Selection tests

Plant Genetic Resources (3502-470)

23 May 2025

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1 Motivation

The diversity of crops and landraces is the result of artificial selection by humans. Over time, the selection of favorable traits leads to the enrichment or fixation of genes, which control these traits, in the population. One example are different crops from the two species *Brassica rapa* and *Brassica oleracea*. From these two closely related wild ancestors humans selected multiple vegetable crops, which differ by their phenotypic properties (Figure 1)

For example from the wild mustard *Brassica oleracea* cauliflower developed from the selection of flower buds, whereas kohlrabi evolved by the selection of the stem (Figure 2).

Our knowledge of selection during domestication and modern plant breeding has led to the concept that different genes are selected and fixed at different stages of crop evolution. Figure 3 shows a modification of the original concept by Tanskley and McCouch of different funnels influencing genetic diversity.

Genes or genomic regions, which do not control phenotypic traits under selection, remain polymorphic because only genetic drift and bottleneck effects but not selection influence their diversity. In contrast, genes controlling domestication traits are fixed early in crop evolution and remain

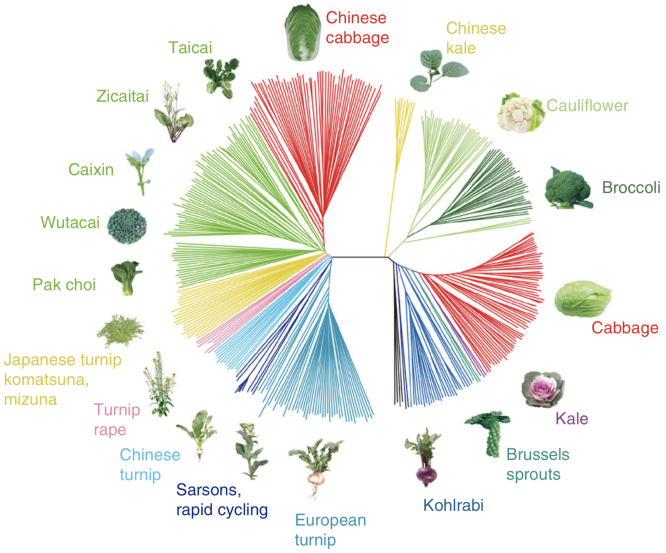


Figure 1 – *Phylogeny of vegetable crops derived from* Brassica rapa *and* Brassica oleracea*. Source: Cheng et al. (2016)*

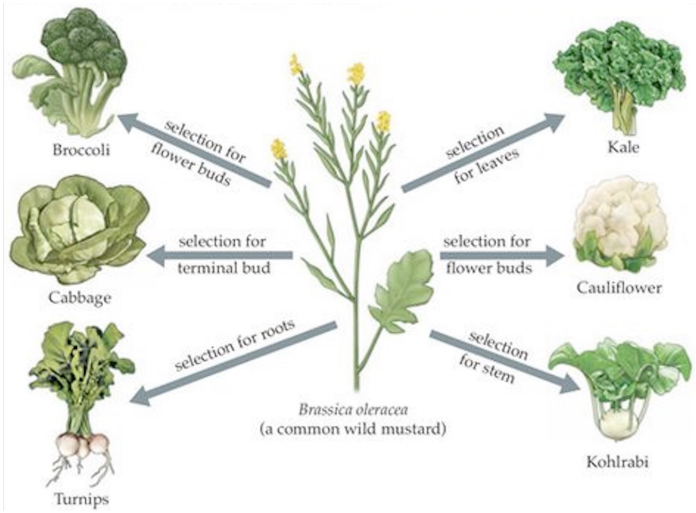


Figure 2 – *Summary of traits that were selected in the different types of* Brassica oleracea *vegetables. Source: Unknown.*

monomorphic (i.e., lack genetic diversity) because they continue to be selected. New mutations, which occur rarely, are removed by selection. A third group of genes are improvement genes, which contribute to agronomic properties and they are selected in modern plant breeding programs. These genes differ from domestication genes and they are diverse among landraces, but strongly selected in modern breeding material and varieties and devoid of variation.

