Genomic insights into the origin of ecotypes

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Abstract

A century ago, Göte Turesson introduced the ecotype concept to describe populations of species that are phenotypically and genetically differentiated by adaptation to contrasting habitats. His simple idea—that ecological divergence can occur below the species level—has had lasting influence, inspiring experimental tests of local adaptation across taxa. Today, ecotypes are described throughout the tree of life and even beyond biology, but their origin and nature remain debated. Genomics has brought new life to Turesson's concept, revealing variable levels of divergence, complex demographic histories, and a prominent role for pre-existing variation, chromosomal inversions, and gene regulation. These findings refine our understanding of ecotypes and raise new questions about predictability, parallelism, genomic architecture, plasticity and their role in speciation.

Pioneering studies of ecological divergence and the birth of a new concept

In 1922, Swedish botanist Göte Turesson conducted pioneering field and greenhouse experiments on wild plants [1]. He demonstrated that populations often classified as species were in fact interfertile yet displayed striking phenotypic differences when occupying contrasting habitats. These differences persisted in **common garden** (see Glossary) experiments, demonstrating a genetic basis, and were repeatedly observed across similar environmental transitions, suggesting that natural selection was driving divergence.

From these findings, Turesson coined the term ***ecotype***, defining it as "the ecological unit arising as a result of the genotypical response of a species to a particular habitat" [1] (See glossary for our modern take). His ideas and methods quickly gained traction among plant biologists, inspiring ambitious common garden studies in diverse taxa [2]. Over the last century, the concept has become widely adopted, with ecotypes now described from across the tree of life (Fig. 1) and invoked beyond evolutionary biology, from agriculture to cancer research (Box 1).

Since the 1980s, molecular markers have increasingly shaped ecotype research, and recent access to genomic data has caused a step-change in our understanding how and why ecotypes form. Here, we review these insights, reflect on criteria for diagnosing ecotypes in light of Turesson's definition, and highlight exciting new research directions.

What are ecotypes and how can we identify them?

Although Turesson's definition of the ecotype is straightforward, it can be challenging to apply. Difficulties arise both from the inherent complexities of studying wild populations and the broader conceptual debates about how to delimit units of biological diversity. Here, we outline five criteria that provide a strong basis for recognising ecotypes.

Criterion 1: Ecotypes are subunits of a species

Ecotypes are ecological subdivisions within species, making them subspecific units alongside varieties, host races, or morphotypes (see Box 1 where we contrast concepts). Turesson emphasized that, unlike species—which are separated by "bridgeless gaps"—ecotypes remain reproductively connected, a point demonstrated by their ability to hybridize [1].

This criterion can be satisfied by demonstrating reproductive continuity, either through **controlled crosses**, or through evidence of interbreeding in natural hybrid zones. For example, Swedish Crab and Wave ecotypes of the intertidal snail *Littorina saxatilis* readily produce fertile hybrids, and intermediates can be observed at contact zones in both morphology and molecular markers [3].

