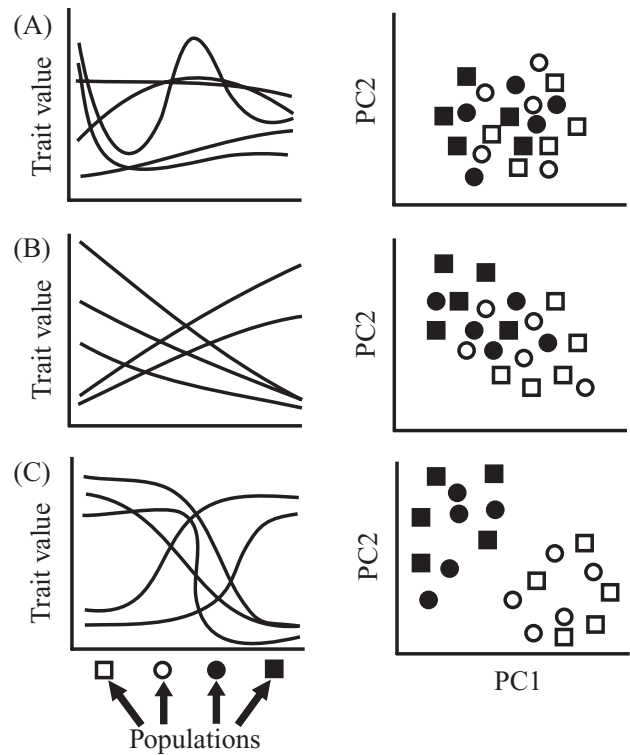


Figure 1. The distribution of trait variation over space as it relates to divergence of populations within species. Hypothetical trait values across two dimensions of space are displayed in the left column. The first two principle components (PC) of all traits are displayed in the right column. Four populations occur along the hypothetical gradients and are indicated in the left column. A, trait variation resulting from uncorrelated environmental gradients or genetic drift. B, trait variation arising from gentle correlated environmental gradients, such as across the Great Plains of North America. C, trait variation at the border of two very divergent ecoregions, such as coastal and inland habitats that give rise to widespread ecotypes.

will never go on to become distinct phylogenetic lineages.

A major corollary to the debate over stages in the evolution of species is the question of whether the process of speciation is reversible, and if so, under what conditions? Clausen (1951) was well aware that ecological barriers to gene flow could be reversed if environmental conditions changed, and thus dedicated a large portion of his book *Stages in the Evolution of Plant Species* to different degrees in the severity of intrinsic postzygotic isolation and his belief that such barriers are necessary to complete the process.

The present review focuses on the history of the controversy over stages in the process of speciation. To better understand the details of the historical

debate, the scope of the review is limited primarily to nonpolyploid speciation and the views of historical botanists. Excellent comprehensive reviews on polyploidy speciation and historical narratives of the views of zoologists toward speciation are available elsewhere (Ramsey & Schemske, 1998, 2002; Coyne & Orr, 2004; de Queiroz, 2005; Mallet, 2008a, b; Fitzpatrick, Fordyce & Gaverilets, 2009; Sobel *et al.*, 2010).

THE BIRTH OF ECOTYPE

The idea to classify stages of speciation is about as old as the Theory of Natural Selection. Not long after Darwin's publication of 'The Origin of Species', Wallace (1865) laid out a classification scheme for distinct stages in the formation of species based on observations of swallowtail butterflies. Mallet has recently suggested that this may have been 'the first attempt by a Darwinist to enumerate and classify the . . . "varieties" that Darwin argued were the fore-runners of species' (Mallet, 2009: 40). Darwin, Wallace, and their followers thus represented the first wave of interest in understanding the stages of the speciation process. However, it was not until the second wave that data from well-designed field experiments would help clarify the population genetic processes involved in the stages of speciation.

Even though Darwin (1859) rejected Platonic–Aristotelian essentialism with regard to the immutability of organisms, early plant evolutionary biologists were confused about the nature of varieties and species in relation to adaptation to local environmental conditions. Much of this confusion resulted from an incomplete understanding of the mechanisms of heredity and a persistence of Lamarckian thinking well into the 20th Century (Stebbins, 1980; Hagen, 1984; Smocovitis, 2006). A big shift occurred after the publication of a set of experiments conducted by Göte Turesson in the 1920s. Those studies convinced many plant evolutionary biologists that a large portion of the phenotypic differentiation among populations has a genetic basis (Turesson, 1922a, b, 1925; Clausen, 1951; Hagen, 1984), launching the second wave of interest in studying the stages of speciation.

Turesson's interest in heritable variation led to the concept of ecotype. To test whether there was a genetic basis to population differences, Turesson (1922a, b, 1925) set up common garden experiments, where plants collected from across Sweden were grown under one set of environmental conditions. After conducting similar such experiments across multiple species, Turesson recognized a pattern: plants collected from coastal and inland habitats differed from each other by a large suite of traits (Table 1). Having confirmed genetically-based differ-

ences among groups of plants growing in particular habitats, Turesson (1922a) coined the term 'ecotype' as way to refer to a set of ecologically distinct populations. In the same paper, Turesson also employed the term 'ecospecies' (Turesson, 1922a) to refer to a widespread set of ecotypes of the same species with similar genetically based traits. For example, he considered multiple ecotypes growing along the coast of Sweden, whether from dunes, bluffs or cliffs, as one ecospecies. Many researchers around the world have now observed the divergent morphology of coastal and inland ecotypes across multiple species in common garden experiments (Table 1).

Although Turesson may have originally described ecotypes as rigid abstract entities, he quickly came to see them as continually evolving groups (Turesson, 1925, 1929, 1931). This is an important point. For, as we will see later, Mayr (1958) and Stebbins (1980) both focused on his initial definition to label Turesson as a 'typologist'. Turesson's initial definitions made abstract distinction between the ecological versus genetic aspects of taxonomic groups and presented ecotypes as discrete units of organization (Turesson, 1922a, b; Hagen, 1983, 1984). However, this distinction between ecology and genetics quickly dissolved by the next year. In a follow-up paper, Turesson (1923) coined the term 'genecology' to refer to this field of inquiry, defined as a focus on understanding heritable genetic difference between taxonomic groups through breeding and transplant experiments. He also clarified that ecotypes are the product of natural selection (Turesson, 1925).

Turesson's work established foremost that phenotypic differences among populations derived from particular ecoregions were not a result of chance. Instead, these genetic differences were now considered to be a result of adaptations to specific sets of environmental factors that define habitats.

The biosystematists that followed Turesson utilized his functional definitions and increasingly stressed the importance of reproductive isolating barriers that limited gene flow (Gregor, 1931, 1939; Gregor, Davey & Lang, 1936; Clausen, Keck & Hiesey, 1939). For example, Gregor *et al.* (1936: 325) provided a definition for ecotype based on reproductive isolation as 'a population distinguished by morphological and physiological characters, most frequently of a quantitative nature; interfertile with other ecotypes and ecospecies, but prevented from exchanging genes by ecological barriers'. The redefinition of the genecological terminology reflected empirical results of researchers who found that partitioning heritable genetic variation within species was more complex than Turesson (1922a) originally envisioned (Gregor, 1930; Gregor *et al.*, 1936; Clausen *et al.*, 1939).

Table 1. Examples of coastal and inland ecotypes from around the world

Species	Location	Coastal ecotype traits	Inland ecotype traits	Reference
<i>Achillea borealis</i>	Western North America	Compact short stature Late flowering	Tall stature Early flowering	Clausen (1951)
<i>Achillea lanulosa</i>	Western North America	Compact short stature Late flowering	Tall stature Early flowering	Clausen (1951)
<i>Achillea millefolium</i>	California	Compact short stature	Tall stature	Clausen (1951)
<i>Agrosti stolonifera</i>	Great Britain	Less salt retained on leaves	More salt retained on leaves	Ahmad & Wainwright (1976)
<i>Armeria vulgaris</i>	Sweden/Norway	Late flowering Thick leaves	Early flowering Thin leaves	Turesson (1922b)
<i>Geranium robertianum</i>	Great Britain	Prostrate growth Red stems	Erect growth Green stems	Baker (1957)
<i>Gilia capitata</i>	California	Short stem Late flowering	Long stem Early flowering	Grant (1950, 1952) Nagy & Rice (1997)
<i>Hemizonia multicaulis</i>	California	Short central stem Short internodes	Long central stem Long internodes	Clausen & Hiesey (1958)
<i>Hemizonia paniculata</i>	California	No central leader Short central stem	Central leader Long central stem	Clausen & Hiesey (1958)
<i>Hieracium umbellatum</i>	Sweden	Short internodes Prostrate growth	Long internodes Erect growth	Turesson (1922b)
<i>Hordeum spontaneum</i>	Israel	Thick leaves Late flowering	Thin leaves Early flowering	Verhoeven <i>et al.</i> (2008)
<i>Layia chrysanthemoides</i>	California	Low relative growth rate Larger seeds Prostrate growth Late flowering	High relative growth rate Smaller seed Erect growth Early flowering	Clausen (1951)
<i>Layia platyglossa</i>	California	No central leader Short internodes Smooth akenes Prostrate growth Late flowering	Central leader Long internodes Hairy akenes Erect growth Early flowering	Clausen (1951) Clausen <i>et al.</i> (1947)
		No central leader Succulent	Central leader Not succulent	