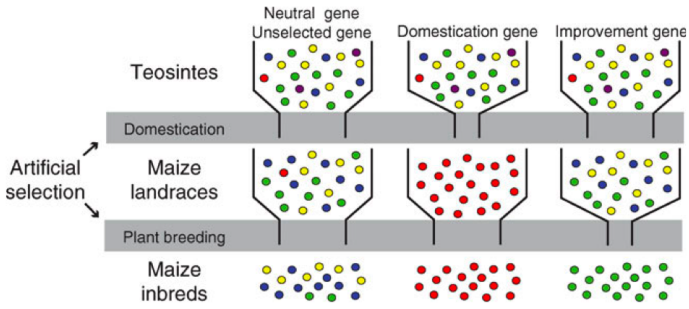


Figure 3 *– Loss of genetic diversity in different stages of crop evolution. Source: Yamasaki et al. (2005)*

Genes controlling phenotypic traits can be identified by two approaches:

Genetic mapping Parents that differ in interesting phenotypic traits are crossed. In the resulting offspring genomic regions controlling these traits are identified by QTL mapping. In genetically diverse material, genome-wide association studies (GWAS) can also be conducted to identify marker-trait associations.

Selection scans This approach scans the genome for regions with unusual patterns of genetic variation using so-called tests of selection. The function of genes with a signature of selection is then further investigated using mutagenesis or genome editing.

Genetic mapping starts with the phenotype of interest, whereas selection scans start with genetic diversity and is agnostic with respect to the phenotype.

One example is shown in Figure 4, where genetic diversity in modern breeding material, landraces and the wild ancestor of maize were compared in hundreds of genes and those genes identified, which had no genetic diversity in modern material. Several of these genes were known to control traits that are selected in modern breeding programs.

Generally, an analysis of genetic variation in the context of plant genetic resources is useful to determine the patterns and levels of genetic variation in genes and genomic regions of interest for plant breeding, and to identify patterns of genetic relationships in genetic resources. Knowledge about patterns of genetic variation helps to

- identify groups of genetically distinct individuals for breeding (**core collections**)
- find geographic regions with high levels of genetic variation, for additional collections of genetic resources
- identify suitable populations for **introgression** into modern breeding populations
- establish **heterotic groups** for hybrid breeding programs

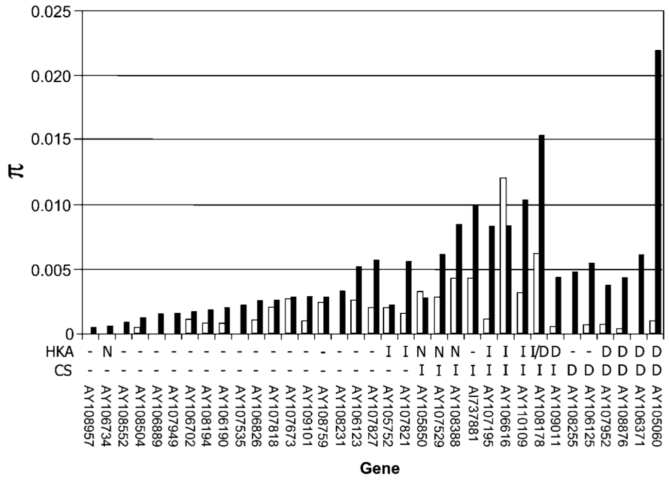


Figure 4 *– Nucleotide diversity π in landraces and teosintes for each of 35 genes with zero diversity ($\pi = 0$) in modern inbred lines. Black bars indicate genetic diversity in the teosintes, and white bars indicate genetic diversity in the landraces. If no white bars are present, genetic diversity in the landraces was zero. Letter below row: I, improvement candidate; D, domestication candidate. Source: Yamasaki et al. (2005)*

- estimate the number of accessions in a gene bank collection that need to be sequenced to identify nearly all alleles segregating at a locus (**allele mining**)

Molecular population genetics is concerned with the genome-wide analysis of observed genetic variation. This branch of population genetics developed in the 1960s with the invention of techniques such as gel electrophoresis that brought about the possibility of monitoring genetic variation within and between species, and to characterize evolutionary forces such as selection, recombination and genetic drift. The rapid progress of DNA sequencing technologies allows the resequencing of the complete genome of numerous genotypes and the characterization of genome-wide genetic variation.

The basic theory of molecular population genetics is the **Neutral theory of molecular evolution**, which was mainly developed by the Japanese scientist Motoo Kimura (Figure 5). The theory states that the majority of polymorphisms segregating in a population are neutral and have no fitness effects. This statement is based on two main assumptions:

1. Strongly **advantageous** mutations are **fixed** rapidly in the population and therefore do not segregate.
2. Strongly **deleterious** mutations are **removed** rapidly in the population and therefore do not segregate.

Figure 5: Motoo Kimura (1914-1994) Source: Wikipedia

The neutral theory allows simple and testable predictions of expected patterns of genetic variation in a population. For this reason, the neutral theory is frequently used as a **null hypothesis** in tests of natural selection.

