Ecological Genetics – HS18

v1.1

Gleb Ebert

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This document aims to summarize the lecture Ecological Genetics as it was taught in the autumn semester of 2018. It is heavily based on the slides and often contains passages verbatim. Unfortunately I cannot guarantee that it is complete or free of errors. You can contact me under `glebert@student.ethz.ch` if you have any suggestions for improvement. The newest version of this summary can always be found on my website: `http://www.glebsite.ch`

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1 Introduction

"Nothing in biology makes sense except in the light of evolution"
— Theodosius Dobzhansky

Ecological genetics is the study of the process of phenotypic evolution occurring in present-day natural populations and is concerned with the genetics of ecologically important traits, that is, those traits related to fitness. In other words, ecological genetics deal with the adjustments and adaptations of wild populations to their environment.
Phenotypic evolution is the change in the mean or variance of a trait across generations due to changes in allele frequencies. **Ecologically important traits** are closely tied to fitness and are important in determining an organisms adaptation to its natural environment.
Adaptation is a heritable phenotypic trait that has evolved in a population in response to a specific environmental factor and improves the survival or reproduction of its carriers. It can also be seen as a process whereby the members of a population become better suited to some feature of their environment through change in a characteristic that affects their survival or reproduction. Of the four key evolutionary processes, only natural selection consistently leads to adaptation (mutations, genetic drift and gene flow do not).

Uses of ecological genetics include

- agriculture (crop improvement)
- medicine (e.g. antibiotics)
- conservation measures (assisted migration)
- geographical differences between populations
- changes in species composition
- habitat adaptation & speciation

2 Species

Species are the fundamental unit in ecology, evolution and conservation legislation. Depending on the research question, adequate species identification, assignment of samples to populations or discrimination of individuals may be of relevance.

2.1 Species concepts

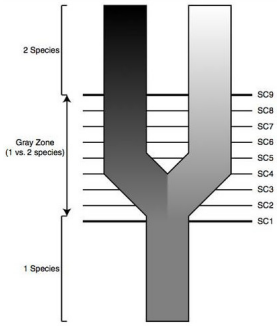
- **Morphological / typological species concept**: focus on similar morphology
- **Biological species concept**: group of potentially or actually interbreeding populations which are **reproductively isolated** from other such groups
- **Phylogenetic species concept**: focuses on monophyletic lineages

2.2 Operational Taxonomic Unit

An **OTU** is a group of organisms that is treated as a distinct evolutionary unit for the purposes of research underway. They are often applied when one or several species concepts fail. Once identified and research has been completed, OTUs should receive full taxonomic treatment and be given a scientific name if possible. OTUs are sometimes called **molecular operational taxonomic unit** (MOTU) when molecular methods are used.

2.3 Unified Species Concept

The only necessary property of species is that they form a separately evolving matepopulation (involves dynamics of gene flow and separation) lineage. The concept separates species conceptualization and separation. All criteria can be used for species delimination and any one of the properties is accepted as evidence for the existence of a species. More properties provide a higher degree of corroboration.



2.4 Identification of Species

Difficulties may include

- species-specific traits are not (always) visible
- differences are cryptic
- direct observation may be difficult and traces may be confused
- undescribed species may occur

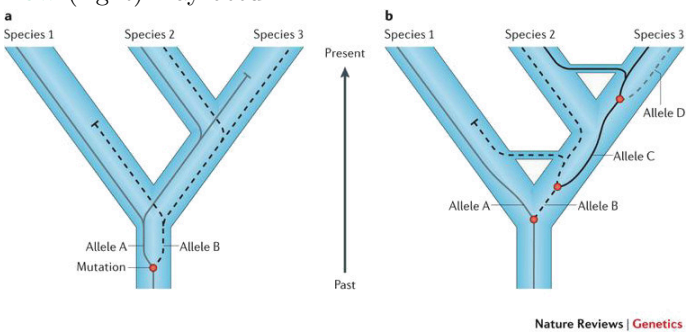
Parataxonomy may be used when identification is difficult. It sorts the material to species on the basis of external morphology without considering taxonomy.

2.5 Species delimination

Species delimitation is the act of identifying species-level biological diversity (independent evolutionary lineages). Most methods fit models to collected data to make (often different) simplifying assumptions. Incongruence across methods may occur due to differences in the power to differentiate lineages or due to violations of one or several assumptions made by a given method. Fundamentally there are two approaches. Some models can assign samples to groups without being given information first (STRUCTURE, Structurama, Geneland). Others need the user to assign samples to putative lineages (BPP, iBPP, spedeSTEM, DISSECT, tr2).

2.5.1 Problems of Species Trees

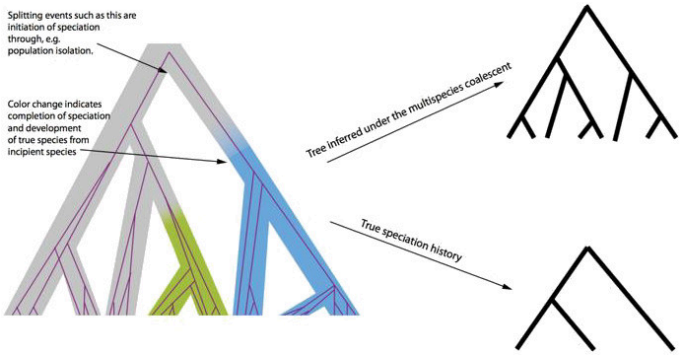
Problems like **imcomplete lineage sorting** (left) or **gene flow** (right) may occur.



2.5.2 Bayesian Species Identification under the Multispecies Coalescent

This method is currently the most used approach for species delimination. The **multispecies coalescent** describes the genealogical relationships between DNA samples from several species. Simplifying assumptions include:

- species phylogeny unknown
- complete isolation after divergence
- no recombination



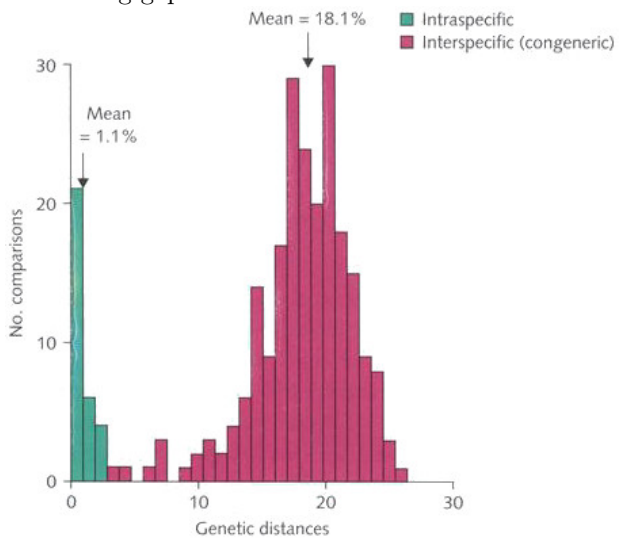
The above graph shows that the MSC approach identified populations as separate species from a simulated data set. MSC delimits structure, not species.

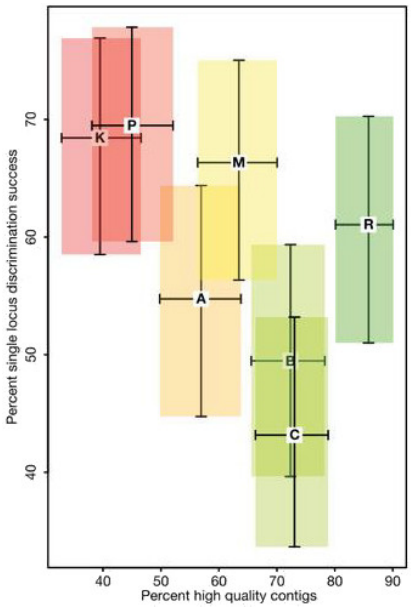
2.5.3 Recommendations for Species Delimination

- use at least 10 samples from all lineages
- simulate before analysis
- use several complementary methods
- combine genetic with nongenetic data
- define the used species concept

2.5.4 DNA Barcoding

DNA barcoding is a method for rapid identification of species through the analysis of short, standardized gene regions. These have to be universal for all animals or plants, have to be amenable to the production of bidirectional sequences with little ambiguity and allow for discrimination of most species. The barcoding gap defines the distance between species. Genetic distances are based on the extent of nucleotide sequence devergence. Some groups like orchids lack the barcoding gap.





Examples of application are the identification of amphibian species diversity and abundance after epidemic diseases in Panama or restriction of shaving brushes to only use hair from the Hog badger instead of the Eurasian badger.

3 Molecular Markers

Molecular markers are polymorphic proteins or DNA sequences and reveal different alleles within individuals, populations or species. Ideally they can be used as **indicators of genome-wide variation**.

- **Chromosome based markers**
  - Numbers and staining patterns
- **Enzyme based**
  - Allozymes
- **DNA based**
  - Restriction fragment length polymorphisms (RFLPs)
- **DNA & PCR based**
  - Random amplified polymorphic DNA (RAPD)
  - Amplified fragment length polymorphisms
  - Microsatellites
  - DNA sequencing and SNPs

3.1 Genome

The size of genomes can differ between species by large factors. Size and complexity are not coupled, as non-coding regions are the main reason for big genomes. These regions contain repeated sequences like tandem repeats or interspersed repeats (transposable elements). The number of chromosomes is limited, because at some point there would be problems with the spindle apparatus. Introns are spliced after transcription while intergenic regions are not.
Mitochondria and chloroplasts have their own genome (mtDNA and cpDNA respectively). Depending on the species, different **organelle genomes** should be used for comparisons. There is much more organelle DNA in a cell than there is nuclear nDNA and it is easier to amplify because of its high conservation.