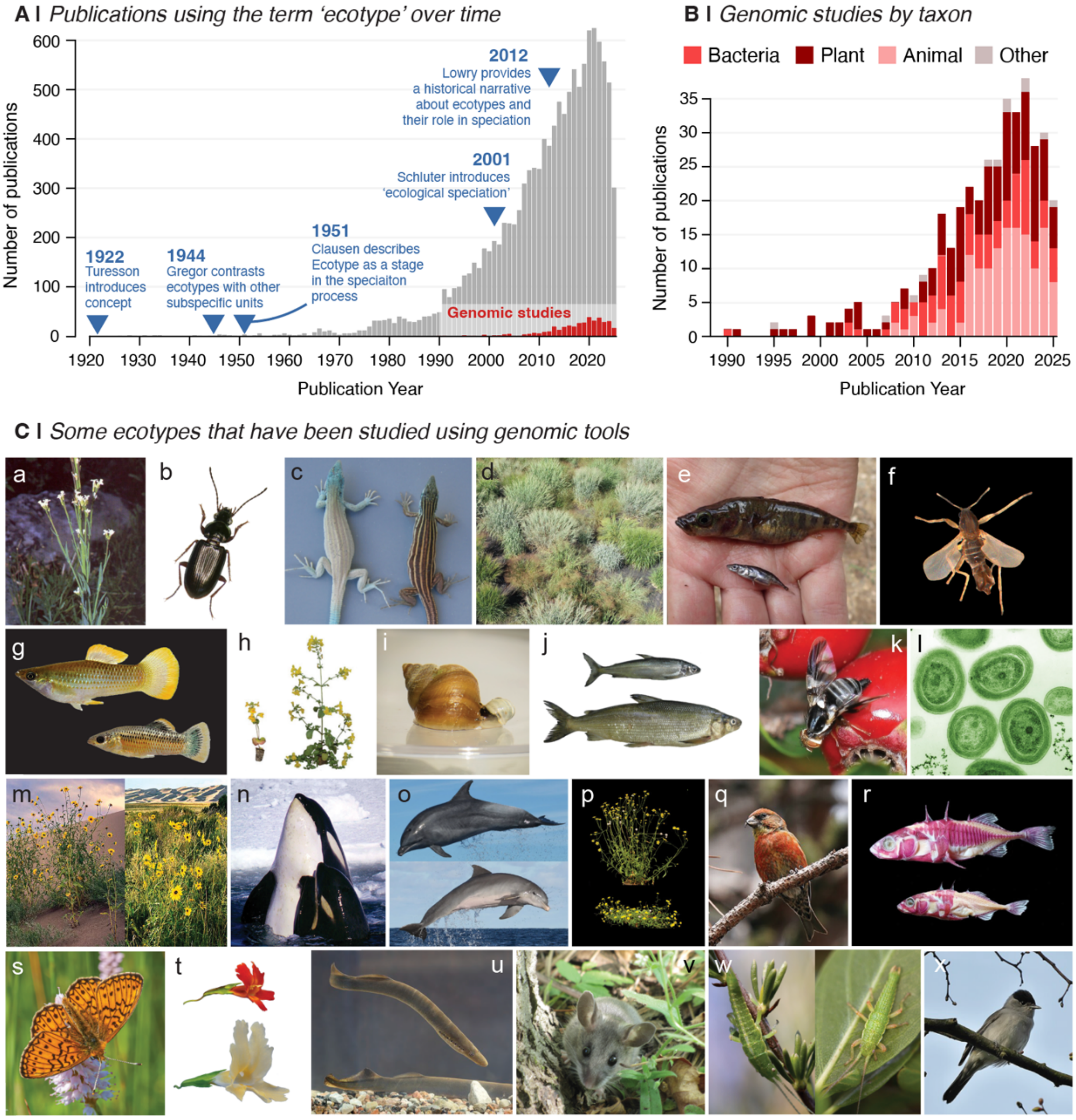


Figure 1. The use of the ecotype concept in the literature and examples of ecotypes that have been studied with genomic tools. (A) Number of papers using the term ‘ecotype’ over time according to Web of Science. The timing of some highly influential reviews is indicated by arrows [1,5,91–93]. (B) Literature search refined to include articles that used the term ‘genomic data’ (also shown as red bars in A). These were screened for accuracy and classified based on the organism studied. (C) Examples of species that show ecotypes and have been studied with genomic tools. More information on these species and ecotype pairs can be found in Table 1. (a) Drummond’s rockcress (*Boechera stricta*; photo: public domain). (b) Saltmarsh beetle (*Pogonus chalceus*; photo: José Muñoz-Santiago, Vicente M. Ortuño, CC BY 4.0). (c) Little striped whiptail (*Aspidoscelis inornata*; photo: Erica Rosenblum). (d) Switchgrass (*Panicum virgatum*; photo: Robert Goodwin). (e) Three-spined stickleback (*Gasterosteus aculeatus*; photo: Ken Thompson). (f) Marine midge (*Clunio marinus*; photo: Tobias Nimpf). (g) Shortfin molly (*Poecilia mexicana*; photo: Michael Tobler). (h) Seep monkeyflower (*Mimulus guttatus*; photo: Alex Twyford). (i) Rough periwinkle (*Littorina saxatilis*; photo: David Carmelet-Rescan). (j) Lake whitefish (*Coregonus clupeaformis*; photo: Louis Bernatchez). (k) Apple maggot fly (*Rhagoletis pomonella*; photo: Phil Huntley-Franck, public domain). (l) Marine cyanobacterium (*Prochlorococcus* sp.; photo: Luke Thompson, CC0 1.0 Universal). (m) Prairie sunflower (*Helianthus petiolaris*; photo: Patrick Myers, public domain). (n) Killer whale (*Orcinus orca*; photo: public domain). (o) Bottlenose dolphin (*Tursiops truncatus*; photo: Laurent Bouveret. (p) Coast groundsel (*Senecio lautus*; photo: Greg Walter). (q) Red crossbill (*Loxia curvirostra*; photo: public domain). (r) Three-spined stickleback (*Gasterosteus aculeatus*; photo: Carl Smith). (s) Bog fritillary (*Boloria eunomia*; photo: Pjt56, CC BY-SA 4.0). (t) Bush monkeyflower (*Mimulus aurantiacus* ssp. *puniceus*; photo: Sean Stankowski). (u) European lamprey (*Lampetra* sp.; photo: Tiit Hunt, CC BY-SA 3.0). (v) Deer mouse (*Peromyscus maniculatus*; photo: Dawn Marsh, CC BY-SA 2.0). (w) Cristina’s timema (*Timema cristinae*; photo: Aaron Comeault). (x) Eurasian blackcap (*Sylvia atricapilla*; photo: CC0 1.0 Universal).

Table 1. Ecological and phenotypic traits distinguishing ecotypes, with evidence for parallel evolution.

Species (Photo ref. in Fig. 1)	Ecotype pair	Ecological differences	Phenotypic differences	Parallelism	Refs
Drummond's rockcress ***Bouchera stricta* (a)**	East / West	Water availability higher in the West	West ecotype has faster growth rate, larger leaf area, less succulent leaves, delayed reproductive time, and longer flowering duration.	—	[50]
Saltmarsh beetle ***Pogonus chalceus* (b)**	Tidal / Seasonal	Tidal salt-marshes are inundated on daily basis for a few hours. Salt-marshes are subject to seasonal inundations lasting several months,	Tidal ecotype has smaller body size, reduced wings and submerges during inundation. Seasonal ecotype disperses upon inundation.	Parallel tidal / seasonal divergence across multiple location in Europe.	[33]
Little striped whiptail ***Aspidoscelis inornate* (c)**	Blanched / Regular	Blanched ecotype camouflaged on white sand dunes; dark ecotype matches adobe soil	Dorsal colouration	Similar colour difference in two other species	[17]
Switchgrass ***Panicum virgatum* (d)**	Upland / Lowland	Upland habitats are drier with colder climates. Lowland areas are wetter and often near rivers and lakes.	Size, growth habit, leaf traits, flowering time, drought tolerance, cold tolerance, root biomass.	—	[83]
Three-spine stickleback ***Gasterosteus aculeatus* (e)**	Benthic / Limnetic	Benthic ecotype feeds predominantly on invertebrates, while limnetic fish feed mostly on zooplankton	Body size, shape, coverage of bony plating, gill raker count, jaw morphology, growth rate, behaviour and territoriality.	Multiple lakes in British Columbia	[11]
Marine midge ***Clunio marinus* (f)**	Full moon / New moon	Low tides suitable for midge reproduction occur at both full and new moon	New moon and full moon ecotypes swarm and mate around the new moon and full moon, respectively.	Different populations show independent evolution of ecotypes.	[13]
Shortfin molly ***Poecilia mexicana* (g)**	Sulfidic / Non-sulfidic	Toxic sulphide springs have lower oxygen and pH, higher salinity, and lower biotic diversity.	Detoxification mechanisms, organ size, metabolic rate, aggression, fecundity, offspring size.	Ecotypes locally evolved throughout western North America.	[84]
Seep monkeyflower ***Mimulus guttatus* (h)**	Annual / Perennial	Annual ecotype grows in habitats that experience summer drought; Perennial ecotype grows in permanently moist sites.	Size, growth habit, time to flower and senescence, potential for clonal spread, flower size.	Local evolution of ecotypes across western North America	[20]
Rough periwinkle ***Littorina saxatilis* (i)**	Crab / Wave	Crab ecotype lives in habitats rich in predatory crabs. Wave ecotype is found on wave-swept rocks.	Wave ecotype has smaller, thinner shells, a larger shell aperture, and bolder behaviour than the Crab ecotype	Across Europe with well-studied examples in Spain, UK and Sweden	[28]
Lake whitefish ***Coregonus clupeaformis* (j)**	Regular / Dwarf	Regular ecotype occupies the benthic zone while Dwarf whitefish occupy the limnetic zone.	Dwarf ecotype is smaller has more gill rakers, slower growth, reduced fecundity, higher metabolic rate and swim more actively.	Multiple lakes in North America	[7]
Apple maggot fly ***Rhagoletis pomonella* (k)**	Apple / Hawthorn	Associated with different host plants that fruit at different times.	Different olfactory preferences for fruit odours. Different eclosion times.	—	[85]
Marine cyanobacterium ***Prochlorococcus sp.* (l)**	Low B/A / High B/A	Low B/A occupies the ocean surface waters with high light. B/A occupies the deep euphotic zone with low light.	Have Chlorophyll b/a$_2$ ratios optimised to different light intensities and wavelength at different depth.	Multiple genetically divergent strains of both ecotypes are known.	[86]
Prairie sunflower ***Helianthus petiolaris* (m)**	Prairie / Dune	Sand dune environments are nutrient poor, have lower water retention, and are less stable.	Dune ecotype has larger seeds, has a shorter time to flower, and use soil nutrients more efficiently.	Independent origins of dune ecotype in Colorado and Texas	[42]
Killer whale ***Orcinus orca* (n)**	Transient / Resident	Sympatric in coastal waters. Resident ecotype feeds on fish while the transient ecotype feeds on mammals.	Subtle differences in colouration and dorsal fin position. Marked differences in acoustic patters and group dynamics	—	[87]
Bottlenose dolphin ***Tursiops truncates* (o)**	Coastal / Pelagic	Coastal ecotype inhabits shallow waters and eats benthic/reef fish. Pelagic ecotype inhabits open water, feeding on squid/pelagic fish.	Pelagic ecotype is more robust, has thicker blubber, smaller flippers. Coastal ecotype forms smaller cohesive groups.	Evidence for multiple origins of coastal ecotype.	[53]
Coast groundsel ***Senecio lautus* (p)**	Dune / Headland	Headlands are rocky and more exposed to wind. Dunes are more protected with deep sandy soil.	Dune ecotype is erect with few branches. Headland ecotype is prostrate with many branches.	Ecotypes maintained at many locations along the Australian coast	[15]
Red crossbill ***Loxia curvirostra* (q)**	Ecotype 5 / ecotype 10	Type 5 specialized to extract seeds from cones of Lodgepole pine. Type 10 feeds on Sitka Spruce.	Different calls, bill size and structure.	—	
Three-spine stickleback ***Gasterosteus aculeatus* (r)**	Marine / Freshwater	Differences in salinity, predation pressure, temperature, food types.	Number of bony plates, pelvic spine and gill raker morphology, head and body shape, body size, schooling behaviour	Many transitions across the northern hemisphere	[35]
Bog fritillary ***Boloria eunomia* (s)**	Meadow / Bog	Meadow ecotype inhabits wet meadows. Bog ecotype inhabits boggy pine forests.	The meadow ecotype has a longer adult lifespan. Larvae prefer different host plants.	—	[88]
Bush monkeyflower ***Mimulus aurantiacus* (t)**	Red / Yellow	Hummingbird and hawkmoth pollinators prefer to visit the Red and Yellow ecotypes, respectively.	Flower colour, flower size, exsertion of stigma.	Evidence for independent origins of the red ecotype.	[52]
European lamprey ***Lampetra* sp. (u)**	Brook / River	Brook ecotype is entirely freshwater while the river ecotype is anadromous. Distributions overlap during mating.	Brook ecotype is smaller and does not feed after metamorphosis. River ecotype is parasitic and has mouthparts for attaching to fish.	—	[29]
Deer mice ***Peromyscus maniculatus* (v)**	Forrest / Prairie	Semiarboreal forest mice occupy dark-soil habitats. Terrestrial prairie mice occupy light substrates.	Forest mice have longer tails, longer hind feet, and darker, redder coats compared with prairie mice.	—	[45]
Cristina's timema ***Timema cristinae* (w)**	Striped / un-striped	*Timema cristinae* occupies two different host plants, *Ceanothus* and *Adenostoma*.	The green-striped morph is more cryptic on the leaves of *Adenostoma*. The un-striped morph is more cryptic on *Ceanothus*.	Stripe has evolved multiple times in *Timema* species that occupy needle leaves	[89]
Eurasian blackcap ***Sylvia atricapilla* (x)**	SW / NW	Both ecotypes breed in central Europe, but SW over-winters in Iberia & northern Africa, while NW migrates to Britain & Ireland.	Ecotypes have genetically controlled migratory differences. SW blackcaps have long-wings, narrow-bills, and return later.	—	[90]