2 Application of the theory

A key feature of the neutral theory of molecular evolution (often called *neutral model*) is that it allows simple predictions.

Assume that you want predict the expected of level of genetic variation at a locus under the neutral model using a sample of n sequences. Remember that $\theta = 4N_e\mu$ is the scaled (by population size) mutation rate. Let S be the number of segregating sites and Π the number of mismatches per pair of sequences. The expected values can be computed from coalescent theory,

$$E(S) = \theta \underbrace{\sum_{i=1}^{n-1} i^{-1}}_{:=a_n} \tag{1}$$

and

$$E(\Pi) = \theta \tag{2}$$

Since S and Π can be observed in a sample, estimates $\theta_S = S/a_n$ (Watterson's estimator) and $\theta_\Pi = \Pi$ (Tajima's estimator; note that θ_{Pi} and π are the same) can be calculated.

Other properties of genetic variation like the expected site frequency spectrum of polymorphisms can also be derived under the neutral theory.

3 Types of natural selection

In the analysis of genetic polymorphisms, three major types of natural selection are distinguished:

Purifying or negative selection With this type of selection, deleterious mutations are removed from the population. This is likely the most frequent type of selection because most new mutations are assumed to be deleterious.

Directional selection It causes the replacement of alleles with a new advantageous mutation. This type of selection is also called positive or Darwinian selection.

Balancing selection It is observed if more than one allele of a locus is advantageous. As a consequence, two or more alleles are maintained by selection at a locus.

These different types of selection leave different footprints of genetic variation in the genome and therefore can be differentiated from each other, and also from neutral evolution.

Each type of selection leaves a different footprint of genetic variation in the genome, and tests of selection identify these footprints by analysing patterns of polymorphisms.

3.1 Outline of a selective sweep

In the following, we describe a model for the fixation of a new, advantageous mutation by selection, or a **selective sweep**. During a sweep the following parameters change:

- Level of genetic diversity
- Changes in allele frequencies
- Extent of linkage disequilibrium (LD)

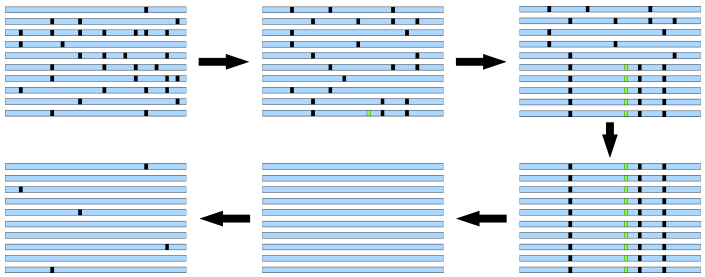


Figure 6 – *Example of a selective sweep. Shown is a sample of 10 chromosomes from a population. The black squares indicate neutral polymorphisms. An advantageous polymorphism arises by mutation and sweeps to fixation. During this process, linked neutral variants are also fixed or removed, if they are not linked to the advantageous mutation. After the sweep, new mutations arise, which originally segregate at low frequency in the population.*

Figure 6 shows a typical selective sweep and shows that a sweep can be divided into several stages:

- *Early stage:* Intermediate, "normal" LD
- *Intermediate stage:* Strong LD, high proportion of derived polymorphisms, strong differentiation between haplotypes of ancestral and derived (selected) polymorphism
- *Final stage:* Lack of polymorphism, very strong LD
- *After sweep:* Excess of rare polymorphisms

4 *Discussion questions*

1. How does a selective sweep influence the diversity statistics Π and P? Are they influenced differently?
2. Are there other genetic/demographic mechanisms which could cause a diversity pattern resembling a selective sweep (in the sweep region)?

4.1 The “genomic neighborhood” of a sweep

Differences in genetic diversity throughout the genome can be investigated with a **sliding window** approach. With this method, regions with a strongly reduced level of neutral polymorphism in a region can be detected (Figure 7). The length of the window with reduced variation depends on

- the *rate of recombination*, c
- the *strength of selection*, s

Discussion questions

1. How does a high recombination rate affect the window size of low polymorphism?
2. How does a high selection coefficient affect the window size of low polymorphism?
3. How does the level of linked polymorphisms look with balancing selection?