3.2 Widely used genetic markers

Marker	Advantages	Disadvantages
Allozymes	• Cheap • Universal protocols	• Requirement for fresh or frozen material • Potentially direct target of selection • Limited number of available markers • 'No longer used' (<1998)
Microsatellites	• Informative (large number of alleles, high heterozygosity) • Easy to isolate	• High mutation rate • Complex mutation behaviour • Difficult to automate • Cross-study comparisons are difficult
AFLPs	• Cheap • Produces a large number of markers • Easy to establish in the lab	• Mainly dominant • Difficult to analyse • Difficult to automate • Cross-study comparisons are difficult
DNA sequencing	• Highest level of resolution possible • Not biased • Cross-study comparisons are easy • Data repositories already exist (e.g. NCBI)	• Sanger sequencing: significantly more expensive than the other techniques • NGS: cost per base (bp) very low • NGS: computational intense analyses
SNPs arrays	• Low mutation rate • High abundance • Easy to type • Cross-study comparisons are easy; data repositories already exist	• Expensive to isolate • Ascertainment bias • Low information content of a single SNP

3.2.1 Microsatellites

- SSR (simple sequence repeat) / STR (short tandem repeat)
- highly polymorphic: mutation rates between 10^{-6} and 10^{-2} per locus per generation
- widely used to assess genetic variation in animals, plants and fungi as they are highly variable between individuals
- codominant
- mostly evolutionary neutral, as they are in intragenic regions
- PCR-based (primers, agarose-gel electrophoresis)
- capillary sequencers use fluorescence labelled primers
- 10-20 SSRs per study
- the mutation mechanism is called **slipped-strand mispairing**: polymerase slips of and when rejoining does not know which repeat it already copied; insertions and excisions happen, but the latter seem to be corrected in nature

3.2.2 Structural variation (SV)

- Microsatellite repeats
- 1bp indels
- More complex insertions and deletions
- Copy number variants (CNVs) (> 1kb)
- ...

3.2.3 Amplified fragment-length polymorphisms (AFLPs)

100-4'000 random markers per individual that are dominant (homo- and heterozygotes indistinguishable).

1) DNA extraction
2) Digestion by restriction enzyme
3) Ligation of adaptors to half-sites
4) Amplification of adaptor-half-sites (+3/+3 bp)
5) Sequencing
6) Binary data matrix (peak present/absent)

3.2.4 SNP microarrays

DNA is hybridized to predefined probes. Only common and known SNPs are called. This is the ascertainment bias.

4 Sampling

Sampling determines...

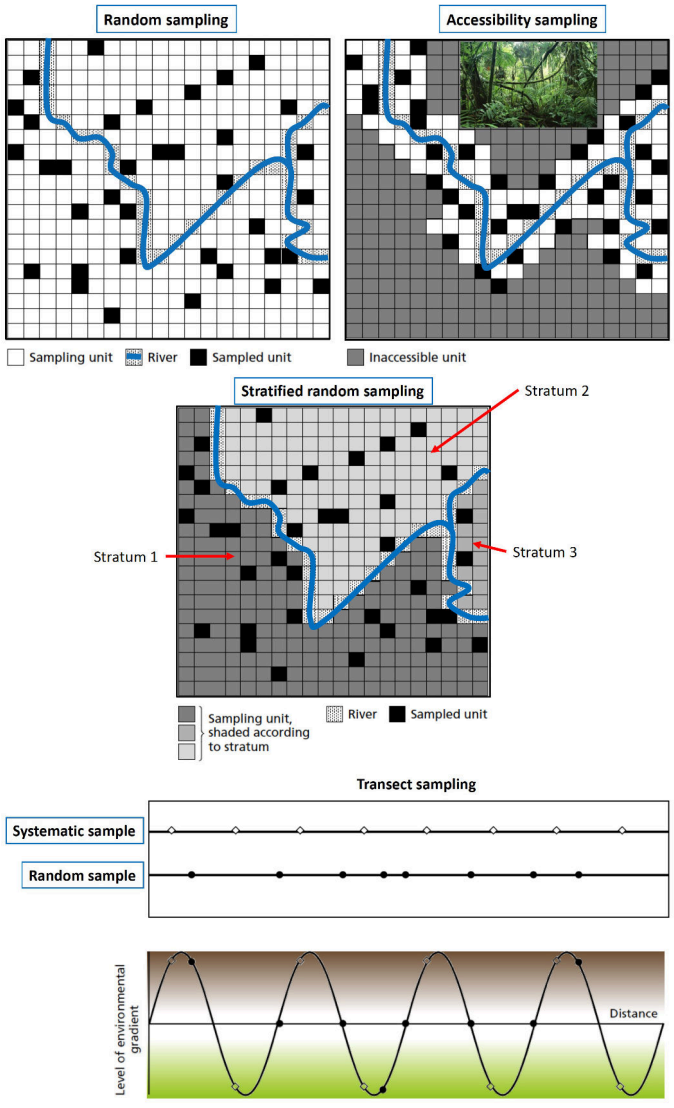
- the chances of answering your research questions
- the time spend on research
- research costs
- the likelihood of obtaining research permits

4.1 Populations

Population type	Definition
Genetic	All individuals which are connected by gene-flow
Ecological	Group of organisms occuring in a particular area at a particular time
Statistical	The universe of items that are under study

4.2 Individuals

Do you sample proportionally or equally across strata? Randomly or systematically? There is no single right solution. One should always consider the circumstances. Roughly 20-30 individuals represent a population (> 80% of all alleles).



4.3 Important considerations

- Documentation: archived, reproducable, verifiable, new technologies
- Storage: adequate storage and transport, test in advance!
- Permits: sampling, handling animals, export and import

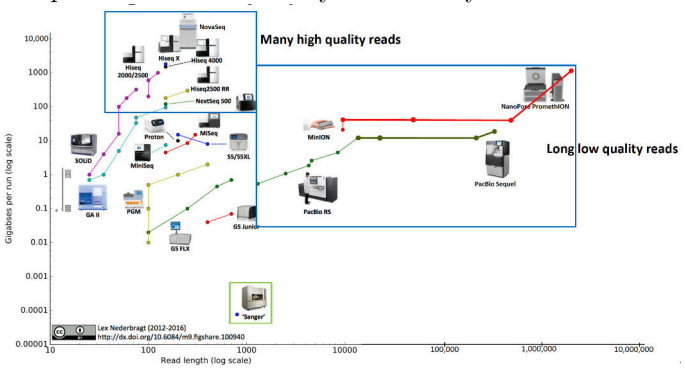
4.4 Nagoya protocol

The convention on biological diversity from 1993 had the objectives of conservation and sustainable use of biological diversity as well as the sharing of benefits arising from the utilisation of genetic resources. The Nagoya protocol from 2010 is a supplementary agreement that expands on the fair and equitable sharing of benefits arising out of the utilisation of genetic resources.

- **Access obligations**: Provide fair and non-arbitrary application procedures and issue permits when access is granted
- **Benefit-sharing obligations**: Share the value of genetic resources and traditional knowledge with developing countries
- **Compliance obligations**: Ensure that genetic resources and traditional knowledge have been accessed in accordance with prior informed consent

5 Genomic Methods

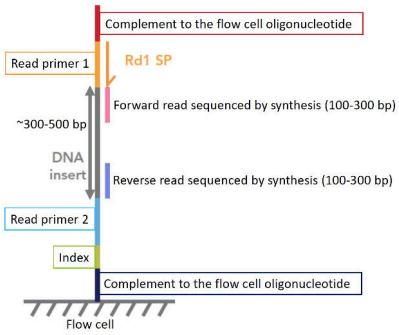
Sequencing methods evolved rapidly since the development of pyrosequencing in 1993. The cost per raw megabase is sinking rapidly as well. Data is being gathered faster and cheaper than it can be analysed nowadays.



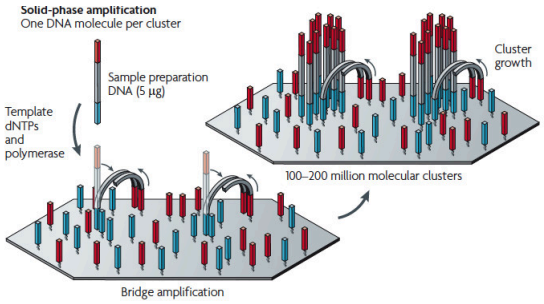
5.1 Illumina high-throughput sequencing

5.1.1 Sample / library preparation

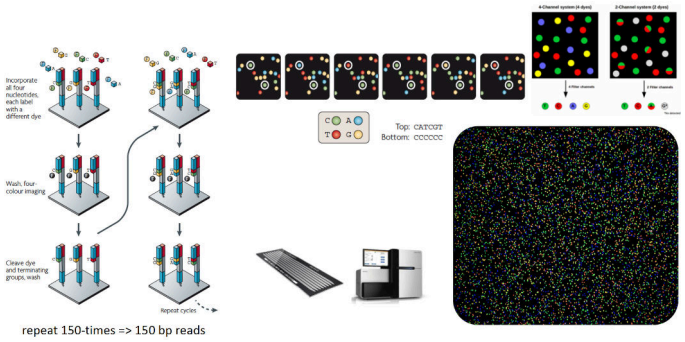
1) Fragmentation of genomic DNA
   1) Mechanical shearing: e.g. sonification
   2) Tagmentation: enzymes
2) Size selection: 300-500bp
3) Adapter ligation
   1) PCR amplification
   2) Individual barcoding



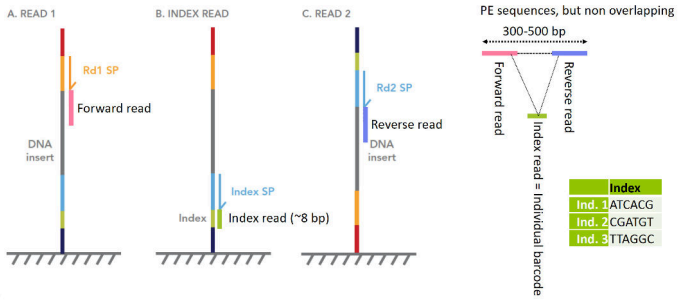
5.1.2 Bridge amplification and cluster generation



5.1.3 Sequencing by synthesis



5.1.4 Paired-end sequencing



5.2 3rd generation sequencing

3rd gen methods sequence single molecules and generate long reads of 10'000 - 2 million bp. They do not use PCR and thus avoid PCR artefacts. One player in the market is **Pacific Biosciences**. They use single molecule real-time analysis (SMART). Another one is **Oxford NanoPore**, whose **MinION** costs only around $300 and generates even longer reads than PacBio is able to. Sequencing happens through voltage changes in the membrane when DNA passes the nano pore. NanoPore sequencing is useful for *de novo* genome assembly, CNV detection, real-time identification of samples and mRNA splicing variant identification.