<i>Matricaria indora</i>	Sweden	Perennial Prostrate growth Isolateral leaves	Annual Erect growth Palisade cells only on lower side	Turesson (1922b)
<i>Melandrium rubrium</i>	Sweden	Thick fleshy leaves Short blunt leaves	Thin leaves Long segmented leaves	Turesson (1925)
<i>Melandrium rubrium</i>	Sweden	Thick leaves	Thin leaves	Turesson (1922b)
<i>Mimulus guttatus</i>	Western North America	Succulent leaves Prostrate growth Perennial	Thin leaves Erect growth Both annual and perennial	Clausen & Hiesey (1958) Hall & Willis (2006) Lowry <i>et al.</i> (2008b)
<i>Nigella arvensis</i>	Israel	Late flowering Large flowers Short internodes Prostrate growth Rounded lobed leaves	Early flowering Small flowers Long internodes Erect growth Linear dissected leaves	Waisel (1959)
<i>Plantago major</i>	Sweden	Robust growth form	Diminutive growth form	Turesson (1925)
<i>Rumex crispus</i>	Great Britain	Late flowering Dense inflorescences	Early flowering Sparse inflorescences	Akeroyd & Briggs (1983)
<i>Sedum maximum</i>	Sweden	Few flowering stems Short stature	Many flowering stems Tall stature	Turesson (1922b)
<i>Silene cucubalus/maritima</i>	Northwestern Europe	Prostrate growth	Erect growth	Marsden-Jones & Turrill (1928)
<i>Solanum dulcamara</i>	Sweden	Late flowering Thick fleshy leaves	Early flowering Thin leaves	Turesson (1922b)
<i>Solidago sempervirens/rugosa</i>	Eastern North America	Hairy leaves Succulent leaves Large rosette leaves More disk florets per head	Smooth leaves Thin leaves Small rosette leaves Less disk florets per head	Goodwin (1937a,b,c)
<i>Spiraea ulmaria</i>	Sweden	Large seeds More auxin Short stature Thick leaves Prostrate growth	Small seeds Less auxin Tall stature Thin leaves Erect growth	Turesson (1925)
<i>Viola tricolor</i>	Denmark	Perennial Succulent leaves Purple stems No central leader Thick leaves	Annual Thin leaves Green stems Central leader Thin leaves	Clausen (1951)

STAGES IN THE EVOLUTION OF PLANT SPECIES

The classic works of Jens Clausen, David Keck, and William Hiesey at the Carnegie Institution at Stanford University further developed an experimental framework to understand the process of speciation (Hagen, 1983, 1984, 1986; Núñez-Farfán & Schlichting, 2001). Over a 20-year period, Clausen, Keck, and Hiesey examined the adaptive differentiation of numerous plant species through a series of experiments (Clausen, Keck & Hiesey, 1940, 1947, 1948; Hiesey, 1940; Clausen, 1951; Clausen & Hiesey, 1958; Hiesey, Nobs & Bjorkman, 1971). Instead of using a single common garden as Turesson had done previously, Clausen, Keck, and Hiesey conducted reciprocal transplant experiments. Here, common garden sites were located in three ecoregions of California: Stanford, a low elevation site in the coastal hills; Mather, a mid-elevation (1400 m) site in the Sierra Nevada foothills; and Timberline, an alpine site (3000 m) high in the Sierra Nevada Mountains. Plants from populations across California, including many far from the transect, were transplanted into all three of the field sites. In this way, Clausen, Keck, and Hiesey were not only able to determine heritability of phenotypic variation, but also to compare the morphological, physiological, and fitness responses of different populations from major ecoregions.

The greatest strength of Clausen, Keck, and Hiesey's research, like Turesson's, was the repeatability of findings over a broad range of taxonomic groups. Across plant families, they found the parallel evolution of coastal, inland, and alpine ecotypes. Instead of calling these widespread groups ecotypes, Clausen (1951) termed them as 'ecological races'. In *Stages in the Evolution of Plant Species*, Clausen (1951: 9) stated his belief that 'the ecological race is now commonly recognized as a basic element in evolution'. Clausen thought of ecological races as the product of local adaptation processes and as partially reproductively isolated groups on the path to speciation (Fig. 2).

Although Clausen (1951) hypothesized that suites of locally adapted traits can result in reproductive isolation and eventual species formation, he did not see species formation as the end of the process. Instead, he followed the classic view that it could only be completed through the eventual formation of complete intrinsic postzygotic reproductive isolation. Clausen (1951) used the term 'cenospecies' to refer to a group of related species (i.e. species complexes) that can only form sterile F_1 hybrids when crossed to members of other cenospecies. Further, the term 'comparia', originally coined by Danser (1929), was reserved for a final stage where groups of plants could no longer form an

F_1 hybrid (Fig. 2; See also Appendix of Terms). Clausen believed that intrinsic postzygotic isolation was ultimately necessary for phylogenetic lineages to become distinct because they would not be affected by shifting environmental conditions: 'The most permanent barriers are the internal, because they persist even through the environmental changes in a changing world' (Clausen *et al.*, 1939: 104). However, he did not consider that species boundaries had to be defined by such barriers: 'An impression has been prevalent that we [Clausen, Keck, and Hiesey] believe hybrid sterility or genetic incompatibility to be the sole criterion of specific distinctness, an erroneous impression that we would like to correct' (Clausen, 1951: 158). Instead, Clausen recognized the importance of many ecological barriers including temporal flowering isolation and pollinator isolation: 'Examples of pollinating systems in wild plants that may keep natural entities distinct morphologically even though there are no genetic barriers. Such internal genetic barriers are not needed to keep inheritances apart when selection by external pollinating agents is constantly at work' (Clausen, 1951: 93).

INFLUENCE OF AND CONTROVERSY WITH ZOOLOGISTS

When Turesson used the term ecotype and Clausen, Keck, and Hiesey used the term ecological races, they were referring to their belief that geographically widespread groups exist within species and that those groups are often the precursors to new species. Some animal evolutionary biologists of the same period also supported the idea of ecotype and were influenced by the plant biosystematists. This is apparent in the first edition of *Genetics and the Origin of Species* in which Dobzhansky praised the work of Turesson and the Carnegie group:

'The work of the botanical school of experimental taxonomy (Turesson, J. Clausen, and others) has brought to light the fact that even the small local variations in plants are frequently hereditary' (Dobzhansky, 1937: 169).

To Dobzhansky, the contribution of the plant biosystematists was obvious and thus, 'the adaptations of the plant species to the climatic and other conditions of their habitat are too well known to necessitate any extended discussion here' (Dobzhansky, 1937: 167). Unfortunately, without a more detailed description of the accomplishments of the plant biosystematists in his landmark book, Dobzhansky may have inadvertently undermined the perceived historical value of their research to the modern synthesis and ideas regarding the process of speciation.