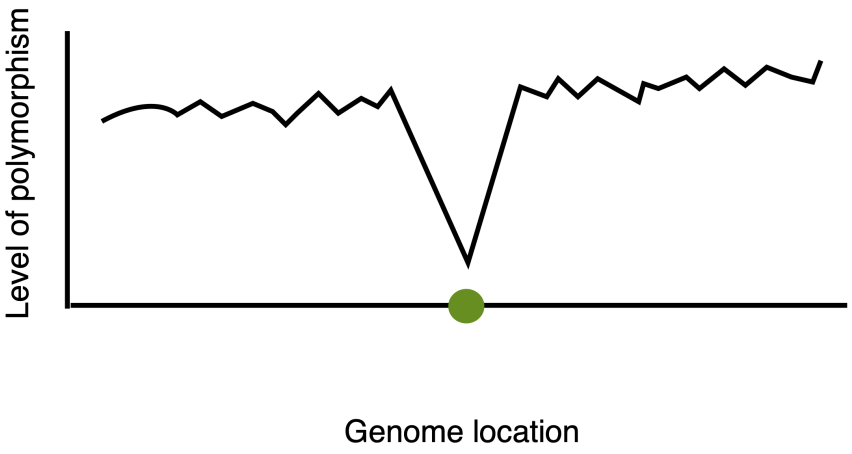


Figure 7 – *Genomic neighborhood of a selective sweep. A selective sweep is indicated by a drop in genetic diversity in a region, which harbors the advantageous polymorphism. Walsh (2008)*

5 Tests of neutral evolution

There are several types of neutral evolution, but they all have in common that the null hypothesis is a **model of neutral evolution** that assumes that no selection occurred in a population. Several **tests of neutral evolution** were developed to analyse whether the observed patterns of genetic variation have been influenced by non-neutral evolution (i.e., selection). Tests are differentiated by the types of polymorphisms that are investigated:

- Polymorphisms *within* species (e.g. Tajima's D)
- Polymorphisms *within* and *between* species (e.g. HKA-test)
- Synonymous and nonsynonymous polymorphisms, and synonymous and nonsynomyous substitutions between species (e.g. McDonald-Kreitman-Test)
- Synonymous and nonsynonymous substitutions between species (e.g. d_N/d_S ratios)

In the following the widely used Tajima's D statistic is further described.

5.1 Tajima's *D*

Tajima's D, one of the simplest tests of natural selection, is based on different estimators of the population mutation parameter $\theta = 4N\mu$.

As we already discussed, it was shown by Watterson (1975) that θ can be estimated with an **infinite sites** model as

$$\hat{\theta}_S = \frac{S}{a_n} \tag{3}$$

with S as the number of polymorphisms at a locus and

$$a_n = 1 + \frac{1}{2} + \ldots + \frac{1}{n-1}$$

as a constant that contains the sample size. In an infinite sites model, each mutation hits a different nucleotide position. For this reason, there are only two alleles at each polymorphic site, which is the case for the vast majority

of single nucleotide polymorphisms. Tajima (1983) showed that θ can be estimated from the nucleotide diversity, π, as

$$\hat{\theta}_{\Pi} = \pi L = \Pi \quad (4)$$

with L as the sequence length. The two estimators differ in one important property: θ_S depends only on the number of segregating (i.e., polymorphic) positions in the sample, whereas θ_{Π} is also affected by the *relative frequency* of the polymorphisms. If there is no selection at a locus, both estimators should give the true value $\theta = 4N\mu$, hence $\theta_{\Pi} = \theta_S$ (Tajima, 1989). If one locus evolves under non-neutral evolution, both estimators are affected differently and this information can be used to identify the type of selection.

If an advantageous mutations is fixed by directional selection, linked but neutral polymorphisms also go to fixation by a process called **genetic hitchhiking**. As a consequence, the level of polymorphism is reduced. After such a selective sweep, new polymorphisms arise by new mutations, which initially segregate at low frequencies in a population (Figure 6). Therefore, some time after the completion of a selective sweep, an excess of rare polymorphisms is expected at a locus. Since θ_S depends only on the number of polymorphisms at a locus, it is not influenced by the frequency of the polymorphisms. On the other hand, polymorphisms of low frequency have little effect on the θ_{Π} estimator. For this reason θ_{Π} is lower than θ_S ($\theta_{\Pi} < \theta_S$).

A similar pattern is expected with purifying selection because disadvantageous alleles are removed from the population and therefore occur at a lower frequency in the population than neutral polymorphisms. If there is balancing selection, polymorphisms are retained at intermediate frequencies in the population, and for this reason, θ_{Π} is expected to be larger than θ_S ($\theta_{\Pi} > \theta_S$).