5.3 Uses of NGS data

NGS inferences require fully annotated high quality reference genomes, draft reference genomes, reference transcriptomes or *de novo* assembled STACKS (RADseq). Methods used for high-throughput marker discovery include:

- Sequencing of individuals and populations
- Whole-genome re-sequencing
- Transcriptome sequencing (RNA-seq)
- Reduced representation sequencing (RADseq; no reference genome required)
- Target capture sequencing methods
  - Sequencing of ultra-conserved elements
  - Exome capture

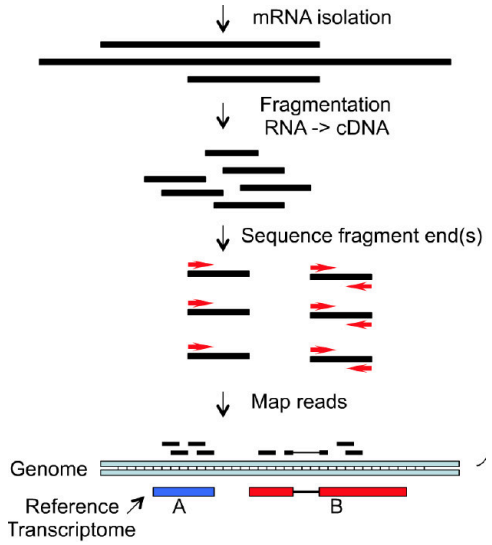
5.3.1 Whole genome re-sequencing

No bias from insufficient marker density or distribution occurs when re-sequencing whole genomes.

- **Individual sequencing**: 1 Flow cell NovaSeq S4 ($\sim$ 35'000CHF+ $\sim$ 100CHF per individual library)
- **Sequencing pools of individuals** (Pool-seq)
  - Cost effective: population libraries ($\sim$ 100CHF per pool library)
  - Lower coverage per individual
  - Population allele frequenciy estimates (no individuals genotypes)

5.3.2 Transcriptome sequencing (RNA-seq)

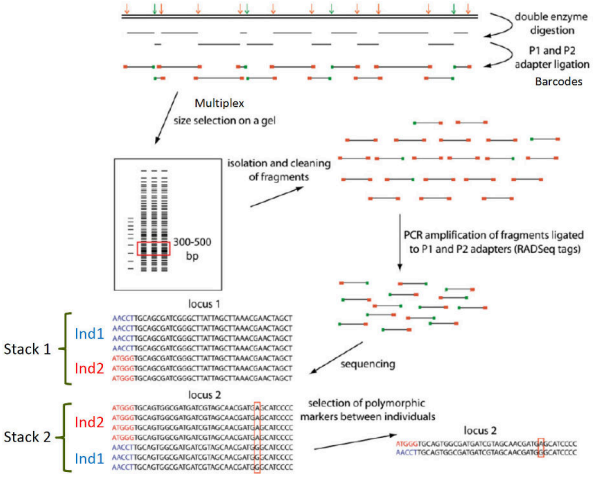
This method reduces the complexity of the genome through only sequencing the expressed exons (mRNA).



5.3.3 Reduced representation sequencing (RADseq)

One method of reduced representation sequencing is **Restriction site-Associated DNA Sequencing (RADseq)**. Restriction enzymes are used to obtain DNA sequences adjacent to a large number of restriction cut sites. As these site are conserved and no reference genome is required, RADseq is used extensively for high-throughput SNP discovery and genotyping in ecological and evolutionary studies. The sequencing depth is considered to be high and the cost per sample lower. 1-200 loci per 1 Mb of genome with lengths of 100-150bp result in a $\frac{100 \text{ loci} * 100 \text{ bp}}{1'000'000} = 100\text{x}$ reduction of genome complexity.

ddRAD: double digest RADseq



5.3.4 Marker identification

If possible, reads are mapped to a genome (e.g. through Burrows-Wheeler Alignment). Other algorithms then do the **SNP calling**. They take into account the **coverage** (# of reads per base), base and mapping qualities as well as many other factors.

5.3.5 Gene expression differences

Differentially expressed genes (DEGs) can be inferred from RNAseq data. Expression is measured in **FPKM**, which stands for **F**ragments (reads) **P**er **K**ilobase per **M**illion mapped fragments. It is then corrected for sequencing depth and gene / exon length.

5.4 The question of usefullness

The computational and storage needs when dealing with NGS data are enormous. Whether a few microsatellites are enough is a valid question. SSRs (see chapter 3.2.1) only reflect a limited portion of the genome and have very different mutation rates compared to SNPs. There is also a lot less microsatellites. In general, they suffer from **ascertainment bias**. It is of statistical nature and is introduced during collection of the data when only markers that were found to be polymorphic are used. When markers with little variance are ignored, genetic diversity is generally overestimated. Furthermore, rare alleles are often missed which may lead to incorrect inferences of demographic parameters.
Additional benefits of whole genome re-sequencing information include

- More than only anonymous markers
- Candidate genes can be studied
- Signatures of adaptation / selection can be detected
- Genetic and genomic diversity (e.g. estimates through exome-wide diversity)
- Demographic history

5.5 NGS pro and contra

	Advantages	Disadvantages
Data quantity	huge amounts of data	huge amounts of data
Data quality	high quality	multiple sequencing required; storage costs
Costs	cost per bp relatively cheap	high costs for individual reads; expensive IT infrastructure
Data analyses	almost everything possible	lots of IT infrastructure

6 Genetic variation

The ultimate source of genetic variation are **mutations**. They occur at random positions in the genome but rates can vary across genomes. In sexual life cycles, existing genetic variation is being re-shuffled continuously through random gamete fertilization, random chromosome segregation and recombination.

6.1 Forms of genetic variation

6.1.1 Singe-nucleotide polymorphism

SNP refers to variation in a single nucleotide at a specific position in the genome. Adjacent nucleotides or indels (insertions and deletions) can have substantial effects on SNP mutation rates. Roughly 15% of all polymorphisms are small indels.

6.1.2 Structural variation

- Balanced nucleotide variation
  - Inversion
  - Intrachromosomal translocation
  - Interchromosomal translocation (up to whole arms of chromosomes)
- Unbalanced nucleotide variations
  - Duplication
  - Deletion

6.1.3 Sizes of variations

- SNP: 1bp
- Microsatellites and minisatellites: 14-200bp
- Indels: <1kb
- Copy number variations (CNVs): >1kb

6.1.4 Other forms of genetic variation

- Gene expression variation
- Methylation variation
- Post-transcriptional modification

6.2 Levels of genetic variation

The main axes of variation in diversity are among species and within genomes. The neutral theory of molecular evolutions postulates that the vast majority of evolutionary change at the molecular level is maintained by the interaction between mutation, which creates variation, and genetic drift, which eliminates it. It also predicts, that in a population of constant size, diversity should be proportional to N_e. The neutral theory is usefull as a null hypothesis for test whether natural selection is occuring.

6.2.1 Population size

In an idealized, panmictic (randomly mating) population, also known as **Wright-Fisher population**, with an equal expected contribution of individuals to reproduction and equal survival, the strength of genetic drift is inversely proportional to the size of the population. Real populations depart from the concept. Therefore the following two concepts are used. The **census population size** N_c is the number of individuals in a population. It varies by several orders of magnitude across taxa. The **effective population size** N_e is the size of an idealized population that would show the same amount of genetic diversity as the population of interest. It varies over time, with long-term N_e explaining current levels of genetic diversity in populations but contemporary N_e explaining how strong drift currently is.

The observed differences in population size are expected to determine differences in genetic diversity across species. However, across-species variation in genetic diversity is much narrower than the variation in abundance. This conflict has been termed the **paradox of variation** and is also known as **Lewontin's paradox**. Possible solutions include

- Demographic fluctuations
- Natural selection and genetic hitchhiking
- Molecular constraints on heterozygosity (in yeast, recombination is impeded when heterozygosity is too high)
- Variation in mutation rate

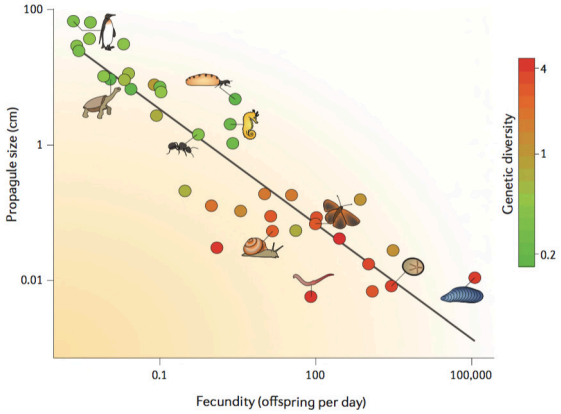


Figure 1 | **Genetic diversity and the *r*/*K* gradient in animals.** The average per-day fecundity is on the *x* axis and the average size of eggs or juveniles is on the *y* axis; each dot is for a family (one to four species each). The colour scale indicates the average nucleotide diversity at synonymous positions, expressed in per cent. The negative correlation reflects a trade-off between quantity and size of offspring. *r*-strategists (bottom right; for example, blue mussels, heart urchins and lumbricid earthworms) are more polymorphic than *K*-strategists (top-left; for example, penguins, Galapagos tortoises and subterranean termites). Figure from REF. 37, Nature Publishing Group.