Box 1. Broader uses of the ecotype concept

The term ecotype has been widely adopted beyond evolutionary biology, sometimes in ways that stretch or depart from Turesson's original meaning. In animal agriculture, ecotype is often used for local breeds of livestock, such as chickens or sheep (Fig. IA). Some cases show clear phenotypic and genetic differentiation [80], but others reflect little more than geographic labels, shaped by breeding programmes rather than natural selection. For this reason, we recommend caution in applying the term to modern breeds, though traditional landraces of crops and livestock may more closely resemble true ecotypes.

In cancer research, distinct lineages of tumour cells with unique cellular compositions and clinical outcomes have been labelled as ecotypes [81]. This usage makes sense if one accepts cancer lineages as parts of a 'cellular ecosystem', and these lineages meet several ecotype criteria, including repeated occurrence across patients (Fig. IB). However, gene flow is absent or irrelevant in this context, since cells propagate asexually.

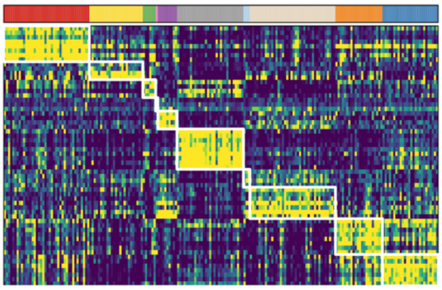
By contrast, using ecotype at the interspecific level is conceptually misleading, as it obscures the idea of ecological divergence maintained within species by selection despite gene flow. Referring to groups of species, such as different bird guilds (e.g., wading birds, swimming birds, climbing birds), as ecotypes should be avoided. However, it is sometimes useful to compare or refer to similar ecotypes across species (e.g. migratory and resident fish in different taxa), provided each remains defined within its own species.

In folklore studies, the term ecotype has been adopted to describe local variations of a folktale, legend, or parable that have been modified to suit different cultural settings (Fig. IC). Much like biological ecotypes, these different stories retain a shared ancestry but have become adapted to local social or ecological conditions [82]. While metaphorical, this use captures the spirit of Turesson's idea, though it is clearly distinct from biological usage.

There are other subspecific categories in biology that we think are synonyms of ecotype. For example, insect host races meet all of our criteria for being ecotypes. Ecomorph is another term that is sometimes used interchangeably with ecotype. Other subspecific categories, like varieties, morphotype, biotype, and subspecies are sometimes used to refer to units that we would recognised as ecotypes, and in other cases not.

A | *Savannah and humid ecotypes of West African Dwarf Goats*

B | *Cell states assigned to carcinoma ecotypes*



C | *Ecotypes of an eastern parable*

Figure I. Uses of the ecotype concept beyond ecology and evolution. (A) West African Dwarf (WAD) goats in Nigeria are distributed widely throughout the northern savannah and southern humid zones of the country (Photo: Samuel N. Chiejina, Jerzy M. Behnke and Barineme B. Fakae; CCBY4.0). (B) In cancer research, tumor 'ecotypes' have been defined as recurrent cellular communities distinguished by their transcriptional states and microenvironmental composition (image courtesy of Andrew Gentles). Depictions of 'ecotypes' of an eastern parable about a group that come across an elephant and who try to understand what it is only by touching it. It some versions the group consists of blind men, and in others, the elephant obscured by the night (images: Public domain).