Tajima's D is then calculated as

$$D = \frac{\theta_{\Pi} - \theta_S}{\sqrt{\hat{V}(\theta_{\Pi} - \theta_S)}}, \quad (5)$$

which represents the *standardized* difference of both estimators. Under neutral evolution, $D = 0$, after a selective sweep and during purifying selection, $D < 0$. Under balancing selection, $D > 0$.

The null hypothesis of the test of selection is then $H_0 : D = 0$. Now it has to be tested whether the observed value of D at a locus differs significantly from D, which can be tested with coalescence simulations of several thousand values of D under a neutral model. For these simulations, only the sample size and the number of observed polymorphisms is needed. Subsequently, a frequency distribution of simulated D values is generated and the critical values that describe the outer 5% of the distribution are identified. If the observed value is located in the outer 5% of the distribution, the difference from the neutral model is considered to be significant and the hypothesis of a neutral evolution at a given locus is rejected.

This test is shown using an example of the *white* locus from the fruit fly *Drosophila melanogaster* using RFLP (Restriction fragment length polymorphism) data (Table 1)

Table 1 – *Estimates of $\hat{\theta}_S$, $\hat{\theta}_\Pi$ and Tajima's D for three types of polymorphism at the* white *locus of* Drosophila melanogaster *($n = 64$). Source: Gillespie (2004)*

Type of polymorphism	S	$\hat{\theta}_S$	$\hat{\theta}_\Pi$	D
RFLP	53	11.21	11.92	0.213
Small insertions/deletions	40	8.46	10.02	0.607
Large insertions/deletions	15	3.17	0.94	-2.071

The D values for the RFLP polymorphisms and the small insertions/deletions (indels) are close to zero, whereas the value for the large indels are highly negative. However, it has to be tested whether this value differs significantly from $D = 0$.

To summarize, a Tajima's D value that is significantly lower than expected under neutrality ($D = 0$) may originate from three causes:

1. **Selective fixation of advantageous mutations ("selective sweep")**: After the fixation of an advantageous allele and the removal of most genetic variation at a locus, new mutations arise that segregate initially at a low frequency.
2. **Background or purifying selection:** Deleterious alleles and any polymorphisms that are linked to it are dragged towards a lower frequency by selection.
3. **Demographic history:** Recent population growth, presence of a migrant individual or strong self-fertilization can all contribute to a significantly negative Tajima's D.

On the other hand, a Tajima's D value that is significantly higher than expected under neutrality ($D = 0$) may originate from these causes:

1. **Balancing selection**: Multiple alleles are maintained in the population which segregate at intermediate frequencies; special cases include heterozygote advantage, frequency-dependent selection, and also spatial variation
2. **Demographic history**: Population shrinkage or population substructure can result in a signifcantly positive Tajima's D

All causes together can cause a significant deviation of Tajima's D statistic from the expected neutral value. For this reason, if it has been shown that the observed value is significantly different, further analyses are required to test which of the processes is mainly responsible for the observed pattern.

5.2 Coalescent simulations

The critical values for a significant difference of expected and observed values of D can be obtained with coalescent simulation. For this, several thousand simulated samples with the same number of alleles (n) and polymorphisms (S) are generated.

The following is an example of a simulation of 10 chromosomes with a $\theta = 10$.

```
ms 10 1 -theta 10
59243

//
segsites: 15
positions: 0.0125 0.1566 0.2156 0.2630 0.3059 0.3209 0.3631 0.3861 0.4501 0.4708 0.7044 0.7493 0.8514
101000110000110
101000000010011
000000000100000
101010000001010
000001001000000
101000000000010
101010000001010
111100000000010
000000001000000
101100000000010
```

From these simulated samples, the expected distribution of Tajima's D values is generated The value `segsites` gives the number of polymorphisms, the line `positions` gives the relative positions of the polymorphisms in the sequence. They are distributed randomly. The `0` and `1` characters are the simulated polymorphisms from which Tajima's D is calculated. The expected distribution fo Tajima's D with $n = 64$ and $S = 15$ (which corresponds to the values of the large indels in Table 1) for 10,000 simulations is shown in Figure 8.