By the mid-20th Century, the views of Clausen (1951) and Dobzhansky (1951) appear to be very

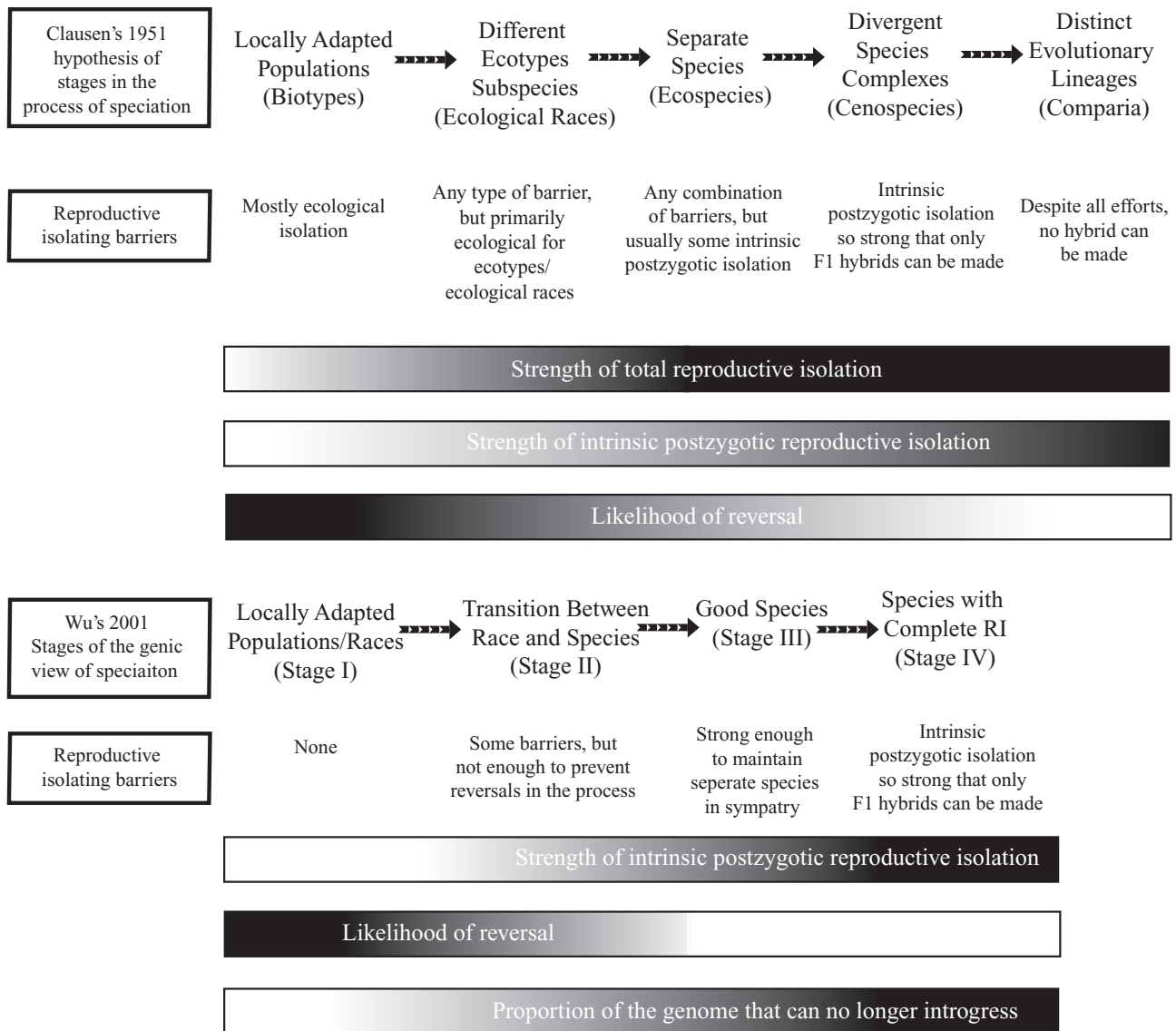


Figure 2. A comparison of stages in the process of speciation according to Clausen (1951) and Wu (2001). Figure based on a compilation of writings of Clausen and Wu's genic view of speciation. For Clausen, any combination of reproductive isolating barriers was thought to be sufficient for species formation, although lineages were not thought to be distinct and irreversible until complete intrinsic postzygotic isolation was achieved. Wu thought that reversals in speciation were impossible once 'good species' had evolved but that a few regions of the genome could still introgress between those species.

similar. Dobzhansky, like Clausen, was focused on the evolution of widespread races in the formation of species. Dobzhansky defined races here as 'Mendelian populations of a species, which differ in the frequencies of one or more genetic variants, gene alleles, or chromosomal structure' (Dobzhansky, 1937: 138) and noted that 'most races are ecotypes in the Turesson's sense' (Dobzhansky, 1937: 147). For Dobzhansky, races were also stages of speciation: 'the evidence for continuity between races and species is overwhelming' (Dobzhansky, 1940: 314) and 'a race

becomes more and more of a "concrete entity" as the process goes on; what is essential about races is not their state of being but that of becoming. But when the separation of races is complete, we are dealing with races no longer, for what have emerged are separate species' (Dobzhansky, 1951: 177). However, Clausen's (1951) views did differ from Dobzhansky's (1951) in that he felt many of the genecological categories beyond ecotype were of importance to understanding the process of speciation. This difference may reflect the fact that Dobzhansky saw

speciation as the end of the process, whereas Clausen was concerned about reversibility after speciation through the breakdown of ecological reproductive isolation.

Mayr levelled much harsher criticism of stages in the process of speciation: 'A species is not a stage of a process, but the result of a process' (Mayr, 1942: 119) and stated that the ecotype concept is 'typological and has itself become a straight-jacket for thinking and for experimentation in the field' (Mayr, 1958: 16). Mayr (1958) thus argued at a meeting in Sweden that the ecotype concept was an attempted revival of Platonic–Aristotelian essentialism and a static view of species. Clausen responded to this accusation at the meeting: 'I should like to state that the experimental taxonomists do not share Professor Mayr's contention that the ecotype is a typological concept' (Clausen, 1958: 20). Clausen argued instead that Turesson viewed species as dynamic and evolving entities that adapted to the features of the natural landscape, as can be seen clearly in the later work of Turesson (1925, 1929, 1931). Overall, it is unclear how different Mayr's views, beyond terminology, actually were from those of biosystematists given his advocacy for a polytypic species concept (Mayr, 1942, 1982), where each species is made up of interfertile geographical races. Mayr even stated a decade earlier: 'All geographic races are also ecological races, and all ecological races are also geographical races' (Mayr, 1947: 280). However, it has been well documented that Mayr's views shifted dramatically on these issues from 1942–1959 (Chung, 2003), and his contradictory quotes likely reflect that change.

CONTROVERSY OVER 'STAGES' AMONG BOTANISTS

The concept of intermediate stages of speciation was particularly controversial among botanists (Faegri, 1937; Turrill, 1946; Heywood, 1959; Langlet, 1971; Raven, 1976). Several factors, both theoretical and empirical, led critics to question the validity of the terms ecotype and ecological race.

ISSUES WITH CLASSIFICATION BASED ON EXPERIMENTATION

As the modern synthesis coalesced, the biological species concept (BSC) (Dobzhansky, 1935; Mayr, 1942) also gained wide acceptance. Although the BSC created an experimentally verifiable mechanistic definition of species (Coyne & Orr, 2004), the concept did not help to resolve the issue of the nature of intermediates in the process of speciation.

Classic taxonomists felt threatened by the idea of classification below the species level as well as the

biological species concepts because both were predicated on experimentation being necessary for classification. Peter Raven was adamantly opposed to the idea that experiments might be used as a method to classify groups of populations and declared: 'The period of 1935–1960 in particular was marked by a "conflict of categories" in which some workers attempted to substitute experimental criteria for morphological–ecological ones in plant classification, and we are not yet completely free of the effects of this confusing and naïve effort' (Raven, 1976: 288). In other words, the biological species concept and therefore any categorization scheme based on gene flow and reproductive isolation threatened the established taxonomic rules dictating the categorization of plant species.

EMPIRICAL RESULTS REVEAL COMPLEXITY OF THE ECOTYPE CONCEPT

More importantly than issues of taxonomy, other plant evolutionary biologists found results inconsistent with the concept of ecotype. Over the second half of the 20th Century, it became clear that the distribution of adaptive genetic variation within a plant species could range from a smooth distribution along an environmental gradient to extremely discrete. The classic works by Gregor (1930, 1931, 1938, 1939) on the distribution of phenotypic variation of *Plantago maritima* along the coastline of Great Britain, by Langlet (1936, 1971) on the Scots Pine (*Pinus sylvestris*), and by McMillan (1959, 1965, 1967, 1969) on grasses across the Great Plains of North America contrasted with that of Clausen, Keck, and Hiesey. In these three systems, phenotypic variation was not associated with discernable ecotypic groups but, rather, was distributed continuously. Huxley (1938) was concerned that this type of variation was overlooked by classification systems of his era and thus introduced the term 'cline' to describe graduation of variation in traits over space. Langlet (1963, 1971) argued that all intraspecific plant variation should be described by clines and that Turesson had received far too much credit for what he believed to be the false concept of ecotype: 'a term automatically and unavoidably gives an impression of uniformity within and disparity between the groups to which it is applied' (Langlet, 1971: 706). Just as Langlet found gentle clines in traits in pine trees, Calvin McMillan (1969) found that trait variation of the grass species he studied across the Great Plains of North America was distributed gently (Fig. 1B). However, McMillan (1967) argued that this was a function of the distribution of environmental variation, and that Clausen, Keck, and Hiesey were conducting research over much steeper ecological gradients, which were more

likely to drive ecotype formation (Fig. 1C). Notably, recent research on one of McMillan's grasses, *Panicum virgatum*, has revealed that, although many traits are distributed clinally across Eastern North America, there are also phenotypically divergent upland and lowland ecotypes that are interspersed across much of this gradient (Casler, 2005; Zhang *et al.*, 2011). Thus, even in regions such as the Great Plains of North America, there may be sufficient topographical variation to drive the formation of discernable ecotypes.