6.3 Determinants of genetic variation

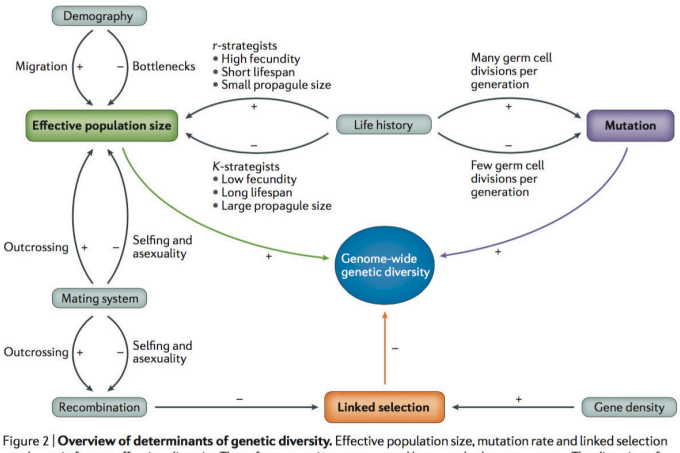


Figure 2 | **Overview of determinants of genetic diversity.** Effective population size, mutation rate and linked selection are the main factors affecting diversity. These factors are in turn governed by several other parameters. The direction of correlation is indicated by the + and − symbols. Selfing, self-fertilization.

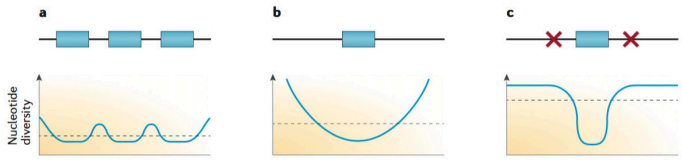


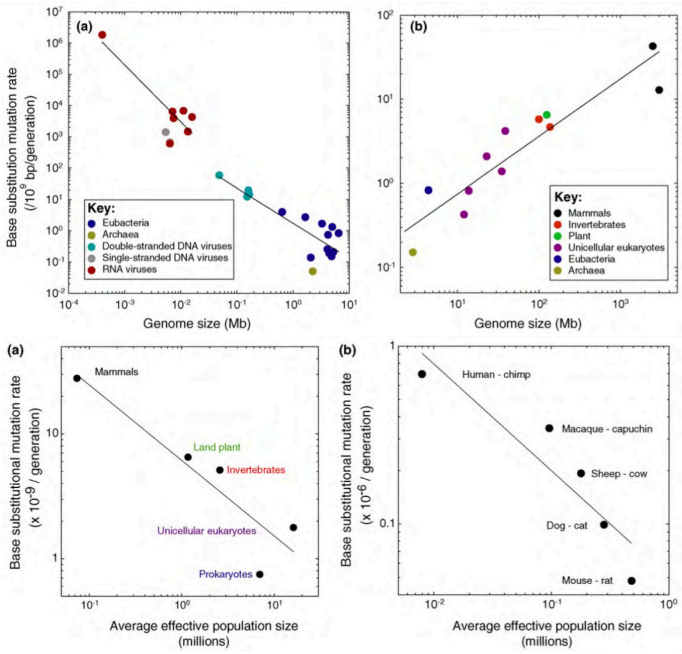
Figure 3 | **Genetic diversity affected by the density of targets for selection and by recombination rate.** A schematic illustration of the effects of linked selection on genetic (nucleotide) diversity around genes or other functional elements (boxes; upper panels). In the lower panels, solid lines indicate the local variation in diversity level and dashed lines indicate the average diversity in the whole region in question. In regions with a high density of targets of selection (part **a**), linked selection is pervasive and significantly reduces diversity compared with regions with a lower density of selection targets (part **b**). When the recombination rate is high (part **c**), the effect of linked selection becomes less prevalent, allowing maintenance of high diversity levels.

6.4 Loss of variation

Relative population size	Rate at which variation is lost each generation
Haploid	$1/N_e$
Diploid	$1/(2N_e)$
Tetraploid	$1/(4N_e)$
Plastid DNA	$1/N_{ef}$*
mtDNA	$1/N_{ef}$*

*True for taxa in which plastid DNA (including cpDNA) and mtDNA are maternally inherited, since N_{ef} is the effective number of females in the population.

6.5 Mutation rate variation



Mutation accumulation experiments generate multiple lines of one ancestor that reproduce through selfing or inbreeding to accumulate mutations. They are useful to estimate mutation rates and mutation variation.

6.5.1 Direct sequencing of families

Analysis of Genetic Inheritance in a Family Quartet by Whole-Genome Sequencing *(Roach et al, Science (2010), 328, 636-639.)* We analyzed the whole-genome sequences of a family of four, consisting of two siblings and their parents. Family-based sequencing allowed us to delineate recombination sites precisely, identify 70% of the sequencing errors (resulting in > 99.999% accuracy), and identify very rare single-nucleotide polymorphisms. We also directly estimated a human intergeneration mutation rate of approximately 1.1×10^{-8} per position per haploid genome. Both offspring in this family have two recessive disorders: Miller syndrome, for which the gene was concurrently identified, and primary ciliary dyskinesia, for which causative genes have been previously identified. Family-based genome analysis enabled us to narrow the candidate genes for both of these Mendelian disorders to only four. Our results demonstrate the value of complete genome sequencing in families.

7 Genetic architecture of adaptive traits

7.1 Linking phenotype with genotype

A fundamental problem in evolutionary biology and ecological genetics is to understand the genetic basis of adaptation and adaptive traits in natural populations.

7.1.1 Forward genetics

A forward genetics approach investigates the genetic basis of a phenotypic trait. Classical forward genetic screens start by mutagenizing individuals. Those with the phenotype of interest are sought and the mutated gene is identified. Detailed studies of the mutant together with molecular analyses of the gene allow identification of gene function.

7.1.2 Reverse genetics

The goal of a reverse genetics approach is to identify the phenotype(s) that are associated with particular nucleotide sequences. Using various techniques, a gene's function is altered and the effect on the development or behaviour of the organism is analysed. Methods include gene inactivation through RNA interference (RNAi), gene silencing mediated by virus infection (virus-induced gene silencing, VIGS) or gene inactivation via CRISPR/Cas9.

7.1.3 Reverse ecology

Reverse ecology is analogous to reverse genetics and is the application of genomic approaches to living systems to uncover the genetic bases of functional variation in nature. By identifying genetic polymorphisms that are associated with a particular habitat or phenotypic trait, one can find targets of natural selection – without a priori knowledge about how selection acted and without knowing the trait that was the target of selection.

7.2 QTL Analysis

The term **quantitative trait locus (QTL)** refers to a specific DNA region that influences the expression of a quantitative phenotype (trait). They are typically identified in the offspring of crosses between parental individuals that differ clearly in the traits of interest. QTL analysis can be considered a forward genetics approach. Results of QTL analyses provide information about the genetic architecture of traits, including:

- The number of loci that contribute to trait variation
- The positions of these loci in the genome
- Their effect sizes
- Interactions (additive and epistatic) among loci

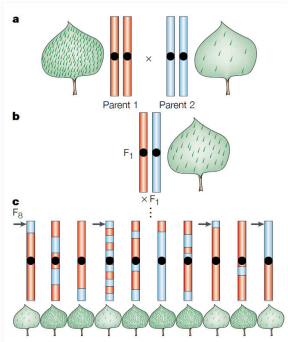
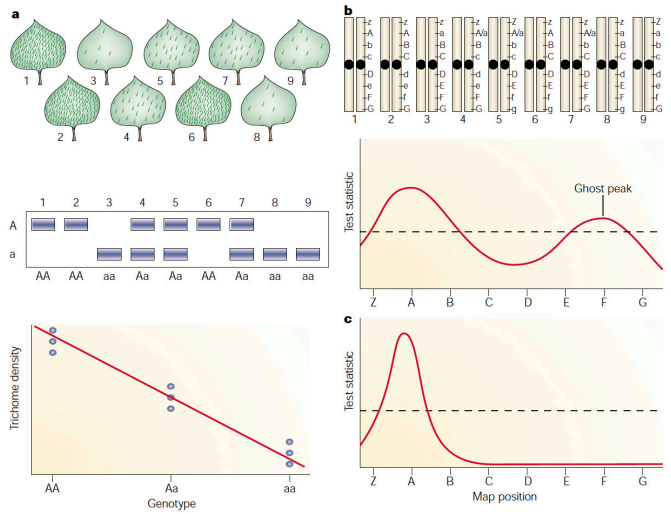


Figure 1 | **Principles of mapping quantitative trait loci.** The basic strategy behind mapping quantitative trait loci (QTL) is illustrated here for **a** | the density of hairs (trichomes) that occur on a plant leaf. Inbred parents that differ in the density of trichomes are crossed to form an F_1 population with intermediate trichome density. **b** | An F_1 individual is selfed to form a population of F_2 individuals. **c** | Each F_2 is selfed for six additional generations, ultimately forming several recombinant inbred lines (RILs). Each RIL is homozygous for a section of a parental chromosome. The RILs are scored for several genetic markers, as well as for the trichome density phenotype. In **c**, the arrow marks a section of chromosome that derives from the parent with low trichome density. The leaves of all individuals that have inherited that section of chromosome from the parent with low trichome density also have low trichome density, indicating that this chromosomal region probably contains a QTL for this trait.



Limitations of QTL mapping include

- Controlled crosses are either impossible or time-consuming in many species
- Genetic variation in the mapping population is restricted with only two (or four, e.g. in outcrossing species) parents used to initiate the QTL mapping population
- Resolution is limited because typically crosses from earlier generations are used and the number of recombination events per chromosome is small.
- QTL intervals often span tens of centiMorgans and thus several Megabases and correspondingly many genes (tens to thousands).
- Many QTL regions contain multiple, closely-linked QTLs that may have smaller or even opposite effects.
- Phenotypes and their QTL are frequently affected by interactions, e.g. epistatic interactions, genotype-by-sex, genotype-by-environment,but most QTL studies do not allow testing for such effects.
- Effect sizes are difficult to estimate and alleles with small effect sizes are typically not identified. The former is related to the Beavis effect in which the smaller the sample size the stronger the effect size is overestimated.