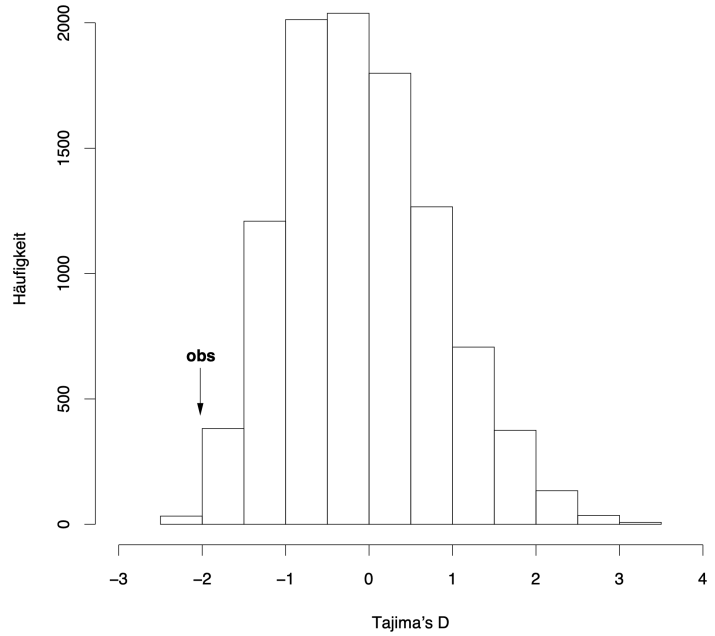


Figure 8 *– Histogram of 10,000 coalescent simulations of a neutral standard model (no population structure, no recombination, constant population size) with parameters $n = 64$ and $S = 15$. The arrow indicates the observed value of Tajima's D for the large indels.*

There are two possibilities to test for significance. In a **one-sided test** one investigates whether the observed value is larger than the one from a null hypothesis. In this case, the critical values are obtained from the right edge of a simulated distribution that covers the outer 5% of the distribution (95% percentile). If one wants to test whether the observed value is significantly

smaller than the observed one, the left margin of the distribution is used (5% percentile).

Alternatively, a **two-sided test** tests whether the observed value differs significantly from the expected value, irrespective of the direction. In this case the critical values are calculated for the left 2.5% and the right 2.5% of the distribution and a subsequent test of whether the observed value is located in these regions.

In our example, the observed values for the large indels ($D = -2.071$) is smaller than the 2.5% percentile of the simulated distribution ($D = -1.66$) and therefore can be considered as significantly different from $D = 0$ in a two-sided test.

Table 2 *– Results of the coalescent simulation assuming a neutral model with the Drosophila melanogaster RFLP data*

Polymorphism	Observed	2.5% Percentile	97.5% Percentile
RFLP	0.213	-1.618	2.172
Small indels	0.607	-1.672	1.758
Large indels	-2.071	-1.400	2.539

Discussion questions

1. Why do coalescent simulations produce a *distribution* of Taima's D values and not the same value in all simulations?
2. Observe Tajima's D at many loci. How can it be explained if a *large proportion* of values deviates from the null distribution?

6 Interpretations of deviations from the null hypothesis

The interpretation of tests of neutrality needs to consider that not only natural selection but other evolutionary processes as well affect patterns of genetic diversity and the statistics that describe this variation. These processes include variation in recombination rate between genes, the presence of a population structure and past changes in population size. The latter two processes, in addition with rates of outcrossing are frequently summarized as the **demographic history** of a species.

For example, are rapid population growth in the past leads to gene genealogies with long terminal branches. In this case, an excess of rare polymorphisms and a negative value of Tajima's D is expected (Figure 9). Since all loci of a genome are affected in a similar fashion from population growth, one expects a negative value for Tajima's D for most loci in the genome. By comparing multiple loci with a particular locus, on can determine whether the pattern of genetic variation at a locus differs significantly from the rest of the genome and therefore results from selection. In *Arabidopsis thaliana*, there is a significant deviation from the neutral standard model (no population structure and population growth, random mating).

Menawhile, similar studies were also conducted in other plant species (Table 3).

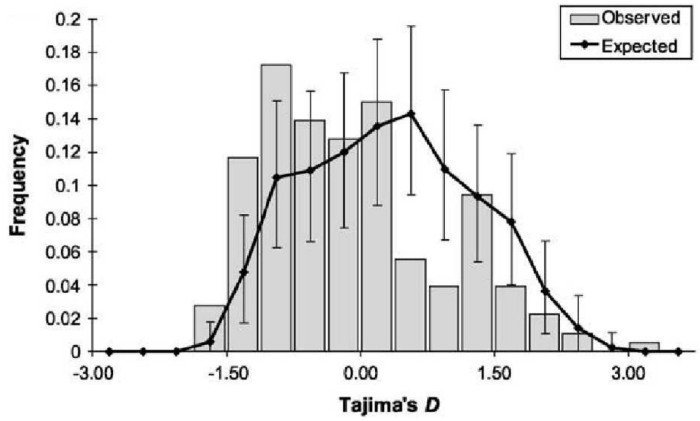


Figure 9 *– Observed and expected distribution of Tajima's D values in 195 short genomic regions of 400 bp in 12 individuals of the model species* Arabidopsis thaliana*. The expected distribution corresponds to the mean value of 10,000 simulations with the same number of loci and the same number of individuals and polymorphisms in each of the individual loci. Source: Karl J. Schmid et al. (2005a)*