G. Ledyard Stebbins, in his grand review *Variation and Evolution in Plants*, appears to make a similar argument to that of McMillan in that widespread distinct ecotypes could evolve under highly heterogeneous conditions but not across gentle environmental gradients:

'In species occupying an area like the eastern United States, which is comparatively uniform in many climatic characteristics and where a single set of factors, such as temperature and length of the growing season, varies gradually and continuously, continuous or clinal ecotypic variation will be particularly prevalent. On the other hand, diversity and discontinuity of the available habitats will promote the differentiation of more distinct, easily recognizable groups of biotypes within the species, and therefore distinct ecotypes' (Stebbins, 1950: 47).

Stebbins concluded that: 'clines and ecotypes are not mutually exclusive concepts, but merely express different ways of approaching the same problem' (Stebbins, 1950: 48). However, three decades later, Stebbins expressed deep doubts about ecotypes: 'as Mayr has commented, Turesson was definitely a typologist' and 'where the gradients were continuous he [Langlet] had continuous clines, and where the gradients were abrupt, as between central Sweden and Lapland, he had sudden change. Langlet's careful, more complete survey showed that Turesson was incorrect' (Stebbins, 1980: 141). Stebbins makes no caveat about wind pollination promoting clinal instead of ecotypic variation here as he did in 1950. Similarly, Stebbins criticized the Carnegie group: 'Clausen found clines that he would not recognize because he saw discontinuities in them' (Stebbins, 1980: 143). Here, Stebbins failed to recognize that clines describe single characters, whereas ecotypes reflect multidimensional trait variation across space as Clausen had argued: 'The term cline can be used only for individual characters and not for an assemblage of characters of group' and 'clines are therefore not commensurate with natural entities, and are oversimplified abstractions' (Clausen, 1951: 28).

Stebbins' rejection of intermediates in the stages of speciation may have had considerable influence on

how others perceived the views of botanists because he was the primary spokesperson of plant evolution biologists for much of the 20th Century. It is thus unfortunate that neither Turesson, nor Clausen were still alive to respond to Stebbins in the 1980s. Both Keck and Hiesey far out lived both of them and their views were unwavering. In a set of interviews conducted by Joel Hagen in 1981, they made their support clear:

Keck: 'Turesson did great work. He was a keen observer with good imagination. His ecotype, ecospecies, cenospecies distinctions were indeed valid and extremely helpful. They could be applied in nature to genus after genus'

Hiesey: 'Perhaps the greatest impact of Experimental Taxonomy on orthodox taxonomy in the 1930s and 1940s was (1) to bring an increasing awareness of the importance of variation within species in the description and delimitation of species and (2) a realization of the significance of cytology and genetics in throwing light on species relationships'

At the opposite extreme from Langlet and McMillan, another set of researchers discovered adaptation of plants to extremely local edaphic conditions, such as mine-tailings (Jain & Bradshaw, 1966; Antonovics & Bradshaw, 1970; Snaydon, 1970) and serpentine outcrops (Kruckeberg, 1951). Kruckeberg (1951) found evidence for discrete serpentine-adapted ecotypes of *Achillea* within the range of ecological races previously documented by Clausen *et al.* (1948). Given this result, Kruckeberg argued: 'In light of the case of *Achillea borealis* where edaphic races appear to be superimposed upon climatic races . . . ecotype seems appropriate only when a single environmental factor is under scrutiny' (Kruckeberg, 1951: 415) because analysis under multiple environmental conditions 'would render the term "ecotype" synonymous with either a local population or a small segment of a population' (Kruckeberg, 1951: 416). He concludes: 'Natural populations might best be visualized as consisting of a continuous or discontinuous array of ecotypic variation in response to the sum total of environmental factors in an area' (Kruckeberg, 1951: 416). Although sets of serpentine and mine adapted populations are ecotypes in the sense that they share composite of many similar traits (heavy metal tolerance, drought tolerance, flowering time, etc.), they do not negate the more regionally widespread ecotypes within which they occur.

GENE FLOW AND THE COHESIVENESS OF SPECIES

Given the mounting evidence that functional genetic variation within plant species could be distributed continuously or extremely discretely, multiple reviews (Heywood, 1959; Langlet, 1963, 1971; Quinn, 1978) dismissed the utility of the term ecotype. Quinn's

(1978) main contention with ecotypes was rooted in his disbelief that widespread ecotypes could ever form because: (1) Quinn believed that near uniform environments were necessary for ecotype formation and argued that such environments are never geographically widespread and (2) gene flow is too low among plant populations to maintain the cohesiveness of widespread ecotypes or species. The second argument is at least partially rooted in the views of Ehrlich & Raven (1969), who, upon reviewing the data showing patterns of restricted gene flow among populations, argued that gene flow was insufficient to hold species together and, thus, the biological species concept itself was flawed. The proliferation of species concepts emerging during that era that followed has also been attributed to Ehrlich & Raven's viewpoints on this issue (Morjan & Rieseberg, 2004).

In response to the arguments that widespread ecotypes or species cannot persist as a result of low levels of cohesive gene flow, Verne Grant reasoned that 'extensive interbreeding within the population system is not an essential property of biological species; non-interbreeding with other population systems is' (Grant, 1981: 91). In other words, it does not make sense to make arguments about what holds a species together when it will continue on as a species unless reproductive isolation breaks it apart. Grant then goes on to write (Grant, 1981: 92), 'Biological species represents a stage in divergence . . . and other stages of uncompleted speciation and secondary refusion of species also exist. Consequently the array of population systems at any given time consists of both biological species and semispecies'. Thus, Grant, similar to Keck and Hiesey, was a holdout supporter of stages in the formation of plant species.

RECENT DISCUSSIONS OF CLINES AND ECOTYPES

During the 1980s and 1990s, there was less discussion in the literature regarding ecotypes and stages in speciation. Briggs & Walters (1997), in the final edition of *Plant Variation and Evolution*, presented a brief summary of Clausen's ideas on stages in the process of speciation, although they did not take any particular stand on validity of Turesson and Clausen ideas. Briggs and Walters nonpartisan treatment of the subject viewed variation as being distributed in different ways depending on the characteristics of a species and geographical features of its range, and noted that 'with hindsight one can see in Turesson's own results the possibility that, in common species, variation patterns were more complex than the ecotype concept implied' (Briggs & Walters, 1997: 190). Linhart & Grant (1996), who conducted the most comprehensive review of local adaptation in the 1990s,

suggested that 'the cline versus ecotype controversy has not proved particularly useful and it has mostly faded' because 'some characters can vary gradually, others discontinuously, depending on, for example, gene flow, intensity of selection, number of genes involved, and terrain configuration' (Linhart & Grant, 1996: 241). It is true that individual traits may vary in different ways but, as mentioned above, ecotypes reflect the composite response of multiple traits to the common selection pressures of ecoregions.

LOCAL SPECIATION AND CHROMOSOMAL REARRANGEMENTS

One of the few plant botanists of the 1990s to take a strong stand regarding the question of intermediate stages in the process of speciation was Donald Levin. Levin (1993, 1995, 2000) argued that species formation occurs almost exclusively at the level of the local population or meta-population (Barrett, 2001; Wilson & Kimball, 2001). His arguments against geographical widespread stages in the formation of species were almost exactly the same as those of Quinn (1978) regarding his doubts about the existence of widespread uniform environmental conditions and that sufficient gene flow could occur within widespread ecotypes to facilitate their conversion to species. Levin's (1993, 2000) viewpoints also have deep roots in peripatric founder effect speciation (Mayr, 1954; Coyne, 1994) and quantum speciation (Lewis, 1962; Grant, 1981).

Levin's argument for local speciation are based on the assumption that underdominant chromosomal rearrangements are the most significant source of reproductive isolation among species and that massive ecogenetic reorganizations occur rapidly in bottlenecked populations to facilitate this process. Furthermore, Levin (1993) argued that widespread ecotypes could not be converted to good species because it would be difficult for underdominant chromosomal rearrangements to spread and complete speciation over wide geographical areas. However, recent studies suggest a more limited role for the involvement of underdominant rearrangements, at least in the early stages of speciation (Rieseberg, 2001; Gottlieb, 2004; Rieseberg & Willis, 2007; Lexer & Widmer, 2008; Lowry *et al.*, 2008a; Bomblies, 2010; Lowry & Willis, 2010; Rieseberg & Blackman, 2010). By contrast to underdominant chromosomal rearrangements, there is mounting evidence that chromosomal rearrangements frequently capture adaptive loci, which facilitate the spread of those rearrangements (Kirkpatrick, 2010). Thus, genome repatterning may at least in part be driven by geographically widespread natural selection, which is the same selection as that responsible for the evolution of widespread

ecotypes. The question that remains is whether natural selection or drift processes are more often responsible for the evolution of genic incompatibilities and chromosomal repatterning that Clausen considered to be so important for preventing the reversal of speciation at later stages in the process.