7.2.1 LOD Score

Statistical support for a QTL at a specific position is estimated using the LOD score:

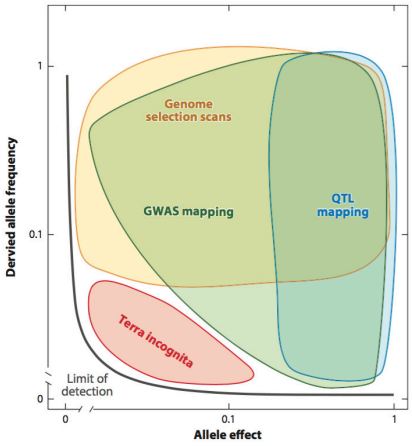
$$\text{LOD} = log_{10}\frac{L_1}{L_0}$$

7.3 Linkage mapping

Linkage maps are constructed using large numbers of segregating markers and information about the recombination rate (linkage disequilibrium) between different markers in a population with known pedigree. Distances between markers do not indicate physical but recombination distances, are estimated from observed recombination frequencies, and are typically given in cM (centiMorgan). Difficulties in constructing such linkage maps include

- Sufficient number of suitable markers
- Estimates of recombination distance may be biased by multiple (e.g. double) crossovers
- Interference: the interaction between neighboring crossover events
- Incomplete information from some offspring genotypes when parents are not fully homozygous. This requires statistical estimate of recombination distance between markers.

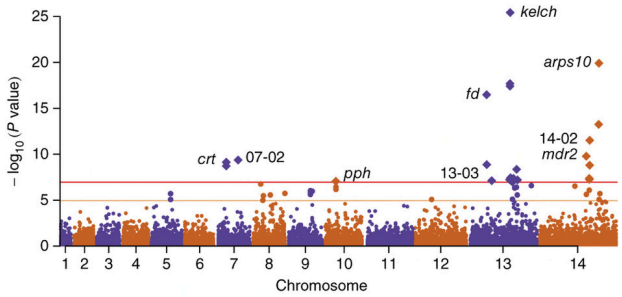
7.4 Methods selection



7.5 Genome-wide assiciation studies

In GWAS, also known as genome-wide association mapping, the **association between each genotyped marker and the phenotype of interest** scored across a large number of individuals is analysed. They can provide insights into trait architecture as well as candidate genes for certain traits or for functional analysis and can compliment QTL analysis. They also provide much higher resolution because associations in natural populations reflect historical recombination events.

1) Select panel of samples / genomes
2) Assess genomic variants
3) Phenotype traits of interest
4) Run statistical methods
5) Rank candidates
6) Validate genes



The power of GWAS to identify a true association is dependent on the phenotypic variance within the population explained by the SNP. The phenotypic variance is determined by how strongly the two allelic variants differ in their phenotypic effect (the effect size), and their frequency in the sample. Problems for GWAS are caused by population structure, rare variants or small effect sizes. The effects of rare variants may be easier to analyse in a QTL experiment because crossing elevates rare variants to intermediate frequency.

Key challenges for GWAS in natural populations are that quantitative phenotypes are often strongly affected by the environment, outcrossing organisms often have low LD, and the lack of *a priori* knowledge of trait architecture complicates planning.

GWAS can only identify a small fraction of causal genes. This is called the problem of **missing heritability**. Possible explanations are

- Complex epistatis interactions among genes are relevant for many traits
- Studies do not have sufficient power to detect small-effect loci
- Importance of epigenetic variation ignored
- Genetic effect due to rare mutations
- Traits are diagnosed incorrectly or inconsistently

7.5.1 GWAS and population structure

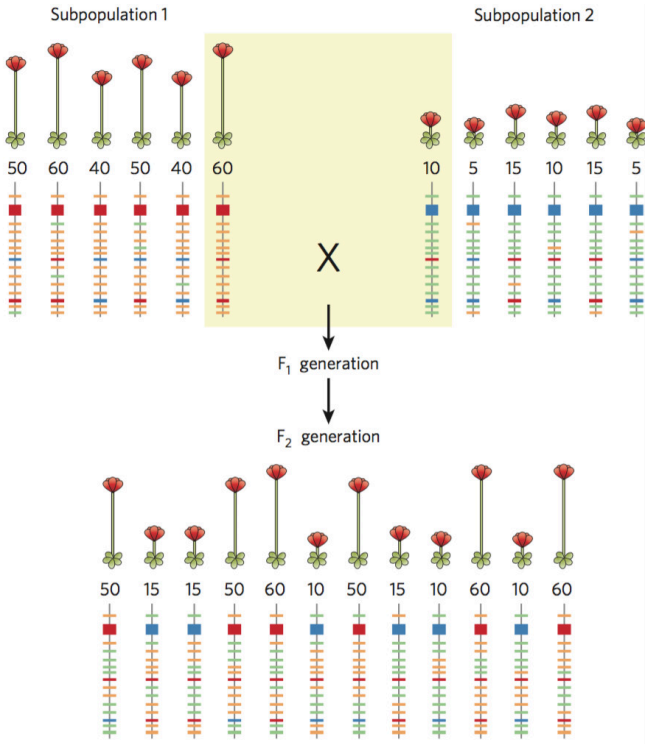


Figure 1 | GWA mapping is ineffective if there is strong genetic differentiation between subpopulations (that is, if there is structure in the population). In this example, two subpopulations of plants are depicted, one tall and one short (as illustrated and indicated by the numerical measurement), together with a schema of the genotype of each plant. The presence of red alleles increases the height of a plant, whereas blue alleles decrease the height; one locus has a major effect, and two have a minor effect. The many background markers (orange and green) are mostly exclusive to a specific subpopulation but are also strongly associated with height, even though they are not causal. By crossing the plants (shaded area) and generating an experimental population of F_2 generation or recombinant inbred lines, any linkage disequilibrium between background markers and causal markers is broken up, and the causal loci can then easily be mapped, albeit with relatively poor resolution.

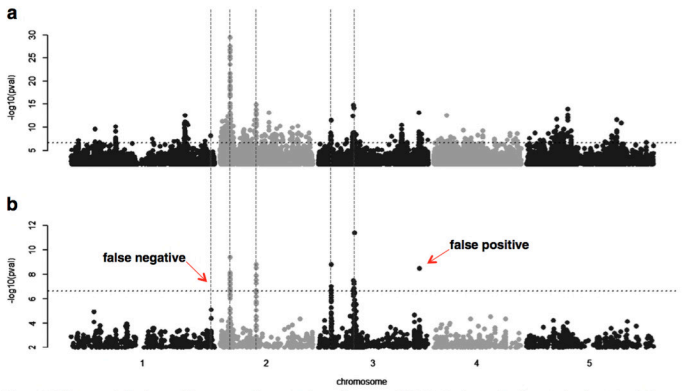


Figure 3 Taking genetic background into account improves the performance of GWAS. Manhattan plots for a simulated trait, in which each data point represents a genotyped SNP, ordered across the five chromosomes of *Arabidopsis*. Five SNPs (indicated by vertical dashed lines) were randomly chosen to be 'causative' and account for up to 10% of the phenotypic variance each. GWAS using **a)** a linear model, and **b)** a mixed model that accounts for population structure and other background genomic factors. The simple linear model leads to heavily inflated p-values and the five causative markers are not the strongest associations. The mixed model is superior, but still leads to one false negative and one false positive. A dashed horizontal line denotes the 5% Bonferroni threshold.

7.5.2 GWAS and trait structure

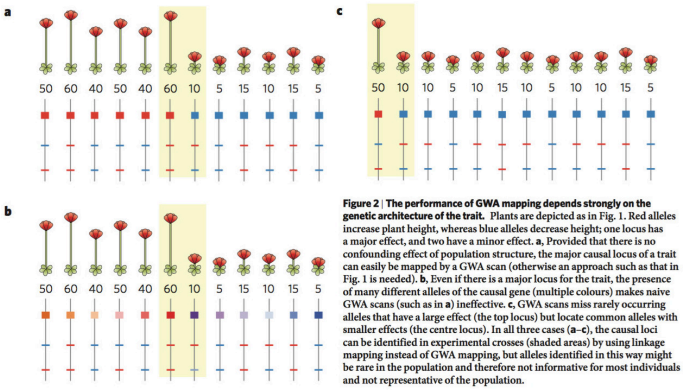


Figure 2 | The performance of GWA mapping depends strongly on the genetic architecture of the trait. Plants are depicted as in Fig. 1. Red alleles increase plant height, whereas blue alleles decrease height; one locus has a major effect, and two have a minor effect. **a**, Provided that there is no confounding effect of population structure, the major causal locus of a trait can easily be mapped by a GWA scan (otherwise an approach such as that in Fig. 1 is needed). **b**, Even if there is a major locus for the trait, the presence of many different alleles of the causal gene (multiple colours) makes naive GWA scans (such as in **a**) ineffective. **c**, GWA scans miss rarely occurring alleles that have a large effect (the top locus) but locate common alleles with smaller effects (the centre locus). In all three cases (**a**–**c**), the causal loci can be identified in experimental crosses (shaded areas) by using linkage mapping instead of GWA mapping, but alleles identified in this way might be rare in the population and therefore not informative for most individuals and not representative of the population.

8 Selection

Selection is nonrandom differential survival or reproduction of classes of phenotypically different entities. An allele that increases fitness experiences **positive selection**. In **negative selection**, also known as purifying selection, rare, deleterious alleles are removed from a population.

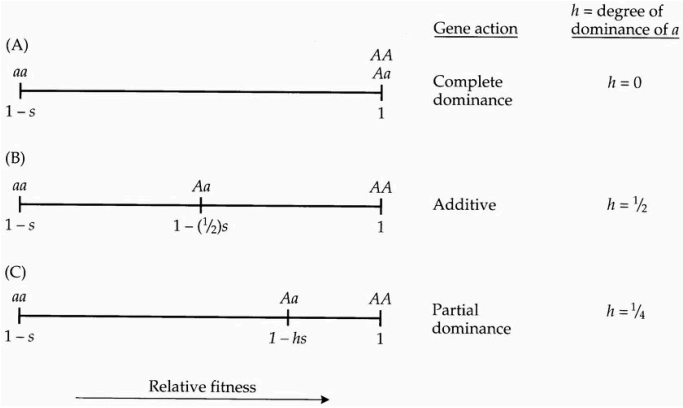
8.1 Fitness

Selection is caused by **differences in fitness** among individuals with different phenotypes or genotypes in a population. Therefore, we are interested in the **relative fitness** w of an individual or genotype but not in its **absolute fitness**. The strength of selection against a given genotype is measured by the **selection coefficient** $s = 1 - w$.