A threshold between ecotypes and species based on complete **reproductive isolation** (RI) is appealing clear, and we view it as the most useful criterion. Yet biologists differ in how much RI is required to recognize species [4]. For some, strongly reduced interbreeding in the lab, a rarity of hybrids, or the ability to coexist is enough to justify species status, whereas others would treat such cases as ecotypes connected by limited gene flow [5].

Many taxa commonly referred to as species could be viewed as ecotypes under these criteria. Darwin's finches, for example, are named species despite frequent hybridization and ecological divergence [6]. Similarly, studies of whitefish taxa have switched between calling them ecotypes and species [7,8]. These decisions likely reflect the personal preferences that biologists have for different species concepts [9].

Criterion 2: *Ecotypes should inhabit different ecological contexts*
A defining feature of ecotypes is that they occupy distinct habitats, which drives the differentiation of adaptive traits (see Box 2). Turesson's classic examples involved plants inhabiting dunes, woods, and fields [1], but today, a broad range of ecological factors are known to separate ecotypes (Table 1).

Ecotypes can be maintained by ecological differences across a range of spatial and temporal scales. Broad ecological differences are often associated with parapatrically distributed ecotypes separated by narrow hybrid zones, as in the bush monkeyflower [10]. In other cases, like shallow and deepwater ecotypes of fish, habitat differences may involve little spatial separation [11]. Temporal processes can also separate ecotypes, examples being spring-spawning and autumn-spawning ecotypes of herring [12], and lunar ecotypes of *Clunio* midge [13].

Ecological differences are often detected through phenotype–environment associations but distinguishing adaptive divergence from chance correlations can be difficult. Evidence is stronger when patterns are repeated—either as parallel phenotypic transitions within a species [14,15] or as similar ecological shifts across multiple species facing the same gradient [16,17]. Thus, while **parallelism** is not itself a criterion, it provides a powerful means of identifying ecological drivers.

Criterion 3. Ecotypes show heritable differences in phenotypic traits
Ecotypes differ in a wide range of traits, from conspicuous features like size and colouration to subtler behavioural and physiological differences (Table 1). Turesson emphasized that phenotypic differences must be at least partly heritable, reflecting genetic divergence rather than a purely **plastic response**. The classic test is a common garden experiment, where individuals are reared for multiple generations under uniform conditions [18]. When such experiments are not feasible, indirect evidence can be inferred from genomic approaches such as **genome-wide association studies (GWAS)**.

Criterion 4. Divergent traits should underpin differential fitness
Criteria 2 and 3 establish ecological and heritable phenotypic differences, but the gold standard is to show that these differences effect fitness. This is typically tested with **reciprocal transplant experiments**, where ecotypes are exchanged between habitats and their performance compared [19]. In *Mimulus guttatus*, for example, annual and perennial ecotypes showed much higher fitness in their home sites and were consistently outperformed by the local ecotype when moved elsewhere [20].

Reciprocal transplants provide compelling evidence for local adaptation but are unlikely to reveal the agents and phenotypic targets of ecologically-based divergent selection. Experiments using natural or experimental hybrids are usually required to establish these links. For instance, field experiments using hybrids of *Mimulus lewisii* and *M. cardinalis*, which segregated for flower colour and nectar volume, showed that bees preferred large, lightly pigmented flowers, whereas hummingbirds preferred nectar-rich flowers with high pigment concentrations [21].

Criterion 5. Ecotypes are discrete and diagnosable units

Ecotypes are a particular outcome of local adaptation in which divergence produces more or less discrete units rather than continuous variation (Box 2) [5]. This discreteness arises when multiple ecological factors coincide to form distinct habitat classes, leading to coincident changes in traits and loci. This makes ecotypes diagnosable as phenotypic clusters, in contrast to the diffuse or fluctuating variation that is more typical of other types of local adaptation.

Whether local adaptation leads to ecotypes depends largely on the scale of the ecological transition (Δ) relative to the scale of dispersal (σ) (Box 2) [22]. When these scales are similar (or when $\Delta < \sigma$), divergent selection generates sharp steps in allele frequencies and adaptive traits; when habitat transitions are much broader than dispersal ($\Delta >> \sigma$), diffuse gradients will form. Diagnosing ecotypes therefore requires careful sampling at the appropriate spatial scale.

Box 2. The relationships between local adaptation, ecotype formation, and speciation

Because ecotypes arise through ecologically based divergent selection, it is natural to ask whether they differ from locally adapted populations. Local adaptation often involves modest shifts in trait means around local fitness optima, producing smooth gradients or local fluctuations that track environmental transitions—for example, gradual change along altitudinal or latitudinal clines (Fig. IA & B). Rather than recognizing ecotypes at every point along an environmental gradient, it may be more useful to describe the overall pattern as an **ecocline**.

By contrast, ecotypes are distinct, diagnosable units that represent a more discrete outcome of ecological divergence. Unlike eco-clines, the boundaries between ecotypes are often sharp, reflecting strong selection across **ecotones** or between discrete habitats (Fig. IC & D). Ecotypes can sometimes be connected by intermediate forms, but these usually arise through hybridization rather than adaptation to an intermediate optimum. Such hybrids often have reduced fitness, as their trait combinations do not fit well into either parental niche. This contrast highlights a key distinction: eco-clines reflect continuous adaptation along gradients, whereas ecotypes embody discrete adaptive solutions separated by fitness valleys as depicted in the metaphor of the **adaptive landscape**.

Whereas ecotype formation emphasizes the evolution of ecologically differentiated units, speciation focuses more on the barriers to gene flow that maintain units of biological diversity as separate lineages. These barriers can arise from local adaptation but also from additional pre- and post-zygotic mechanisms—such as assortative mating or intrinsic incompatibilities—that are not directly ecological (Fig IE). All ecotypes exhibit some degree of reproductive isolation, in some cases supported by processes other than local adaptation. Ecotypes can therefore be seen as undergoing speciation, even if it remains uncertain whether they will ultimately complete the process (see Main Text).

A hypothetical ring species provides a fun thought experiment for considering the relationship between local adaptation, ecotype formation, and speciation (Fig IF). Across most of the range of a ring species, populations form an eco-cline, connected by gradual change and ongoing gene flow. Yet where the ends of the ring meet, accumulated divergence can produce sharp barriers to interbreeding, making the terminal populations appear as if they are distinct ecotypes, or even species. This raises a provocative question: are the ends best described as ecotypes, or is the entire ring simply one extended eco-cline?