Table 3 *– Summary of Tajima's* D *values in different studies.*

Species	Accessions	Loci	Length	π_S	Tajima's D	P Value	Reference
Arabidopsis thaliana	96	846	583	0.007	-0.8	*	Nordborg et al. (2005)
Arabidopsis thaliana	12	185	414	0.010	-0.4	*	Karl J. Schmid et al. (2005b)
Arabidopsis lyrata	140	77	530	0.0135	0.32	*	Ross-Ibarra et al. (2008)
Boechera drummondii	46	86	591	0.0041	-0.46		Song et al. (2009)
Inbred Maize	14	774	?		0.04	n.s.	Wright et al. (2005)
Teosinte	16	774	?		-0.50	*	Wright et al. (2005)
Sorghum bicolor	16	204	671	0.0038	-0.08		Hamblin et al. (2006)

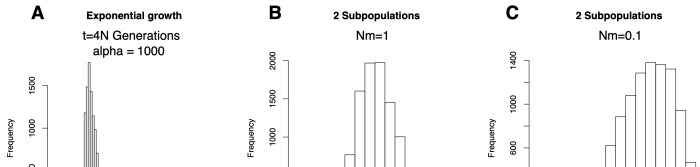
If the standard neutral model is violated, a modified null model must be used that incorporates the particular demographic history of a species to obtain genome-wide distributions of test statistics.

Figure 10 shows simulated distributions of Tajima's D under the assumption that the sample size in the past increased exponentially or that the population from which the sample was taken is subdivided into two equal subpopulations with different migration rates between the populations ($Nm = 0.001$ und $Nm = 1$). The parameter Nm corresponds to the number of migrants per generation.

The three models are defined by the following parameters:

- Growth rate: $N(t) = N_0 \exp^{-\alpha t}$, t is the time *before present*, measured in units of $4N_0$ Generations
- $N(t)$ Population size in the past, N_0 current population size
- Two populations which exchange Nm alleles per generation

To summarize, coalescent simulations are an important tool in the study of genetic variation because they allow the testing of evolutionary hypotheses regarding the history of a sample. In the recent past, coalescent simulations were used mainly for investigating basic population genetic questions, but they are now increasingly used to address more applied questions such in the context of plant breeding and genetic resources.



□ Selective sweep □ Tajima's *D* statistic □ Test of selection

8 Summary

- There are three types of selection at the molecular level: Purifing, positive and balancing selection.
- Several tests of neutral evolution were developed that are based on the comparison of nucleotide variation within and between species, and on the comparison of divergence between species.
- Tajima's D is an important test for the analysis of selection acting on individual genes. This summary statistic measures the frequency distribution of polymorphisms.
- Coalescent simulations are used to test whether the observed levels of Tajima's D and other summary statistics are consistent with the neutral level of sequence variation.

9 Further reading

- Hartl and Clark, Principles of population genetics, Chapter 4
- Nielsen and Slatkin, An introduction to population genetics, Chapters 6 to 9
- Jensen et al. (2007) , Approaches for identifying targets of positive selection. Trends in Genetics 23:568-577 (2007)
- Walsh (2008), Using molecular markers for detecting domestication, improvement and adaptation genes. Euphytica 161:1-17 - A bit dated, but still useful introduction to the topic

10 Study questions

- How is Tajima's D statistic defined and which properties of genetic variation does it measure?
- How is a test of neutral evolution of a locus conducted with the help of the Tajima's D statistic and coalescent simulations?
- Why is the expected D value of a neutral locus zero?
- How does either a selective sweep or exponential population growth cause a negative Tajima's D?
- How does Tajima's D change in the different stages of a selective sweep? (See Figure 6)
- How does either balancing selection or recent population admixture cause a positive Tajima's D?
- How is it possible to differentiate between selection or demography as causes of non-zero values of Tajima's D at a locus?