Genotype	Abs. fitness (# offspring)	w	$s = 1 - w$
AA	60	1.0	0.0
Aa	60	1.0	0.0
aa	48	0.8	0.2

8.2 Modes of gene action

Considering the way genotypes affect phenotypes is important to understand the genetics of phenotypic traits. In the following figure, s measures the strength of selection against the aa phenotype. h is the degree of **dominance for fitness** of the a allele.



8.2.1 Joint effects of mode of gene action and selection

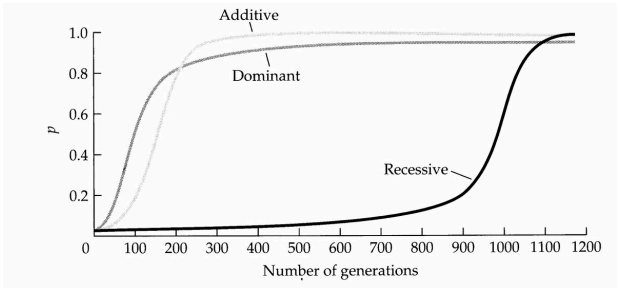
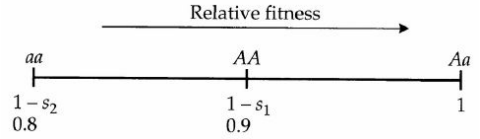


Figure 3.8 Expected change in frequency over time of a favored allele ($s = 0.05$) with dominant, additive, or recessive effects on fitness.

8.2.2 Overdominance or heterozygote advantage

Occur when the heterozygote genotype has higher fitness than both homozygous genotypes.



8.3 Artificial selection

The **selection differential** S or **strength of selection** corresponds to the difference between the population mean and the mean of the selected individuals. The **response to selection** R indicates the difference across generations in population means (short-term phenotypic evolution). Thus the **breeder's equation** can be formulated: $R = h^2 S$. Directional selection leads to a correlated response in other correlated traits. Limits of artificial selection include the depletion of additive genetic variance for the selected trait, opposing natural selection, physiological or intrinsical limits, limiting environments.

8.4 Natural selection

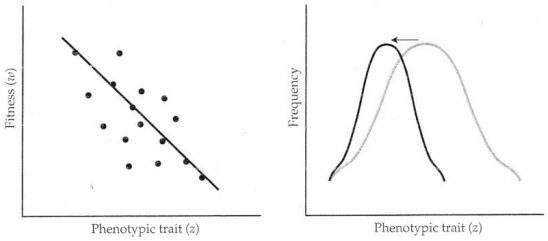
Natural selection acts on phenotypic variation in natural populations. A significant proportion of the phenotypic variation is heritable, i.e. is caused by additive genetice variance. A consistent relationship between phenotypic variation and variation in fitness exists.

8.5 Estimating selection in natural populations

Standardized phenotypic values (z) are the individual value minus the population mean divided by the population standard deviation. Regressing relative fitness on the phenotype (z) allows estimating the strenght of selection and provides a statistical estimate of the fitness function. A **fitness function** describes the relationship between fitness and the phenotype and determines the strength and form of natural selection. Three basic types of phenotypic selection are distinguished according to the shape of the fitness function.

8.5.1 Linear fitness functions

Directional selection changes the trait mean and decreases the trait variance (V_A). The fitness function is linear with the slope of the line measuring the strength of selection, which is known as the **standardized selection differential** (or intensity of selection i).



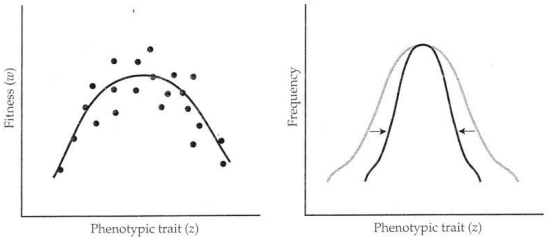
8.5.2 Non-linear fitness functions

When the fitness function has curvature, quadratic regression is used to estimate the strength of selection:

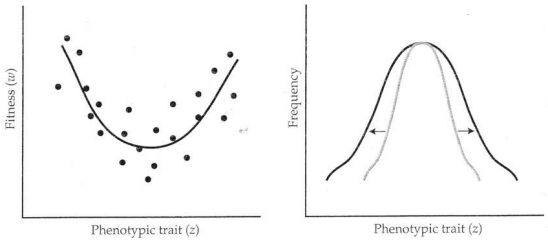
$$\omega = \alpha + \beta z + \frac{\gamma}{2} z^2$$

α is the y-intercept, β is the slope of the fitness function, γ measures the rate of change of the slope with increasing z. The latter also estimates the amount of curvature in the fitness function and is called the **non-linear selection gradient**.

Stabilizing selection does not change the trait mean but decreases trait variance, which means that an intermediate optimum for fitness exists.



Disruptive selection does not change the trait mean but increases trait variance, which means that an intermediate minimum for fitness exists.



8.6 Selective agents and targets of selection

Selective agents are environmental causes of fitness differences among organisms with different phenotypes. **Targets of selection** are phenotypic traits that selection acts upon directly. Changes in the target of selection or the selective agent can change the fitness function.

8.7 Direct selection

Direct selection operates when a causal relationship between a phenotypic trait (the target of selection) and fitness exists. Only direct selection leads to adaptive evolution. The target trait is thus a present-day adaptation.

8.8 Indirect selection

Many phenotypic traits are correlated with other phenotypic traits. Direct selection on one of these traits will also affect the correlated trait(s). Thus **indirect selection** is a covariance between a trait and fitness within a generation caused by a phenotypic correlation between the trait and another trait that experiences direct selection. Indirect selection does not contribute to adaptation.

8.9 Total selection

Selection gradients β measure direct selection on each trait after multiple regression.
Fitness = intercept + β_1 trait$_1$ + β_2 trait$_2$ ... β_k trait$_k$

Total selection on trait 1 is the sum of the direct selection on that trait (β_1) and the direct selection on all correlated traits weighted by the correlations between these traits and trait 1:

$$S_1 = \beta_1 + \beta_2 r_{12} + \beta_3 r_{13} + \dots$$

9 The genomic signature of recent selection

9.1 Locus behaviour

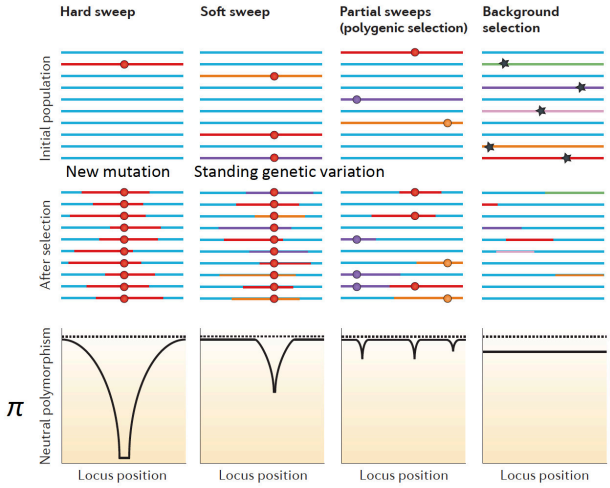
Neutral processes affect all loci similarly. Selection however affects only a single locus (in a simplified case) and can be seen through outlier detection.
A population under selection need to have a minimum N_e to overcome drift, or s needs to be very strong ($s >> \frac{1}{2N_e}$). Selection acts most efficiently on large populations.

9.2 Hitchhiking

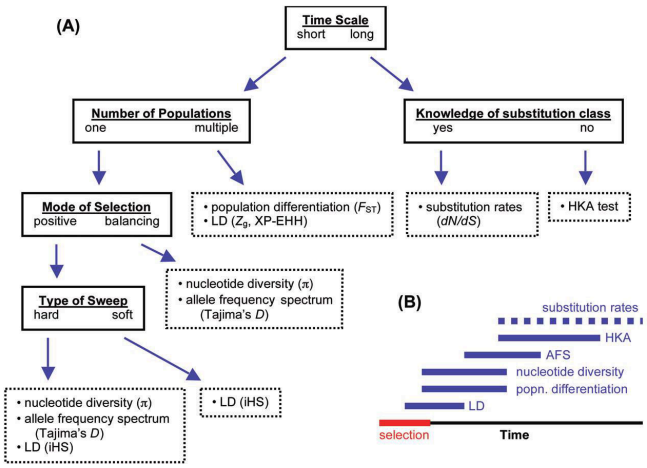
Neutral loci close to a locus under selections hitchhike along and have their allele frequency increased. The signature of selection decays with increasing distance from the locus under selection. These islands of selection can be seen on Manhattan plots. However, marker density is vital to detect outliers when sequencing.

9.3 Selective sweeps

An allele that increases fitness arises and "sweeps" to fixation in a population. This results in skewed allele frequency, linkage disequilibrium, long haplotypes and reduced diversity, the latter three being results of hitchhiking. Because of recombination only a single locus is affected. Single genes or loci also show a more recent MRCA (most recent common ancestor).
Populations with a locus under selection show reduced diversity (π) and increase differentiation $F_{ST} = \frac{\pi_T - \pi_S}{\pi_T}$ (fixation index, measures deficit of heterozygosity).
π is the mean number of nucleotide substitutions per site between any two randomly selected DNA sequences in a population.



9.4 Methods to detect recent selection



9.4.1 Reduced level of genetic variation (π)

Genomes of three cultivated (C) and one wild (W) cucumber groups were re-sequenced. Morphologically they were all different. Comparing π_W and π_C revealed 112 regions with reduced π in cultivated cucumbers. The *Bt* locus, which is responsible for fruit bitterness, showed a sweep of around 2 Mb.