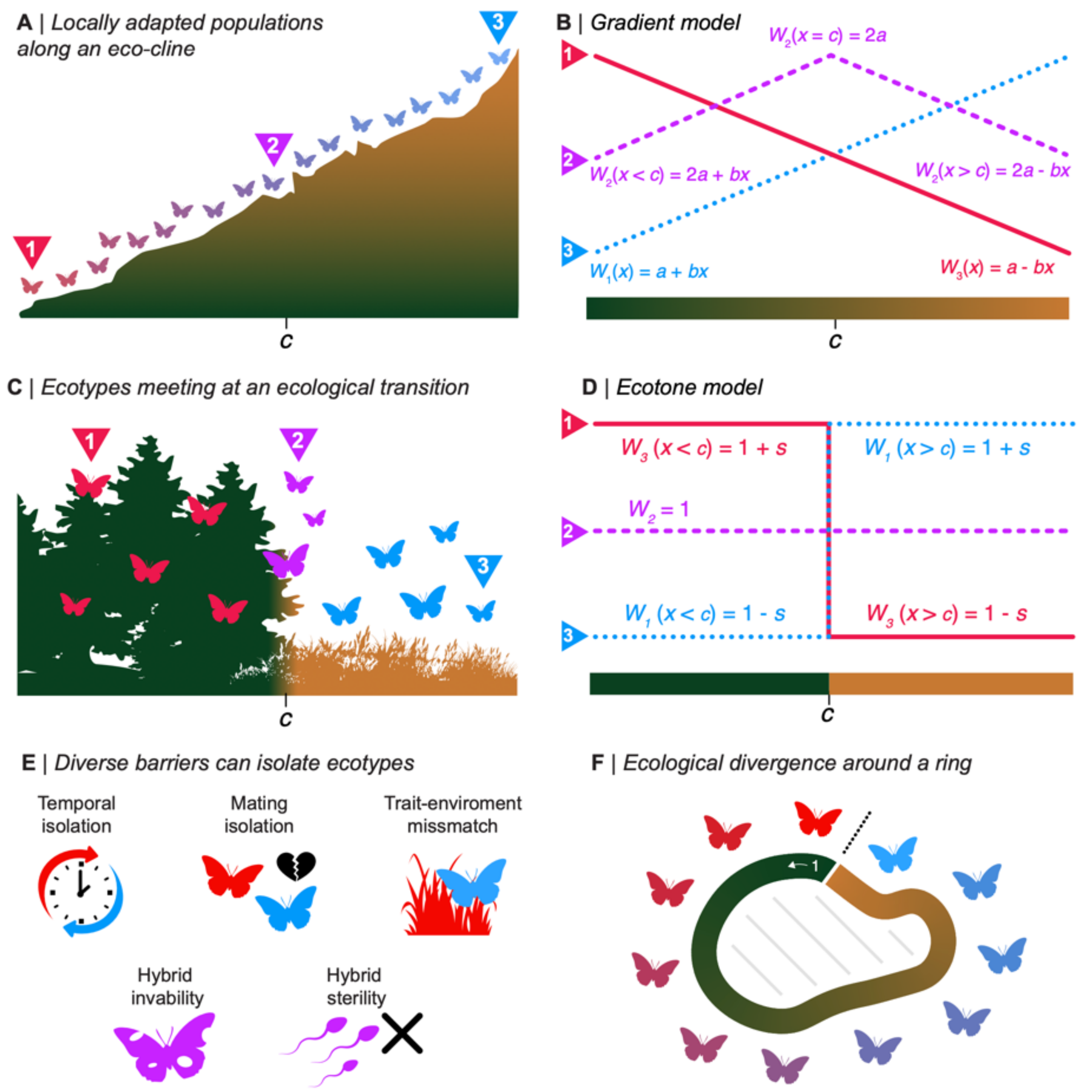


Figure I. Models of ecological divergence and sources of isolation. (A). Butterflies adapted along an ecological gradient. *c* marks the centre of the gradient. The extreme ends are connected gradient of intermediate populations that are adapted to different locations (1, 2, & 3) (B). Fitness functions for populations 1, 2 and 3 (see A). Populations have highest fitness (*W*) where they are located, and this decays with increasing distance. (C) Ecotypes meeting at a sharp ecological boundary, and (D) fitness functions across the transition. Note that F1 hybrids (purple), do not fit into either ecological niche so always have reduced fitness. (E) Ecotypes can be separated by multiple sources of reproductive isolation, not limited to the examples shown. (F) Ecological divergence around a ring. Imagine that red butterflies colonise location 1 and then expand their range around a geographic barrier in an anticlockwise direction until the divergent end points meet. (e.g., a mountain, an island, or a lake).

What has genomics taught us about the origin and nature of ecotypes

We next highlight some of the general findings that have emerged from recent genomic studies of ecotypes. These studies have not only confirmed long-standing expectations but also delivered surprising results that reshaped our understanding of ecological divergence and speciation.

Ecotype pairs show low levels of genome-wide differentiation

A general finding is that ecotype pairs tend to show low levels of genetic differentiation with genome-wide F_{ST} usually falling between 0.01 - 0.15 (Fig. 2A). This is not surprising given that ecotypes are subunits of species. However, named species also fall in this range, highlighting varying use of concepts in some groups of organisms. Moderate to high differentiation (0.2-0.8) is observed between some plant and whale species, but also between ecotypes of marine cyanobacteria which primarily reproduce through binary fission [23].

Variable differentiation can also be seen within defined taxonomic groups, both within and among environments and across broad geographic regions (Fig. 2B & C). F_{ST} between stickleback ecotypes ranges from 0 to 0.4, with higher values observed in interspecific comparisons (Fig. 2B). Across 12 lake–stream pairs, F_{ST} ranges from 0 to > 0.2 [24–26]. Even in three different lake-stream pairs originating from the same lake (Lake Constance), F_{ST} ranges from 0.005 to 0.061 [25].

Understanding what causes varying levels of F_{ST} is challenging because many factors can influence levels of differentiation. In the pairs we examined (Fig. 2A), some variation is explained by their present-day spatial arrangement (i.e., allopatric, parapatric, sympatric; Fig. 2D), which is a proxy for the potential for current gene flow. But other factors, not limited to the time of colonization, strength of selection, and effective population size, may play a role. In the case of the stickleback ecotype pairs at Lake Constance, the magnitude divergence seems to be limited primarily by levels of gene flow between lake and stream habitats [25].