THE THIRD WAVE: CONTEMPORARY VIEWS ON STAGES IN THE FORMATION OF SPECIES

There are three clear periods in the history of evolutionary biology where researchers were intently interested in defining stages in the formation of species and understanding the role of natural selection in this process. The crux of Darwin's long argument in the *Origin of Species* was that natural selection drives the formation of varieties and that these varieties then evolve into species. Although many have argued over Darwin's true beliefs on speciation (Mayr, 1947; Mallet, 2008a, b, 2009; Schemske, 2010), Wallace (1865) articulated the first known classification scheme for stages in the formation of species (Mallet, 2009). The biosystematists, beginning with Turesson (1922a, b) and peaking with the books by Clausen and Dobzhansky in 1951, represent the second wave of interest in 'stages'. Similar to Darwin and Wallace, the biosystematists were motivated by the role of natural selection in the formation of distinct groups and species. A handful of biologists, such as Grant (1981), carried on the torch of the biosystematists into the 1980s.

We are currently in the third wave of interest in studying stages in the evolution of species (Schluter, 2001; Wu, 2001; Mallet, 2008a, b; Hendry *et al.*, 2009; Nosil *et al.*, 2009; Smadja & Butlin, 2011). This renewal has been brought about by new ideas regarding the role of ecology in speciation (Schluter, 1996, 2009; Rundle *et al.*, 2000; Lexer & Fay, 2005; Rundle & Nosil, 2005; Smadja & Butlin, 2011) and the widespread identification of partially reproductively isolated ecotypes and host races within species (Schluter, 2001; Dres & Mallet, 2002; Rundle & Nosil, 2005; Egan & Funk, 2009). A recent review by Nosil *et al.* (2009) laid out the third wave's argument for studying speciation as a process involving stages: 'Notably, different species concepts can disagree on when speciation starts and when it is complete, while still sharing the characteristic of having stages of divergence' (Nosil *et al.*, 2009: 145–146). The above quote echoes the views of Dobzhansky (1937) from the second wave: 'Species is a stage in a process, not a static unit. This difference is important, for it frees the definition of the logical difficulties inherent in any static one' (Dobzhansky, 1937: 312). Thus, one need

not be concerned with classification when the goal is to understand the process by which speciation occurs.

STAGES AND THE GENIC VIEW OF SPECIATION

Early in the last decade, Chung-I Wu (2001) presented a 'genic view of the process of speciation' where he reframed speciation as the product of the accumulation of genic incompatibilities across the genome over time by natural selection. Interestingly, Wu presented the process of speciation as occurring over four stages, which reflected the gradual build up of regions of the genome that could no longer introgress between diverging races or species (Fig. 2). Similar to Clausen, species formation (Stage III) was not seen as the end of the process because some region of the genome could still introgress between what he called 'good species.' In Wu's view, the process was only completed when two species reached a point where all F_1 hybrids were inviable or sterile (Stage IV), which is the definition that Clausen gave for cenospecies. However, Wu's focus on intrinsic postzygotic isolation led him to argue that reversals in the process were no longer possible after the transition from race (Stage II) to species (Stage III). For Clausen, as well as many modern supporters of the BSC, species are defined by isolation involving any combination of possible barriers, and thus are potentially reversible under the right set of environmental conditions.

REVERSIBILITY OF THE PROCESS

Recent empirical studies have renewed interest in the question of the reversibility of the process of speciation. Species collapses have now been observed in Great Lake ciscoes (Todd & Stedman, 1989), cichlid (Seehausen *et al.*, 2008), and stickleback (Gow, Peichel & Taylor, 2006; Taylor *et al.*, 2006) fishes, as a result of changes to lakes brought about by pollution and the introduction of a foreign species. The reproductive isolating barriers that previously maintained these species were ecological and, without strong postzygotic isolation, the species collapsed as a result of changing environmental conditions, as Clausen argued they might. This realization that ecological barriers are reversible should lead contemporary researchers to focus their attention on how intrinsic postzygotic barriers, both genic and chromosomal, eventually evolve to complete the process of speciation.

THE ROLE OF GENE FLOW IN THE PROCESS

One of the major differences between stages of the third wave and the focus of Clausen and Grant is the emphasis of the third wave on speciation with gene

flow: “divergence-with-gene-flow” or “speciation-with-gene-flow” has been spreading in the literature, reflecting interest in a more continuous vision of speciation in time and space’ (Smadja & Butlin, 2011: 5123). However, there is no logical reason that a continuous view on speciation necessarily reflects a sympatric mode of speciation. A majority of the divergent ecological races studied by Clausen, Keck, and Hiesey were geographically isolated. Based on his experiments, Clausen’s (1951) considered that ecological races would collapse in sympatry unless strong

reproductive isolating barriers were already in place. Douglas Schemske, a modern leader in plant speciation, has argued that the strongest barrier to gene flow between diverging plant species is likely ecogeographical isolation (Schemske, 2000, 2010). Ecogeographical isolation is defined as ‘heritable differences in the geographical range of populations or species due to local adaptation’ (Schemske, 2010: S15). A number of recent studies have found that ecogeographical isolation is common and often a very strong barrier to gene flow (Ramsey, Bradshaw & Schemske,

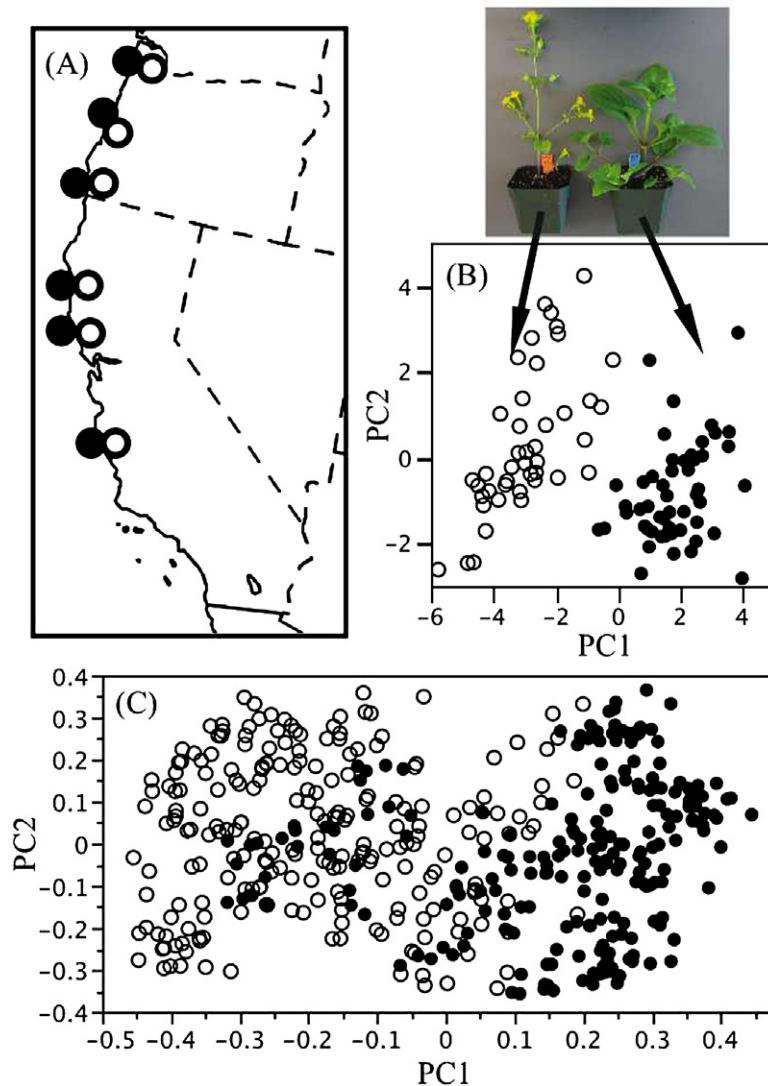


Figure 3. A test of the hypothesis of widespread coastal and inland ecotypes in *Mimulus guttatus* by Lowry *et al.* (2008b). A, pairs of nearby coastal (closed circle) and inland (open circle) populations were collected over 700 km along the Pacific Coast of North America. B, individuals from coastal and inland populations collectively clustered in a principle components (PC) analysis as two groups based on trait data in a common garden experiment ($N = 14$ traits, 94 individuals from six pairs of coastal/inland populations). C, population genetic analysis found evidence suggesting that genomewide divergence is underway between the widespread coastal and inland ecotypes ($N = 10$ microsatellite loci, 463 individuals from 14 coastal and 14 inland populations).