9.4.2 Linkage disequilibrium (LD)

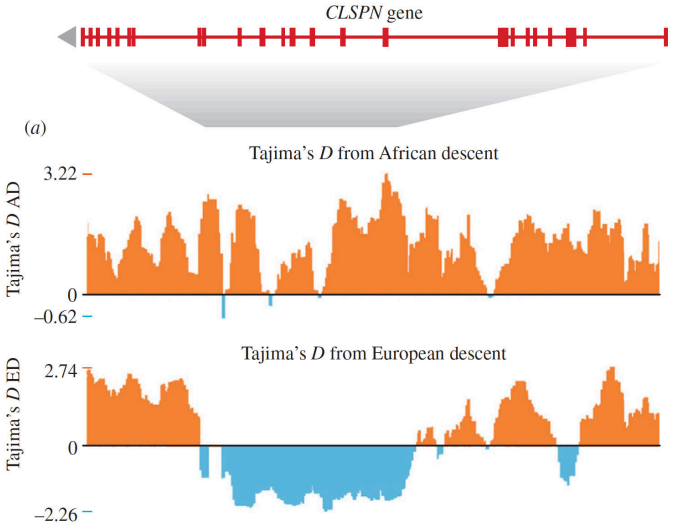
The *dhfr* gene is involved in drug resistance of *Plasmodium falciparum*. SSR heterozygosity is strongly reduced in roughly 100 kb around the locus.
The human gene *LCT* encodes lactase. A particular 2 Mb haplotype causes the lactase persistent phenotype, allowing for continous lactase expression through the whole life. Extended haplotype homozygosity (EHH) measures the decay of homozygosity from a core SNP. The method shows that the lactase persistent phenotype evolved independently in both Africa and Europe.

9.4.3 Skew of allele frequency spectra (Tajima's D)

Tajimas's D is the normalized difference between π and segregating sites (S, θ_W).

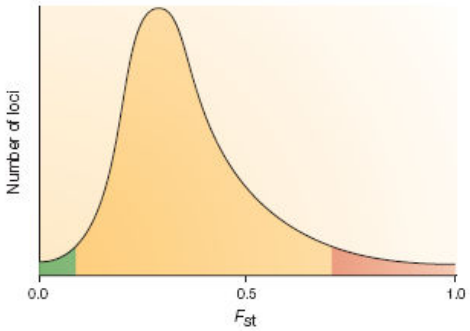
$$d = \pi - \theta_W \qquad D = \frac{d}{\sqrt{V(d)}}$$

Balancing selection (maintenance of multiple alleles within population, excess of intermediate-freq. SNPs): $\pi > \theta_W, +D$
Positive selection (new, non-synonymous mutations selected for, excess of low-freq. SNPs): $\pi < \theta_W, -D$

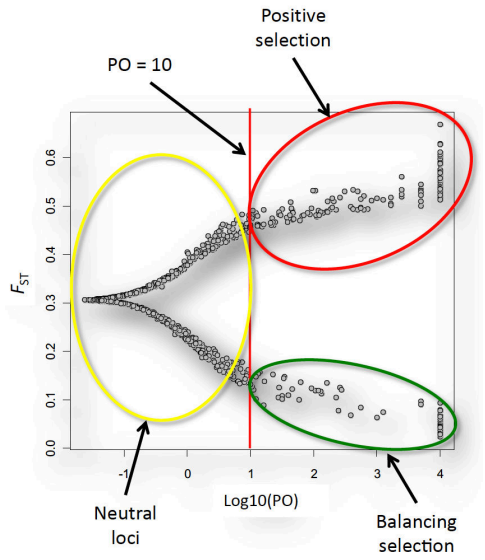


9.4.4 F_{ST} outlier detection

Locus specific population differentiation / fixation is caused by local positive selection, which increases the level of differentiation among populations and leads to high F_{ST} values. Balancing selection causes relatively uniform allele frequencies across populations and low F_{ST} values. Neutral loci can still show intermediate differentiation due to drift and demography.
When working without a model, one can screen many loci (>10'000) and then set an outlier criterium (e.g. 95% quantile of F_{ST} distribution). Balancing selection leads to realtively uniform frequencies across populations and thus low F_{ST} value (green). Positive selection leads to increased levels of differentiation among populations and thus high F_{ST} values (red).



In a model based F_{ST} outlier approach the demographic history is taken into account. The approach estimates the probability of each locus to be under selection (Island model).



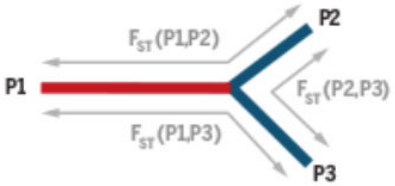
9.4.5 Environmental associations analysis (EAA)

EAA looks for correlations / associations of alleles, SNPs or genes with environmental factors such as temperature. In other words, it looks for environmental factors that drive local adaptation.
The **Partial Mantel test** correlates two distance matrices: pairwise genetic distance of outlier SNPs and pairwise climatic distance of environmental factors.

9.4.6 Population Branch Statistics (PBS)

The PBS metric measures the F_{ST} between three populations and looks for alleles that are particularly extreme in a single population.

$$T^{12} = -log(1 - F_{ST}^{12}) \qquad PBS = \frac{T^{12} + T^{13} - T^{23}}{2}$$



10 Local adaptation and clinal variation

10.1 Phenotypic plasticity and G x E interactions

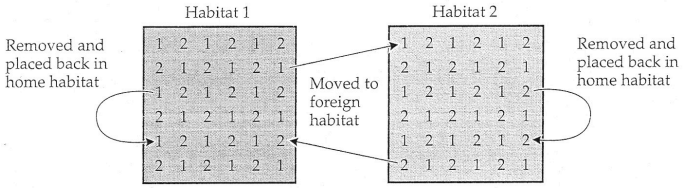
Phenotypes are typically not constant but can vary as a consequence of differences among genotypes and among environments. **Reaction norms** depict the phenotypes produced by different genotypes within a population in two (or more) different environments. **Phenotypic plasticity** occurs when the same genotype produces different phenotypes in different environments. In **genotype-by-environment interactions** (G x E) different genotypes (families) respond differently to different environments.

10.2 Local adaptation

In nature environmental conditions often vary in space. If these differences affect fitness, **spatially divergent selection** may occur. It is expected to cause each local population (deme) to evolve traits that prodive an advantage under its local environmental conditions. This may occur regardless of the consequences of these traits for fitness in other habitats. As a consequence, a pattern may emerge in which resident genotypes in each deme have on average a higher relative fitness in their local habitat than genotypes originating from other habitats. This pattern and the process leading to it are known as **local adaptation**.
Populations (demes) may be discrete units in well-defined habitats or may represent arbitrary sampling units in a continuous species range. Spatial variation in the environment may be discrete with several distinct habitat types or it may consist of continuous environmental gradients, whereby a habitat represents the conditions at a given point of the gradient.

10.2.1 Detecting local adaptation

Local adaptation should be manifested in improved fitness of each deme in its own habitat. Ideal experiments for the study of local adaptation are **reciprocal transplant experiments**.



In each habitat the local deme is expected to show higher fitness than demes from other habitats (local vs. foreign). Each deme is expected to show higher fitness in its own habitat than in other habitats (home vs. away).

10.2.2 Clinal variation

Clines illustrate shifts in phenotypes or allele frequency over geographic space. Clinal variation is often observed in natural populations in the field. Is this variation a consequence of genetic differences or of phenotypic plasticity? Not all clinal variation is caused by natural selection, although there are striking examples of natural selection in action. Clinal variation is frequently being used as a model to investigate how species respond to heterogenous selection pressures in the presence of gene flow.
Adaptive clines represent trade-offs in the selective benefits of traits at different ends of an ecological transect. Variation in the strength and direction of selection is thought to help maintaining the polymorphism. If, through a change in environmental conditions, the selection pressure(s) producing a cline ceases to operate, gene flow, brought about by dispersal, will homogenize the differences, eventually eliminating the cline.

10.3 Fitness

The adaptive value of a trait depends on its effect on the number of offspring produced. The absolute fitness of an organism is the total number of surviving offspring that an individual produces during its lifetime (lifetime reproductive success). For comparison absolute fitness values are standardized to get **relative fitness**. To calculate relative fitness, the genotype with the highest absolute fitness is assigned a relative fitness of 1.0 while for every other genotype relative fitness is calculated as absolute fitness / absolute fitness of fittest genotype.
Fitness can be quantified by using single traits as proxies (e.g. survival, fecundity, etc.). Competition experiments are then performed between genotypes under different conditions and their contribution to the next generation is traced (ideal for species experiencing strong competition). The population growth rate is measured for each deme in a given habitat.

10.4 Antagonistic pleiotropy

Antagonistic pleiotropy is a form of genotype x environment (GxE) interaction in which the alleles have opposite effects on fitness in different environments (habitats). This implies that no single genotype is superior in all habitats leading to trade-offs in adaptation to different habitats. Spatial heterogeneity facilitates the maintenance of polymorphisms that show antagonistic pleiotropy.

10.5 Conditional neutrality

Conditionaly neutrality occurs when an allele is neutral in one environment and beneficial or deleterious in another. Especially in benign habitats where fitness differences can be small antagonistic pleiotropy may be difficult to detect. Consequently, conditional neutrality might be inferred instead.

11 Reproductive isolation, hybridization & introgression

11.1 Speciation

The **biological species concept** states that species are groups of interbreeding natural populations that are **reproductively isolated** from other such groups. Reproductive isolation (RI) between most species is a consequence of multiple isolating barriers.

11.1.1 Ring species

The concept of a ring species consists in the existance of an original ancestor population which migrated around a geographic barrier. While migrating the subpopulations continuously evolve, possibly away from each other. Once the subpopulations meet on the other side of the geographic barrier, they might be sufficiently different to be considered subspecies of eachother. When these interbreed issues like reduced hybrid viability can arise. Examples of proposed ring species are the Greenish Warbler and *Ensatina* salamanders.

11.2 Reproductive barriers

- **Pre-mating stage**
  - Habitat isolation
  - Temporal isolation
  - Behavioral isolation
- **Prezygotic stage**
  - Mechanical isolation
  - Gametic isolation
- **Postzygotic stage**
  - Reduced hybrid viability
  - Reduced hybrid fertility
  - Hybrid breakdown

Only after all these barriers are overcome viable and fertile offspring is produced.