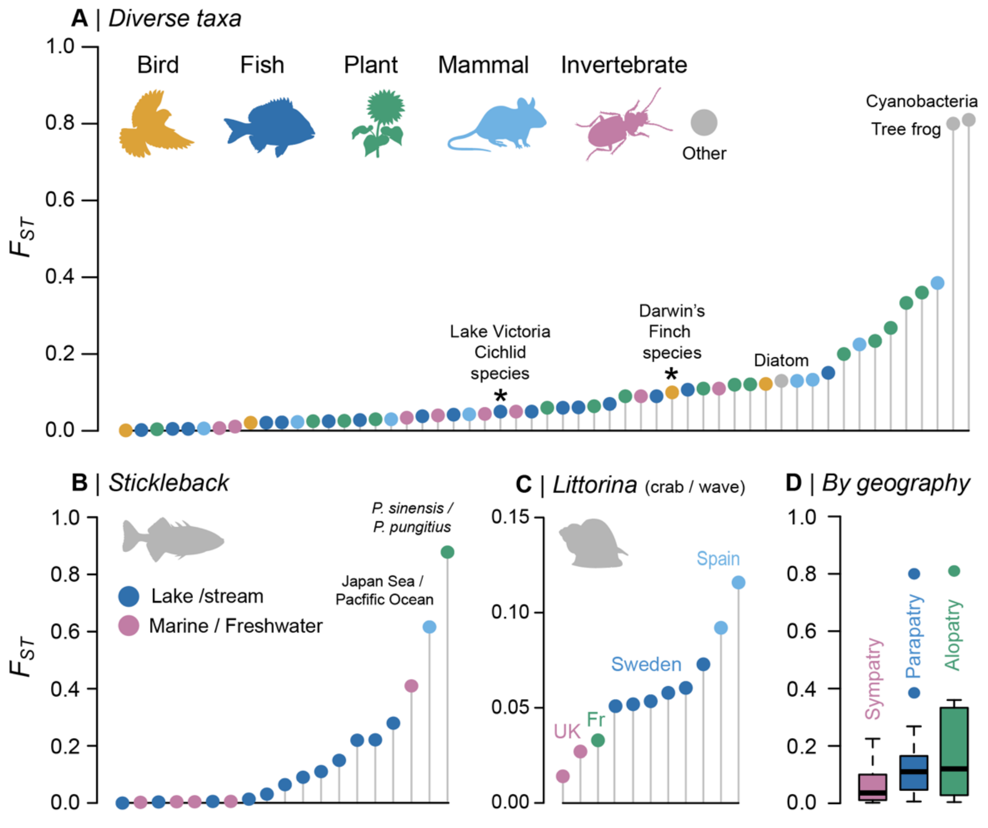


Figure 2. levels of differentiation across multiple ecotype pairs from diverse taxa. (A) Rank-ordered *Fst* between 53 ecotype pairs and two species pairs that fit our criteria for ecotypes. We searched the literature (2015–2025) for papers containing the terms '*ecotype'* or '*ecotypes'* together with '*genetic differentiation*' or '*Fst*'. This returned ~600 hits, of which 161 were classified under '*Evolutionary Biology*.' These were manually curated to identify studies reporting F_{ST} estimates between ecotypes. To avoid redundancy, only one study per species was included, resulting in 42 examples. Studies based solely on mtDNA were excluded. In addition, we incorporated 13 relevant studies

from the same period that were known to us independently. All studies are available in Table S1. (B) Rank order *Fst* in 17 three-spine stickleback ecotype pairs and 2 species pairs (Data from [94]. (C) Rank order *Fst* in 11 *Littorina saxatilis* Crab / Wave ecotype pairs. Data from [95]. (D) Levels of *Fst* from the 55 ecotype pairs is classified according to the level of current geographic overlap (sympatry, allopatry, parapatry).

Ecotype formation often involves periods of geographic isolation

Ecotypes are frequently maintained by selection despite ongoing gene flow, raising the question of whether they form this way. Theory shows this is possible [22], but only if selection is strong enough to counteract gene flow, which prevents the buildup of **linkage disequilibrium (LD)** among adaptive alleles [27]. Thus, there has been much debate about whether ecotypes routinely form with continuous gene flow, or whether geographic isolation often plays a role.

Access to genomic data has allowed researchers to address this question by modelling the **demographic history** of ecotypes formation. In only a few cases do results support formation with continuous gene flow. For example, in *Littorina saxatilis*, Crab and Wave ecotypes appear to have diverged while exchanging genes at three distant locations across Europe [28]. More commonly, demographic models favour scenarios that include periods of isolation [15,29–32]. For example, in sympatric whitefish ecotypes, demographic inference suggests that initial divergence occurred in isolated glacial refugia, with gene flow commencing after the colonisation of post-glacial lakes [30]. Other systems reveal even complex, multi-phase histories involving alternating periods of isolation and gene flow [33].

Thus, evidence suggests that ecotypes commonly diverge through ‘layered’ demographic histories that include periods of isolation and contact. One interpretation is that ecotype formation requires more than ecological selection, including contributions from genetic incompatibilities that evolve in isolation. Another is that geographic isolation is needed to establish LD, which then underpins persistence after contact. It is, however, always important to remember that demographic models are simplified scenarios, and that real histories will always be more dynamic [34]. For example, divergence often unfolds across metapopulations, where adaptation, extinction–recolonisation, and dynamic connections between populations generate demographic patterns and gene histories that do not correspond with traditional categories like primary and secondary contact. Recognising this complexity will be critical for interpreting genomic data and refining models of ecotype formation.

Ecotype formation can involve loci of large effect

Genome scans and genome-wide association studies in diverse taxa have helped to reveal cases where single loci of large effect strongly contribute to ecotypic differences. Examples include, *EDA*, which controls armour plating in stickleback [35], *ALX1*, which controls beak size in Darwin’s finches [36], and *Agouti*, which controls colouration in deer mice [37]. In other systems, or for some traits, no such major effect loci are found, implying that traits are controlled by many loci of small effect. This polygenic effect can be studied and quantified using methods like chromosome partitioning analysis [38].

While powerful, the results of genome scans and GWAS come with caveats. F_{st} outliers may reflect demographic processes or forms of selection that are not related to ecological divergence [39,40], and GWAS can be strongly influenced by population structure. To establish causality, functional validation is needed, using approaches such as CRISPRbased knockouts, transgenics, or expression assays that directly link genetic variants to phenotypic effects.

Chromosomal inversions often control multiple ecotypic differences
A major finding of the last decade is that chromosomal inversions often differentiate ecotype pairs [41], including plants [42], birds [43], fish [44], mammals [45], marine invertebrates [32,46], and insects [47]. Multiple inversions are often involved, and these can encompass a large fraction of the genome [32], and be much older than current populations.

Although initially surprising, inversions are expected to be favoured during adaptive divergence with gene flow, as they suppress recombination between divergent genetic backgrounds [48]. By reducing recombination, inversions preserve LD among sets of locally adapted alleles, thereby facilitating local adaptation and ecotype formation despite ongoing gene flow. The effect if inversions be observed in genome scans, as large blocks of differentiation and LD within inversions relative to the colinear genomic background [32,42].

QTL mapping and GWAS have shown that inversions control variation in multiple adaptive traits [20,38,45]. For example, In *Littorina saxatilis*, inversions control multiple traits that differentiate Crab and Wave ecotypes [38]. Yet, one key aspect of theory has proven difficult to test: whether inversions harbour multiple independent causal loci. This is because genetic mapping approaches rely on recombination to separate causal loci from one another, yet this is supressed in inversions, [49]. An exception is in *Boechera stricta*, where East and West ecotypes differ in phenology, stress tolerance, and fitness-related traits [50]. Initial QTL mapping revealed that many differences localise to a large inversion. Crucially, the inversion occurs only in a hybrid zone where gene flow is substantial, allowing the authors to map QTL between East and West individuals that shared the non-inverted arrangement. This showed that the inversion contains multiple independent QTL, as theory predicts.