2003; Husband & Sabara, 2004; Kay, 2006; Lowry, Rockwood & Willis, 2008b; Sobel *et al.*, 2010; Glennon, Rissler & Church, 2011). Ecogeographical isolation could even facilitate the evolution of irreversible postzygotic isolation through non-ecological mechanisms if it acts to enforce the allopatry of diverging ecotypes and species. Thus, at least for plants, speciation-with-gene-flow may be far less important than regional ecotype formation that leads to heritable geographical isolation.

THE FUTURE OF RESEARCH ON STAGES

Beyond renewed interest that has focused on the role of ecology, the recent expansion of genetic and genomic tools to numerous systems coupled with new methodologies to analyze geographical patterns of population structure (Pritchard, Stephens & Donnelly, 2000; Dyer & Nason, 2004; Novembre & Stephens, 2008; Novembre *et al.*, 2008; Novembre & Di Rienzo, 2009) has revived interest in understanding how genetic variation is partitioned within plant species (Nordborg *et al.*, 2005; Song *et al.*, 2009). The increase in molecular capacity led Baldwin to conclude: 'Although infeasible during Clausen's life, resolving "ecological races" that correspond to natural groups worthy of taxonomic recognition is now possible and desirable' (Baldwin, 2006: 87). Researchers can now readily use population structure analyses as well as principle components analysis of trait variation to detect ecotypes within species. Recently, Lowry *et al.* (2008b) used these methodologies, in conjunction with reciprocal transplant experiments, to show that coast and inland populations of *Mimulus guttatus* constitute widespread regional ecotypes that are in the process of genome-wide divergence as a result of strong ecogeographical isolation (Fig. 3).

Given all the predictions of its demise (Heywood, 1959; Langlet, 1971; Quinn, 1978), the term ecotype persists in the literature. The persistence of the term reflects the reality that there are groups of populations that occupy various ecoregions and share a common suite of morphological and physiological characters. Ecotypes are thus represented best by the distribution of the principle components of adaptive variation over many traits across the natural landscape. Importantly, confirmation of ecotypes requires reciprocal transplant experiments to link their establishment with local adaptation. Clines in individual traits will often vary independently of the distribution of ecotypes and can even occur within ecotypes. However, individual clines are far less important than the combination of all traits because 'the fitness of a particular plant depends not so much upon a single character as upon a combination of several' (Clausen, 1951: 52). Furthermore, ecotypes should not

be viewed as distinct static groups with clearly discernable boundaries; Clausen and Turesson certainly did not. Rather, ecotypes represent the nonrandom organization of genetic variation across the landscape at one cross-section of time along the continuum of the speciation process.

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REFERENCES

- Ahmad I, Wainwright SJ. 1976.** Ecotype differences in leaf surface properties of *Agrostis stolonifera* from salt marsh, spray zone and inland habitats. *New Phytologist* **76**: 361–366.
- Akeroyd JR, Briggs D. 1983.** Genecological studies of *Rumex crispus* L. II. Variation in plants grown from wild-collected seed. *New Phytologist* **94**: 325–343.
- Antonovics J, Bradshaw AD. 1970.** Evolution in closely adjacent plant populations. VIII. Clinal patterns at a mine boundary. *Heredity* **25**: 349–362.
- Baker HG. 1957.** Genecological studies in *Geranium* (Section Robertiana). General considerations and the races of *G. Purpureum* Vill. *New Phytologist* **56**: 172–192.
- Baldwin BG. 2006.** Contrasting patterns and processes of evolutionary change in the tarweed-silversword lineage: revisiting Clausen, Keck, and Hiesey's findings. *Annals of the Missouri Botanical Garden* **93**: 64–93.
- Barrett SCH. 2001.** The life and times of plant species: from metapopulations to mutational meltdown. *Evolution* **55**: 641–646.
- Baum DA. 2009.** Species as ranked taxa. *Systematic Biology* **58**: 74–86.
- Bomblies K. 2010.** Doomed lovers: mechanisms of isolation and incompatibility in plants. *Annual Review of Plant Biology* **61**: 109–124.
- Briggs D, Walters SM. 1997.** *Plant variation and evolution*, 3rd edn. Cambridge: Cambridge University Press.
- Casler MD. 2005.** Ecotypic variation among switchgrass populations from the northern USA. *Crop Science* **45**: 388–398.
- Chung C. 2003.** On the origin of the typological/population

- distinction in Ernst Mayr's changing views of species, 1942–1959. *Studies in the History and Philosophy of Biological and Biomedical Sciences* **34**: 277–296.
- Clausen J, Hiesey WM. 1958.** *Experimental studies on the nature of species. IV. Genetic structure of ecological races.* Washington, DC: Carnegie Institution of Washington.
- Clausen J. 1951.** *Stages in the evolution of plant species.* Ithaca, NY: Cornell University Press.
- Clausen J. 1958.** Discussion of E. Mayr: the evolutionary significance of systematic categories. In: Hedberg O, ed. *Systematics today.* Uppsala, Sweden: Lundequistska Bokhandeln, 20.
- Clausen J, Keck DD, Hiesey WH. 1940.** *Experimental studies on the nature of species I. Effects of varied environments on western North American plants.* Washington, DC: Carnegie Institution of Washington.
- Clausen J, Keck DD, Hiesey WM. 1939.** The concepts of species based on experimentation. *American Journal of Botany* **26**: 103–106.
- Clausen J, Keck DD, Hiesey WM. 1947.** Heredity of geographically and ecologically isolated races. *American Naturalist* **81**: 114–133.
- Clausen J, Keck DD, Hiesey WM. 1948.** *Experimental studies on the nature of species. III. Environmental response of climatic races of Achillea.* Washington, DC: Carnegie Institution of Washington.
- Coyne JA. 1994.** Ernst Mayr and the origin of species. *Evolution* **48**: 19–30.
- Coyne JA, Orr HA. 2004.** *Speciation.* Sunderland, MA: Sinauer Associates, Inc.
- Danser BH. 1929.** Ueber die begriffe komparium, kommissuum und konvivium und uber die entstehungsweise der Konvivien. *Genetica* **11**: 399–450.
- Darwin C. 1859.** *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life.* London: John Murray.
- Dobzhansky T. 1935.** A critique of the species concept in biology. *Philosophy of Science* **2**: 344–355.
- Dobzhansky T. 1937.** *Genetics and the origin of species.* New York, NY: Columbia University Press.
- Dobzhansky T. 1940.** Speciation as a stage in evolutionary divergence. *The American Naturalist* **74**: 312–321.
- Dobzhansky T. 1951.** *Genetics and the origin of species*, 3rd edn. New York, NY: Columbia University Press.
- Dres M, Mallet J. 2002.** Host races in plant-feeding insects and their importance in sympatric speciation. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **357**: 471–492.
- Dyer RJ, Nason JD. 2004.** Population graphs: the graph theoretic shape of genetic structure. *Molecular Ecology* **13**: 1713–1727.
- Egan SP, Funk DJ. 2009.** Ecologically dependent postmating isolation between sympatric 'host forms' of *Neochlamisus bebbianae* leaf beetles. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 19426–19431.
- Ehrlich PR, Raven PH. 1969.** Differentiation of populations. *Science* **165**: 1228–1232.
- Faegri K. 1937.** Some fundamental problems of taxonomy and phylogenetics. *Botanical Review* **3**: 400–423.
- Fitzpatrick BM, Fordyce JA, Gaverilets S. 2009.** Pattern, process, and geographic modes of speciation. *Journal of Evolutionary Biology* **22**: 2342–2347.
- Glennon K, Rissler L, Church S. 2011.** Ecogeographic isolation: a reproductive barrier between species and between cytotypes in *Houstonia* (Rubiaceae). *Evolutionary Ecology* DOI 10.1007/s10682-011-9539-x.
- Goodwin RH. 1937a.** The cytogenetics of two species of *Solidago* and its bearing on their polymorphy in nature. *American Journal of Botany* **24**: 425–432.
- Goodwin RH. 1937b.** The role of auxin in leaf development in *Solidago* species. *American Journal of Botany* **24**: 42–51.
- Goodwin RH. 1937c.** Notes on the distribution and hybrid origin of *X Solidago asperula*. *Rhodora* **38**: 43–51.
- Gottlieb LD. 2004.** Rethinking classic examples of recent speciation in plants. *New Phytologist* **161**: 71–82.
- Gow JL, Peichel CL, Taylor EB. 2006.** Contrasting hybridization rates between sympatric three-spined sticklebacks highlight the fragility of reproductive barriers between evolutionarily young species. *Molecular Ecology* **15**: 739–752.
- Grant V. 1950.** Genetics and taxonomic studies in *Gilia*. I. *Gilia capitata*. *El Aliso* **2**: 239–316.
- Grant V. 1952.** Genetic and taxonomic studies in *Gilia*. II. *Gilia capitata abrotanifolia*. *El Aliso* **2**: 361–373.
- Grant V. 1981.** *Plant speciation*, 2nd edn. New York, NY: Columbia University Press.
- Gregor JW. 1930.** Experiments on the genetics of wild populations. I. *Plantago maritima*. *Journal of Genetics* **22**: 15–25.
- Gregor JW. 1931.** Experimental delimitation of species. *New Phytologist* **30**: 204–217.
- Gregor JW. 1938.** Experimental taxonomy II: initial population differentiation in *Plantago maritima* L. of Britain. *New Phytologist* **37**: 15–49.
- Gregor JW. 1939.** Experimental taxonomy IV. Population differentiation in North American and European sea plantains allied to *Plantago maritima* L. *New Phytologist* **38**: 293–322.
- Gregor JW, McM. Davey V, Lang JMS. 1936.** Experimental taxonomy I. Experimental garden technique in relation to the recognition of the small taxonomic units. *New Phytologist* **35**: 323–350.
- Hagen JB. 1983.** The development of experimental methods in plant taxonomy, 1920–1950. *Taxon* **32**: 406–416.
- Hagen JB. 1984.** Experimentalists and naturalists in twentieth-century botany: experimental taxonomy, 1920–1950. *Journal of the History of Biology* **17**: 249–270.
- Hagen JB. 1986.** Ecologists and taxonomists: divergent traditions in twentieth-century plant geography. *Journal of the History of Biology* **19**: 197–214.
- Hall MC, Willis JH. 2006.** Divergent selection on flowering time contributes to local adaptation in *Mimulus guttatus* populations. *Evolution* **60**: 2466–2477.
- Hendry AP, Bolnick DI, Berner D, Peichel CL. 2009.** Along the speciation continuum in sticklebacks. *Journal of Fish Biology* **75**: 2000–2036.