11 Problems

1. Answer the discussion questions in the text above.

2. A large number of tests of selection were developed. In addition to comparing allele frequencies, one general approach is to compare the haplotype length of selected and unselected alleles at a locus. Check out Figures 1 and 6 of the paper by Voight et al. (2006), which implements the Extended Haplotype Heterozygosity (EHH) test and the integrated Haplotype Score (iHS). Describe the key principle of this test of selection in works.
3. Figure 11 shows an analysis of diversity (ROH: reduction of diversity) and selection (PiHS, a variant of the iHS test) in *Brassica oleracea* (Cheng et al., 2016). The statistics were calculated from the resequencing data of all different cabbage types (cauliflower, etc.) in the study. How big is the correspondence between both selection tests? How do you interpret the results with respect to the numbers of genes and genomic regions involved in domestiation of *B. oleracea* and the subsequent differentiation into crops?

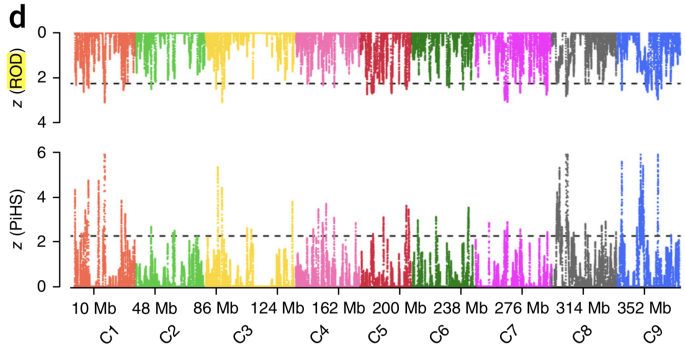


Figure 11 – *Reduction of diversity (ROF) and PiHS tests of selection in the genome of* Brassica oleracea*. The different colors indicate the chromosomes. Source: Cheng et al. (2016)*

4. Figure 12 shows the genome-wide distribution of nucleotide diversity and Tajima's *D* of two wild amaranth species (*A. hybridus* and *A. quitensis*) and three grain amaranth species (*A. caudatus*, *A. cruentus* and *A. hypochondriacus*). *A. hybridus* is the ancestor of the three grain amaranth species. What are the difference in nucleotide diversity between domesticated and wild amaranths? What are the values of Tajima's *D* between the five groups. Do they fit the expectation of a simple domestication and selection model?

Cheng F, Sun R, Hou X, Zheng H, Zhang F, Zhang Y, Liu B, Liang J, Zhuang M, Liu Y, Liu D, Wang X, Li P, Liu Y, Lin K, Bucher J, Zhang N, Wang Y, Wang H, Deng J, Liao Y, Wei K, Zhang X, Fu L, Hu Y, Liu J, Cai C, Zhang S, Zhang S, Li F, Zhang H, Zhang J, Guo N, Liu Z, Liu J, Sun C, Ma Y, Zhang H, Cui Y, Freeling MR, Borm T, Bonnema G, Wu J, Wang X. 2016. Subgenome parallel selection is associated with morphotype diversification and convergent crop domestication in Brassica rapa and Brassica oleracea. *Nature Genetics* **48**:1218–1224. doi:10.1038/ng.3634

Gillespie JH. 2004. Population genetics: A concise guide. Baltimore: John Hopkins University Press.

Hamblin MT, Casa AM, Sun H, Murray SC, Paterson AH, Aquadro CF, Kresovich S. 2006. Challenges of detecting directional selection after a bottleneck: Lessons from Sorghum bicolor. *Genetics* **173**:953–64. doi:10.1534/genetics.105.054312

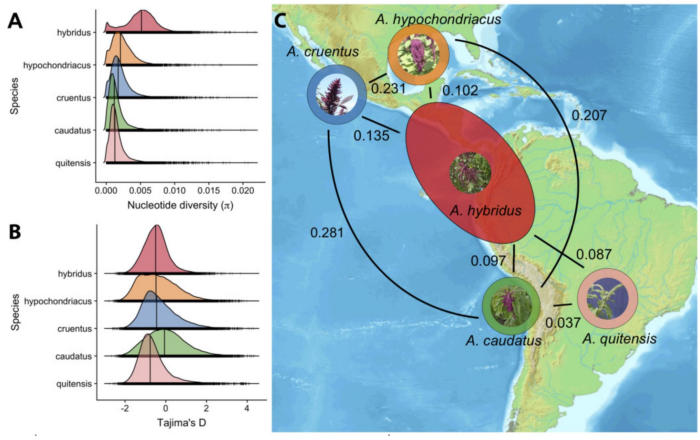


Figure 12 *– Nucleotide diversity (A), Tajima's* D *(B) and evolutionary relationship (C) of wild and cultivated amaranths. Source: Stetter et al. (2020)*

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