Ecotype formation is often fuelled by pre-existing variation.
A recurring insight from genomic studies is that ecotype formation is often driven by genetic variants that predated the formation of contemporary ecotype pairs [51]. Such variants may persist within populations as **standing variation** and enter them via **introgressive hybridization** from other populations or taxa [51]. Recognizing the role of pre-existing variation has helped resolve aspects of ecotype formation that were difficult to explain under models where divergence arises from new mutations.

First, pre-existing variation often underpins the repeated evolution of ecotypes [26,35,52]. In bottlenose dolphins, the parallel evolution of coastal ecotypes drew on ancient alleles present in pelagic populations [53]. In *Helianthus petiolaris*, dune ecotypes in Colorado and Texas share chromosomal inversions that increase seed size and nutrient-use efficiency [42]. Phylogenetic analyses suggest these adaptive haplotypes originated via ancient introgression into *H. petiolaris* and were later re-used during parallel ecotype formation.

Natural and experimental studies show that pre-existing variation can enable ecotypes to arise rapidly when new habitats become available. [54,55]. In three-spined stickleback, uplift during the 1964 Great Alaska Earthquake created freshwater ponds on several islands

[56]. Standing variation in marine colonists enabled fish to evolve into a freshwater ecotype in just 50 generations [56]. In *Littorina saxatilis*, a transplant experiment moved Crab ecotype snails onto a vacant wave-exposed skerry [54]. Within a few dozen generations, selection on pre-existing variation drove changes in shell size, shape, thickness, and colour to close resemblance of the Wave ecotype.

Differences in gene regulation and expression can underpin ecotype formation
Divergence in **gene regulation and expression** have also emerged as an important driver of ecotype formation [57]. Gene expression has heritable and plastic components (66), and heritable trait differences can arise from shifts in gene expression rather than from protein coding changes [58]. This can allow adaptive divergence to proceed with relatively few underlying mutations [59].

Studies of regulation and expression have revealed when in development and in what tissues genes are activated [60]. In seaweed flies, a chromosomal inversion shapes global expression patterns, with stronger effects in adults compared to larvae [61]. Studies of parallel ecotype pairs of Arctic charr showed that repeated phenotypic divergence is accompanied by parallel patterns of differential gene expression [62], suggesting that similar selective pressures can drive convergent regulatory outcomes across independent populations

Although expression differences often persist in common garden experiments, context-dependent regulatory divergence can also play a key role ecotype formation. In stickleback nearly half of the **eQTLs** identified were specific to an environmental treatment [63], while in *Arabidopsis arenosa,* expression of cuticle-associated genes varied with both ecotype and condition [64]. **Alternative splicing** can also lead to regulatory divergence [65] by altering transcript isoforms rather than overall expression levels. For example, alternative splicing of *MSX2A* gene creates shorter spines in the Freshwater three-spined stickleback [66]. Isoform differences have also been observed between ecotypes of Arctic charr [67], sunflowers [68], and butterflies [69].

Hybrids also provide a powerful lens into the role of regulatory divergence in ecotype formation. They often exhibit **misexpression** in parts of the genome which can disrupt pathways and may reduce fitness, thereby limiting gene flow between ecotypes [57]. In lake whitefish, backcross hybrids reared under common garden conditions showed widespread misexpression compared to parental ecotypes, which may generate isolation [70]. Similarly, in *Cyprinodon* pupfish, crosses between a dietary generalist and two specialist forms revealed parallel patterns of misexpression, highlighting the possibility the same disrupted pathways might contribute to reduced hybrid fitness and reproductive isolation [71].

Open questions about the origin and nature of ecotypes

Genomic studies have greatly advanced our understanding of how ecotypes arise and persist yet also highlight the limits of our knowledge. Many fundamental questions remain unresolved and addressing them will require integrating genomic tools with ecological experiments, comparative studies, and new theoretical approaches (also see Outstanding Questions).

Why do some species form ecotypes while others do not?

Some species readily form ecotypes, while others rarely do, and the reasons remain unclear. Ecological specialisation may be limited if genetic variation or ecological opportunity is lacking, or when developmental constraints restrict phenotypic evolution. Life history traits are also likely to matter, because they shape dispersal, and thus gene flow. Ecotypes may be more common in organism with restricted movement or strong site fidelity, as high dispersal and gene flow, typical of wind-pollinated grasses or broadcast-spawning invertebrates, might inhibit ecotype formation. Explaining these patterns will require comparative studies that integrate ecological and genetic data with information on life-history.

How predictable is ecotype formation?

Another question surrounds the predictability of ecotype formation. When populations adapt to similar environments, are outcomes highly consistent? At the genetic level, does parallel adaptation use the same or different alleles and pathways? Replicated field studies, such as those in stickleback, provide valuable insights but are necessarily limited in spatial and temporal scale. Experimental evolution offers a complementary approach, allowing predictability to be tested from the same initial conditions, while manipulating factors that might shape evolutionary outcomes [72]. Such approaches will help clarify when ecotype formation follows consistent trajectories and when outcomes are more contingent.

When and how does plasticity contribute to ecotype formation?

Turesson stressed that ecotypic differences must be partly heritable, but said little about the role of **phenotypic plasticity**. Plastic responses are almost certain to contribute to ecotype formation and may be particularly important in the earliest stages of ecotype formation by buffering mismatches between existing phenotypes and new environments [54]. In this way, plasticity could facilitate the establishment of locally adapted ecotypes [73]. Clarifying when plasticity promotes adaptive divergence, and when it constrains it, remains a central challenge.

What role does structural variation play in ecotype formation?

Large chromosomal inversions have been a major focus of genomic studies, largely because they are relatively easy to detect using polymorphism data [74]. Yet inversions are just one class of **structural variant** (SV), and we still know little about the broader role of SVs in ecotype formation. Other classes of SV, including copy number changes, fusions and fissions, transposable element insertions, may also contribute by coupling adaptive loci or altering regulation, yet have been much less studied. Recent advances in long-read sequencing are now making it feasible to generate multiple population assemblies, enabling us to characterise structural polymorphisms and understand how they shape ecotypes.

Does ecotype formation lead to the completion of speciation?

Ecotypes are often viewed as incipient species, with the expectation that the process will inevitably complete. Yet evidence from several systems suggests that ecotypes can persist for long periods without complete speciation, and can merge rapidly if the balance between gene flow and selection is disturbed [75]. Theoretical work also suggests that ecotypes are not transitional stages, but may represent persistent evolutionary outcomes [76]. There are reasons why partial isolation may be advantageous [77,78]. For example, mechanisms that reduce local recombination (such as inversions) prevent local adaptation from being eroded by gene flow, while allowing the rest of the genome to recombine, maintaining a large effective population size in colinear regions. Partial isolation can also allow beneficial mutations to spread throughout the species range [79]. Identifying the factors that favour the maintenance of partially isolated ecotypes, as opposed to the evolution of complete isolation, remains an outstanding challenge [5].