- Heywood VH. 1959.** The taxonomic treatment of ecotypic variation. *Systematics Association* **3**: 87–112.
- Hiesey WH. 1940.** Environmental influence and transplant experiments. *Botanical Review* **6**: 181–203.
- Hiesey WM, Nobs MA, Bjorkman O. 1971.** *Experimental studies on the nature of species. V. Biosystematics, genetics, and physiological ecology of the Erythranthe section of Mimulus*. Washington, DC: Carnegie Institution of Washington.
- Husband BC, Sabara HA. 2004.** Reproductive isolation between autotetraploids and their diploid progenitors in fireweed, *Chamerion angustifolium* (Onagraceae). *New Phytologist* **161**: 703–713.
- Huxley JS. 1938.** Clines: an auxiliary taxonomic principle. *Nature* **142**: 219–220.
- Jain SK, Bradshaw AD. 1966.** Evolutionary divergence among adjacent plant populations. I. The evidence and its theoretical analysis. *Heredity* **21**: 407–441.
- Kay KM. 2006.** Reproductive isolation between two closely related hummingbird-pollinated neotropical gingers. *Evolution* **60**: 538–552.
- Kirkpatrick M. 2010.** How and why chromosomal inversions evolve. *PLoS Biology* **8**: e1000501.
- Kruckeberg AR. 1951.** Intraspecific variability in the response of certain native species to serpentine soil. *American Journal of Botany* **38**: 408–419.
- Langlet O. 1936.** Studier over tallens fysiologiska variabilitet och dess samband med klimatet. Ett bidrag till kannedomen om tallens ekotyper. *Meddel Statens Skogsforsoksanst* **29**: 219–470.
- Langlet O. 1963.** Patterns and terms of intraspecific ecological variability. *Nature* **200**: 347–348.
- Langlet O. 1971.** Two hundred years of geneecology. *Taxon* **20**: 653–722.
- Levin DA. 1993.** Local speciation in plants: the rule not the exception. *Systematics Biology* **18**: 197–208.
- Levin DA. 1995.** Metapopulations: an arena for local speciation. *Journal of Evolution Biology* **8**: 635–644.
- Levin DA. 2000.** *The origin expansion and demise of plant species*. Oxford: Oxford University Press.
- Lewis H. 1962.** Catastrophic selection as a factor in speciation. *Evolution* **16**: 257–271.
- Lexer C, Fay MF. 2005.** Adaptation to environmental stress: a rare or frequent driver of speciation? *Journal of Evolutionary Biology* **18**: 893–900.
- Lexer C, Widmer A. 2008.** The genic view of plant speciation – recent progress and emerging questions. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **363**: 3023–3036.
- Linhart YB, Grant MC. 1996.** Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology, Evolution, and Systematics* **27**: 237–277.
- Lowry DB, Modliszewski JL, Wright KM, Wu CA, Willis JH. 2008a.** The strength and genetic basis of reproductive isolating barriers in flowering plants. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **363**: 3009–3021.
- Lowry DB, Rockwood RC, Willis JH. 2008b.** Ecological reproductive isolation of coast and inland races of *Mimulus guttatus*. *Evolution* **62**: 2196–2214.
- Lowry DB, Willis JH. 2010.** A widespread chromosomal inversion polymorphism contributes to a major life-history transition, local adaptation, and reproductive isolation. *PLoS Biology* **8**: e1000500.
- Mallet J. 2008a.** Hybridization, ecological races and the nature of species: empirical evidence for the ease of speciation. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **363**: 2971–2986.
- Mallet J. 2008b.** Mayr's view of Darwin: was Darwin wrong about speciation? *Biological Journal of the Linnean Society* **95**: 3–16.
- Mallet J. 2009.** Alfred Russel Wallace and the Darwinian species concept: his paper on the swallowtail butterflies (Papilionidae) of 1865. *Gayana* **73**: 35–47.
- Mallet J, Beltran M, Neukirchen W, Linares M. 2007.** Natural hybridization in heliconiine butterflies: the species boundary as a continuum. *BMC Evolutionary Biology* **7**: 28.
- Marsden-Jones EM, Turrill WB. 1928.** Researches on *Silene maritima* and *S. vulgaris*. I. *Kew Bulletin* **1**: 1–17.
- Mayr E. 1942.** *Systematics and the origin of species*. Ithaca, NY: Columbia University Press.
- Mayr E. 1947.** Ecological factors in speciation. *Evolution* **1**: 263–288.
- Mayr E. 1954.** Change of genetic environment and evolution. In: Huxley J, Hardy AC, Ford EB, eds. *Evolution as a process*. London: Allen and Unwin, 157–180.
- Mayr E. 1958.** The evolutionary significance of systematic categories. In: Hedberg O, ed. *Systematics today*. Uppsala: Lundequistska Bokhandeln, 1–20.
- Mayr E. 1982.** *The Growth of Biological Thought*. Cambridge, MA: Harvard University Press.
- McMillan C. 1959.** The role of ecotypic variation in the distribution of the central grasslands of North America. *Ecological Monographs* **29**: 285–308.
- McMillan C. 1965.** Ecotypic differentiation within 4 North American Prairie grasses. II. Behavioral variation within transplanted community fractions. *American Journal of Botany* **52**: 55–65.
- McMillan C. 1967.** Phenological variation within seven transplanted grassland community fractions from Texas and New Mexico. *Ecology* **48**: 807–813.
- McMillan C. 1969.** Ecotypes and ecosystem function. *Bio-science* **19**: 131–134.
- Morjan CL, Rieseberg LH. 2004.** How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. *Molecular Ecology* **13**: 1341–1356.
- Nagy ES, Rice KJ. 1997.** Local adaptation in two subspecies of an annual plant: implications for migration and gene flow. *Evolution* **51**: 1079–1089.
- Nordborg M, Hu TT, Ishino Y, Jhaveri J, Toomajian C, Zheng HG, Bakker E, Calabrese P, Gladstone J, Goyal R, Jakobsson M, Kim S, Morozov Y, Padhukasahasram B, Plagnol V, Rosenberg NA, Shah C, Wall JD, Wang J, Zhao KY, Kalbfleisch T, Schulz V, Kreitman M, Bergelson J. 2005.** The pattern of polymorphism in *Arabidopsis thaliana*. *PLoS Biology* **3**: 1289–1299.