11.3 Estimating the strength of reproductive barriers

$$RI = 1 - \frac{\#\text{ cross species foraging bouts}}{\#\text{ foraging bouts}}$$

$$RI = 1 - \frac{\text{fitness of F}_1\text{ hybrids}}{\text{fitness of parentals}}$$

RIs vary between zero which means free gene flow and one which means complete isolation and zero gene flow. Individual components of RI act at sequential stages in life-history. The **proportional contribution (PC)** of an individual component of RI at stage n can be calculated:

$$\begin{aligned} PC_1 &= RI_1 \\ PC_2 &= RI_2(1 - PC_1) \\ PC_3 &= RI_3(1 - (PC_1 + PC_2)) \\ PC_n &= RI_n(1 - \sum PC_i)_{i=1}^{n-1} \end{aligned}$$

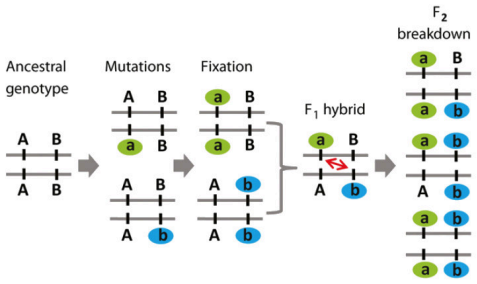
A given reproductive barrier eliminates gene flow that has not already been prevented by previous stages of reproductive isolation. Thus later acting barriers often have a smaller effect on total reproductive isolation even when they are stronger in absolute terms compared to earlier acting barriers.

11.4 Evolution of reproductive barriers

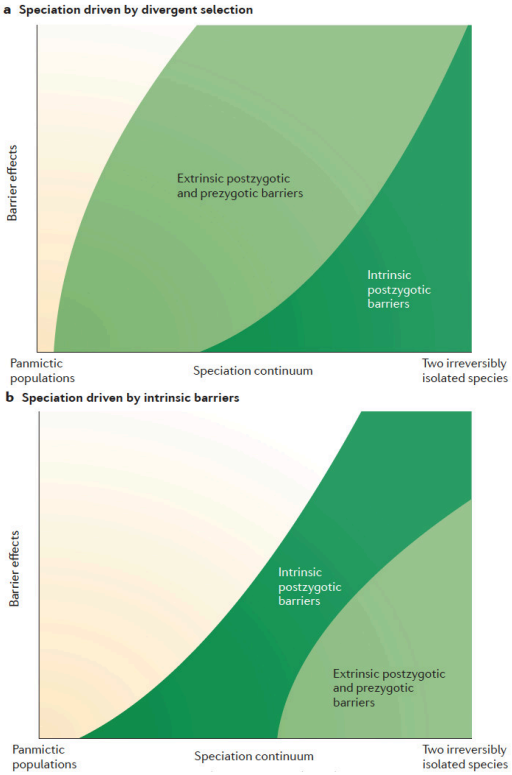
In sympatric situations, reproductive barriers may evolve rapidly by natural and / or sexual selection (reinforcement). Local adaptation to different niches can lead to extrinsic prezygotic isolation. In allopatric situations reproductive barriers often evolve in a clock-like fashion (new mutations and drift). Prezygotic barriers can evolve faster than postzygotic barriers in organisms for which mate recognition plays a central role.

11.4.1 Dobzhansky-Muller incompatbility (DMI)

The Dobzhansky-Muller model of intrinsic hybrid incombatibility (intrinsic postzygotic barriers) aims to explain how genetic incompatibility between species evolve without simultaneously causing defects in pure species. It proposes that two subpopulations acquire a different, mutated allele each. When they interbreed a part of the F_2 hybrids show less fitness because they carry both mutated alleles with at least one being homozygous.



11.4.2 Sympatric vs allopatric



11.5 Inferring reproducive barriers from genomic data

Case study: The hybridization zone between the carrion crow and the hooded crow is very narrow. There is no ecological selection on phenotypes. Gene flow between the two (sub)species is substantial throughout the whole genome except for a small number of narrow genomic islands. The crows seem to prefer assortative (meaning between similar looking individuals) mating. This leads to the two (sub)species to remain largely separated.

11.6 Hybridization

Hybridization is the process during which mating between different taxa leads to the appearance of hybrid offspring. Hybridization is common in both plants and animals. Between 10% and 30% of multicellular animal and plant species are believed to hybridize regularly. Hybridization is however distributed unequally with certain families (e.g. *Orchidaceae*) showing much higher hybridization rates than others. Hybridization is often a local and transient phenomenon that creates evolutionary noise. Many hybrids are sterile. They might be of great importance for conservation biology however. Hybridization can also be a creative evolutionary force with significant ecological consequences.

11.7 Introgression

Introgression is the transfer of genes or alleles from one taxon to another due to hybridization followed by repeated backcrossing of hybrids with one of the parent species. A prerequisite for introgression is that early generation hybrids are at least partially fertile and viable.

11.7.1 Adaptive introgression

One example of adaptive introgression is the DDT (an insecticide) resistance introgressing from *Anopheles gambiae* to *Anopheles coluzzii*. The latter was not resistant before the introgression event, but the population in Ghana became more and more resistant due to the massive selective pressure from insecticides. Frequencies of hybrids between the two species have remained low and stable because reproductive isolation has not been eliminated.

11.7.2 Speed of introgression

The speed of introgression may vary for different parts of the genome as a consequence of various processes:

- Incompatible alleles may fail to introgress
- Neutral alleles may introgress at intermediate speed
- Advantageous alleles may introgress faster due to positive selection

11.7.3 Ecological consequences of introgression

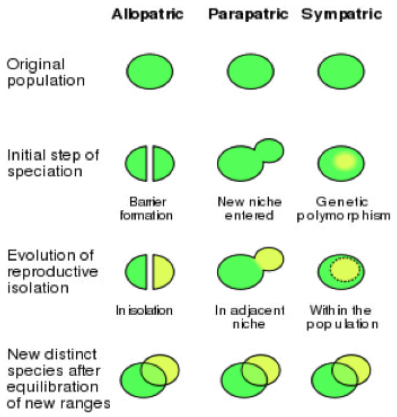
- Porous genomes
- Novel genetic variation enters a gene pool
- Adaptation to novel habitats or changing conditions
- Less inbreeding in small and isolated populations

11.7.4 Genomic consequences of introgression

Loci can show association with either chloroplast or mitochondrial haplotypes (analogous to linkage disequilibrium). This is called **cytonuclear disequilibrium** or **chloroplast capture**. Continued backcrossing to a single parental species may thus lead to the loss of all nuclear genetic variation of the other parental species at certain loci.

12 Speciation

The primary importance of geographic isolation for speciation is the reduction of gene flow between diverging lineages. In biogeographic context, speciation is often divided into **allopatric, parapatric and sympatric speciation**.



12.1 Allopatric speciation

Speciation is thought to occur most easily in allopatry. Consequently, allopatric speciation is often considered the default mode of speciation. It can be subdivided further into vicariant and peripatric speciation. These two speciation modes differ in the relative sizes of the populations involved (N_e) and the strength of selection. Both do not exhibit any gene flow. Experimental studies indicate that divergence in allopatry can be promoted by differential selection.

12.1.1 Vicariant speciation

In vicariant speciation reproductive isolation evolves after the geographic range of a species splits into two or more reasonably large, isolated populations. The following evidence for vicariant speciation exists:

- Geographic concordance of species border with existing geographic or climatic barriers.
- Allopatry of young sister species.
- Geographic coincidence of species borders or hybrid zones among different taxa.
- Absence of sister species in areas where geographic isolation was unlikely.
- Concordance between present or past geographic barriers and genetic discontinuities between species (phylogeography)

12.1.2 Peripatric speciation

Peripatric speciation often involves the invasion of novel habitats that exert strong selection. The other option is that a small population becomes geographically isolated. The number of founders is expected to be small (1-100). Genetic drift may further contribute to speciation. Classic cases take place on single islands or archipelagos.

12.2 Parapatric speciation

Parapatric speciation is midway between allopatric and sympatric speciation. Reproductive isolation evolves between two populations that exchange genes but do not do so freely. The biogeogrphic signature of parapatric speciation are newly formed sister species that have abutting ranges. Parapatric speciation always involves an interplay between genetic drift and selection. The two existing models of parapatric speciation are the **clinal model** (variable environment) and the **stepping-stone model** (discrete populations). A central problem is to reject an allopatric phase. Evidence for parapatric speciation includes

- A pair of closely related species with abutting distributions
- Multiple pairs of related species with abutting distributions especially at ecotones
- Morphological or genetic discontinuities at ecotones

Ecotones are borders between biomes, where communities meet and integrate.

12.3 Sympatric speciation

In sympatric speciation the isolating mechanisms evolve among the members of an interbreeding population. Sympatric speciation is probably not a common speciation mechanism. Even though it is theoretically possible, prerequisites for sympatric speciation are much more specific than for allopatric speciation. Evidence and criteria include

- An allopatric phase must be highly unlikely
- Species must be sister taxa
- Species must occur in sympatry
- Species must demostrate reproductive isolation
- Allopatric populations must not have an influence on sympatric divergence

12.4 Hybrid speciation

Generally, ancient hybridization can fuel accelerated adaptive radiation. In **homoploid hybrid species** the hybrid species has the same chromosome number as both parental species. In **polyploid hybrid species** hybridization is accompanied by genome duplication (polyploiddisation) which results in sterile backcrosses.

12.4.1 Homoploid hybrid speciation in Helianthus

Repeated hybridization between *Halianthus annuus* and *Helianthus petiolaris* led to the formation of three homoploid hybrid species. Strong natural selection was applied through transgressive segregation (occurence of extreme phenotypes in hybrids; can increase or decrease fitness). In experiments only 5% of F_1 hybrids were fertile. However after only five generations hybrid fertility was over 90%

12.4.2 Ancient hybridization fuels rapid cichlid fish adaptive radiations

The Lake Victoria Region Superflock encompasses over 700 cichlid fish species that evolved over the last 150'000 years. "Hybridization between two divergent lineages facilitated this process by providing genetic variation that subsequently became recombined and sorted into many new species. Notably, the hybridization event generated exceptional allelic variation at an opsin gene known to be involved in adaptation and speciation."