Concluding remarks

Genomic research has given new life to Turesson's century-old concept of the ecotype, providing unexpected insights into the origin of ecotypes. Yet genomics cannot replace the ecological experiments that first inspired the concept, as these remain essential for quantifying heritability and local adaptation and provide the foundation for interpreting genomic results. The most exciting opportunities lie in combining these approaches. By integrating next-generation genomic tools, including transcriptomic and comparative genomic methods, with ecological and experimental work, we will be able to tackle new challenges and ensure that the coming decade of research on ecotypes is even more revealing.

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Box 1. Broader uses of the ecotype concept

The term ecotype has been widely adopted beyond evolutionary biology, sometimes in ways that stretch or depart from Turesson's original meaning. In animal agriculture, ecotype is often used for local breeds of livestock, such as chickens or sheep (Fig. IA). Some cases show clear phenotypic and genetic differentiation [80], but others reflect little more than geographic labels, shaped by breeding programmes rather than natural selection. For this reason, we recommend caution in applying the term to modern breeds, though traditional landraces of crops and livestock may more closely resemble true ecotypes.

In cancer research, distinct lineages of tumour cells with unique cellular compositions and clinical outcomes have been labelled as ecotypes [81]. This usage makes sense if one accepts cancer lineages as parts of a 'cellular ecosystem', and these lineages meet several ecotype criteria, including repeated occurrence across patients (Fig. IB). However, gene flow is absent or irrelevant in this context, since cells propagate asexually.

By contrast, using ecotype at the interspecific level is conceptually misleading, as it obscures the idea of ecological divergence maintained within species by selection despite gene flow. Referring to groups of species, such as different bird guilds (e.g., wading birds, swimming birds, climbing birds), as ecotypes should be avoided. However, it is sometimes useful to compare or refer to similar ecotypes across species (e.g. migratory and resident fish in different taxa), provided each remains defined within its own species.

In folklore studies, the term ecotype has been adopted to describe local variations of a folktale, legend, or parable that have been modified to suit different cultural settings (Fig.

IC). Much like biological ecotypes, these different stories retain a shared ancestry but have become adapted to local social or ecological conditions [82]. While metaphorical, this use captures the spirit of Turesson's idea, though it is clearly distinct from biological usage.

There are other subspecific categories in biology that we think are synonyms of ecotype. For example, insect host races meet our criteria for being ecotypes. Ecomorph is another term that is sometimes used interchangeably with ecotype. Other subspecific categories, like varieties, morphotype, biotype, and subspecies are sometimes used to refer to units that we would recognise as ecotypes, and in other cases not.

Outstanding Questions (max 2000 characters including space)

- To what extent is ecotype formation convergent at the molecular level across species? For example, do shallow- and deep-water ecotypes in distantly related fish recruit overlapping sets of genes?
- Do ecotypes typically represent transient stages in the speciation process, or can they persist as long-term evolutionary outcomes? What factors determine these trajectories?
- How predictable is ecotype formation across taxa and environments? Do similar ecological pressures consistently lead to similar phenotypic and genetic solutions, or are outcomes idiosyncratic?
- How often do structural variants like copy number changes and TE insertions underpin ecotype divergence compared to point mutations, and why?
- To what extent do gene flow and hybridization hinder versus facilitate the emergence and persistence of ecotypes?
- How can we disentangle differences in expression that reflect adaptative traits, genetic incompatibilities, and phenotypic plasticity?
- Can we use expression and regulatory data to predict adaptive potential in changing environments or future ecotype formation?
- What ecological, demographic and genetic factors allow species to repeatedly form ecotypes over evolutionary timescale.
- How do plastic responses interact with genetic change during the early stages of ecotype formation, and when does plasticity hinder or facilitate divergence?
- To what extent is expression plasticity responsible for environmentally induced phenotypic plasticity, and how does this interact with genetically fixed differences in gene regulation?

Glossary

Adaptive landscape: a metaphorical surface mapping genotype combinations to fitness, with peaks indicating high-fitness solutions and valleys low-fitness ones.

Alternative splicing: A biological process where a single gene can produce multiple different messenger RNA (mRNA) by including or excluding reading of certain DNA segments, or by using alternative start or end sites.

Common garden experiment: An experiment where individuals from different populations are raised in the same environment to separate genetic from environmental effects on traits.

Controlled crosses: Experimental matings performed in the greenhouse or laboratory.

Chromosomal inversion: A structural rearrangement in which a segment of a chromosome is reversed end to end.

Demographic history: The past changes in population size, structure, and migration that shape genetic variation.

Ecocline: A gradual change in genetic, morphological, or physiological traits of a species along an environmental gradient, reflecting continuous local adaptation.

Ecotone: A transition zone between two distinct ecological communities or habitats, where environmental conditions shift sharply and species from both environments may co-occur.

Ecotype (modernized from Turesson): A population that is locally adapted to a specific ecological setting. Ecotypes exhibit heritable phenotypic differences, are more or less distinct from other ecotypes of the same species yet remain capable of interbreeding with them.

eQTL (expression Quantitative Trait Locus): A genomic region where genetic variation affects gene expression levels.

Fitness: The relative reproductive success of a genotype or phenotype, measured as its contribution to the next generation's gene pool.

Gene regulation and expression: The processes that control when, where, and how much a gene's information is transcribed and translated into functional products.

Genome-wide association study (GWAS): A method that scans genetic variants across the genome to find associations with phenotypic traits.

Hybrid zone: A geographical area where genetically differentiated populations (e.g. ecotypes) meet and generate offspring of mixed ancestry.

Introgressive hybridization: The process by which genetic material is transferred from one genetic background into another through hybridization and backcrossing.

Linkage disequilibrium (LD): A non-random association of alleles at different loci.

Misexpression: Aberrant gene expression, often observed in hybrids, where genes are expressed at inappropriate levels or in the wrong context.

Parallel evolution: Independent evolution of similar traits in related lineages under similar selective pressures.

Phenotypic plasticity: When one genotype produces different traits in different environments

Plastic response: When an organism's physiology, morphology, or behaviour is caused by environmental cues.

Quantitative Trait Locus (QTL): A region of the genome that contributes to variation in a quantitative trait.

Reciprocal transplant experiment: An experiment where individuals from different populations are swapped into each other's environments to test for local adaptation.

Reproductive Isolation: Either a reduction in the production of viable and fertile offspring between, relative to within, populations (organismal view) or a quantitative measure of the effect that genetic differences between populations have on gene flow (genetic view).

Standing variation: The presence of alternative forms of alleles at a locus within a population

Structural variant: A genetic change that alters the structure of a chromosome, as opposed to one that changes the nucleotide sequence.

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