- Nosil P, Harmon LJ, Seehausen O. 2009. Ecological explanations for (incomplete) speciation. *Trends in Ecology and Evolution* **24**: 145–156.
- Novembre J, Di Rienzo A. 2009. Spatial patterns of variation due to natural selection in humans. *Nature Reviews Genetics* **10**: 745–755.
- Novembre J, Johnson T, Bryc K, Kutalik Z, Boyko AR, Auton A, Indap A, King KS, Bergmann S, Nelson MR, Stephens M, Bustamante CD. 2008. Genes mirror geography within Europe. *Nature* **456**: 274–274.
- Novembre J, Stephens M. 2008. Interpreting principal component analyses of spatial population genetic variation. *Nature Genetics* **40**: 646–649.
- Núñez-Farfán J, Schlichting CD. 2001. Evolution in changing environments: the ‘synthetic’ work of Clausen, Keck, and Hiesey. *Quarterly Review of Biology* **76**: 433–457.
- Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.
- de Queiroz K. 2005. Ernst Mayr and the modern concept of species. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 6600–6607.
- Quinn JA. 1978. Plant ecotypes: ecological or evolutionary units? *Bulletin of the Torrey Botanical Club* **105**: 58–64.
- Ramsey J, Bradshaw HD, Schemske DW. 2003. Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution* **57**: 1520–1534.
- Ramsey J, Schemske DW. 1998. Pathways, mechanisms and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* **29**: 477–501.
- Ramsey J, Schemske DW. 2002. Neopolyploidy in flowering plants. *Annual Review of Ecology, Evolution, and Systematics* **33**: 589–639.
- Raven PH. 1976. Systematics and plant population biology. *Systematic Biology* **1**: 284–316.
- Rieseberg LH. 2001. Chromosomal rearrangements and speciation. *Trends in Ecology & Evolution* **16**: 351–358.
- Rieseberg LH, Blackman BK. 2010. Speciation genes in plants. *Annals of Botany* **106**: 439–455.
- Rieseberg LH, Willis JH. 2007. Plant speciation. *Science* **317**: 910–914.
- Rundle HD, Nagel L, Boughman JW, Schluter D. 2000. Natural selection and parallel speciation in sympatric sticklebacks. *Science* **287**: 306–308.
- Rundle HD, Nosil P. 2005. Ecological speciation. *Ecology Letters* **8**: 336–352.
- Schemske DW. 2000. Understanding the origin of species. *Evolution* **54**: 1069–1073.
- Schemske DW. 2010. Adaptation and the origin of species. *American Naturalist* **176**: S4–S25.
- Schluter D. 1996. Ecological speciation in postglacial fishes. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **351**: 807–814.
- Schluter D. 2001. Ecology and the origin of species. *Trends in Ecology & Evolution* **16**: 372–380.
- Schluter D. 2009. Evidence for ecological speciation and its alternative. *Science* **323**: 737–741.
- Seehausen O, Takimoto G, Roy D, Jokela J. 2008. Speciation reversal and biodiversity dynamics with hybridization in changing environments. *Molecular Ecology* **17**: 30–44.
- Smadja CM, Butlin RK. 2011. A framework for comparing processes of speciation in the presence of gene flow. *Molecular Ecology* **20**: 5123–5140.
- Smocovitis VB. 2006. Keeping up with Dobzhansky: G. Ledyard Stebbins, Jr., plant evolution, and the evolutionary synthesis. *History and Philosophy of the Life Sciences* **28**: 9–47.
- Snaydon RW. 1970. Rapid population differentiation in a mosaic environment. I. The response of *Anthoxanthum odoratum* populations to soils. *Evolution* **24**: 257–269.
- Sobel JM, Chen GF, Watt LR, Schemske DW. 2010. The biology of speciation. *Evolution* **64**: 295–315.
- Song BH, Windsor AJ, Schmid KJ, Ramos-Onsins S, Schranz ME, Heidel AJ, Mitchell-Olds T. 2009. Multilocus patterns of nucleotide diversity, population structure and linkage disequilibrium in *Boechera stricta*, a wild relative of *Arabidopsis*. *Genetics* **181**: 1021–1033.
- Stebbins GL. 1950. *Variation and evolution in plants*. New York, NY: Columbia University Press.
- Stebbins GL. 1980. Botany and the synthetic theory of evolution. In: Mayr E, Provine WB, eds. 139–152. in edited by *Evolutionary synthesis: perspectives on the unification of biology*. Cambridge, MA: Harvard University Press.
- Taylor EB, Boughman JW, Groenenboom M, Sniatynski M, Schluter D, Gow J. 2006. Speciation in reverse: morphological and genetic evidence of the collapse of a stickleback species pair (*Gasterosteus*). *Molecular Ecology* **15**: 343–355.
- Todd TN, Stedman RM. 1989. Hybridization of ciscoes (*Coregonus spp.*) in Lake Huron. *Canadian Journal of Zoology* **67**: 1679–1685.
- Turesson G. 1922a. The species and the variety as ecological units. *Hereditas* **3**: 100–113.
- Turesson G. 1922b. The genotypic response of the plant species to habitat. *Hereditas* **3**: 211–350.
- Turesson G. 1923. The scope and import of genecology. *Hereditas* **4**: 171–176.
- Turesson G. 1925. The plant species in relation to habitat and climate. *Hereditas* **6**: 147–236.
- Turesson G. 1929. Ecotypical selection in siberian *Dactylis Glomerata* L. *Hereditas* **12**: 335–351.
- Turesson G. 1931. The geographical distribution of the alpine ecotype of some Eurasiatic plants. *Hereditas* **15**: 329–346.
- Turrill WB. 1946. The ecotype concept. *New Phytologist* **45**: 34–43.
- Verhoeven KJF, Poorter H, Nevo E, Biere A. 2008. Habitat-specific natural selection at a flowering-time QTL is a main driver of local adaptation in two wild barley populations. *Molecular Ecology* **17**: 3416–3424.
- Waisel Y. 1959. Ecotypic variation in *Nigella arvensis* L. *Evolution* **13**: 469–475.
- Wallace AR. 1865. On the phenomena of variation and geographic distribution as illustrated by the Papilionidae of

Malayan region. *Transactions of the Linnean Society of London* **25**: 1–71.

Wilson P, Kimball S. 2001. Review: the origin, expansion, and demise of plant species. *Quarterly Reviews of Biology* **76**: 84–86.

Wu C.-I. 2001. The genic view of the process of speciation. *Journal of Evolutionary Biology* **14**: 851–865. doi: 10.1046/j.1420-9101.2001.00335.x

Zhang Y, Zalapa JE, Jakubowski AR, Price DL, Acharya A, Wei Y, Brummer EC, Kaeppler SM, Casler MD. 2011. Post-glacial evolution of *Panicum virgatum*: centers of diversity and gene pools revealed by SSR markers and cpDNA sequences. *Genetica* **139**: 933–948.

APPENDIX OF TERMS

Biosystematics: An approach to classifying diversity using a range of different methodologies, including experimentation, genetics, cytology, biochemistry, natural history, geographical distributions, and ecological function. This is opposed to classical taxonomy that relies most heavily on morphology.

Cline: The change in a trait or allele frequencies at a single locus over space. Originally coined by Huxley in 1938.

Cenospecies: Defined by Clausen as a complex of interrelated species (i.e. species complex) that will only form sterile F_1 hybrids when crossed to related cenospecies. Originally coined by Turesson.

Comparia: Defined by Clausen as a distinct evolutionary lineages that can no longer form any hybrids with related comparia. Originally coined by Danser.

Ecotype: An intraspecific group of individuals that are partially reproductively isolated from other groups as a result of barriers that have evolved in response to adaptation to local environmental conditions. Definition has changed many times since being coined by Turesson in 1922. Contemporary usage of the term is inconsistent.

Ecological race: Often used synonymously with ecotype, although generally more geographically widespread.

Ecospecies: A distinct species that is distinguished from related species by adaptations to a set of environmental conditions. Each ecospecies is thought to contain many ecotypes. Originally coined by Turesson in 1922.

Essentialism: The idea originally conceived by Aristotle that every object contains a particular essence, with a set of attributes that makes it distinct from objects containing different essences. Before Darwin, species were thought to be distinct because they contained the particular essence of that species.

Experimental taxonomy: The idea that experimentation should be used to classify organisms as opposed to classification based on morphology.

Genecology: The study of the distribution of natural genetic variation in relation to different features of the natural landscape through field experimentation and breeding studies. Originally coined by Turesson in 1923.

Local speciation: The idea that speciation is initiated and completed in a small geographical region (population or metapopulation) as opposed to the gradual evolution of widespread geographical races into species. Coined by Levin in 1993.

Typologist: A person who views the organization of biological groups, such as species or ecotypes as being invariant, and thus, that there is no graduation between types or species. Mayr saw typologists as essentialist in their thinking and in opposition to population thinking.

Quantum speciation: The idea that speciation can rapidly occur in a small geographical area as a result of drift and founder effects. This form of speciation is essentially peripatric speciation and similar to local speciation.