Plant breeding - principles and methods - practical exercises

Inheritance of qualitative traits – Analyze segregation results and predict the genetic basis of a trait

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Literature


Overview

Molecular basis of allele variations

Single gene inheritance: dominant and recessive
incomplete dominance
codominance, overdominance
recessive lethal alleles

Two genes inheritance: epistasis
additive genes

Chi-square Test ($\chi^2$-Test) to test for segregation ratios

Analyze segregation results and predict the genetic basis of a trait
Qualitative versus quantitative traits

**Qualitative traits** – controlled by one (few) major gene(s), phenotypic variation can be separated into distinct classes; generally, the environment has little influence.

**Quantitative trait** – controlled by several to many genes having small, cumulative effects, can be measured in quantitative units that are continuous, and is often considerably influenced by environment.

Some characters difficult to categorized. Major gene(s) modified by minor genes plus environmental effects -> the phenotype of such characters may show continuous variation.

-> The range of variation for a particular trait indicates the mode of inheritance of that trait.

To determine the mode of inheritance: mate plants having contrasting phenotypes and evaluate the performance of their offspring. The proportion of progeny exhibiting different phenotypes provides information about the proportion of progeny possessing different genotypes.
Molecular basis of allele variations

Alleles: alternative forms (of the same gene) at one genetic locus
Caused by mutations of the DNA codes (Substitution/Insertion/Deletion)
Many alleles at one locus possible: the ‘normal functional’ – wild-type allele and mutant alleles.

Point mutation: **SNP** (single nucleotide polymorphism) in gene *Cbd* of cotton

**INDEL** (Insertions-Deletions polymorphism): in gene *Cbd* of cotton

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Molecular basis of allele variations

**Wild type**

The expression of the products of wild-type alleles produces wild-type phenotype.

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous</td>
<td></td>
</tr>
</tbody>
</table>

**Loss of function:** Null/amorphic mutation

Null alleles produce no functional product. Homozygous ‘nulls’ have mutant phenotype - no gene product. Heterozygous produce less functional gene product than homozygous wild-types and may have mutant phenotype.

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous</td>
<td>None</td>
</tr>
<tr>
<td>Heterozygous</td>
<td></td>
</tr>
</tbody>
</table>

**Mutant phenotype visible in heterozygous state?**

- Haplosufficiency of wild type allele (= dominant) – mutant allele not visible
- Haploinsufficiency of wild type allele (= recessive) – mutant allele visible
Molecular basis of allele variations

**Gain of function**: Hypermorphic mutation

Excessive expression of the gene product leads to excessive gene action. The mutant phenotype may be more severe or lethal in the homozygous than in the heterozygous.

**Gain of function**: Neomorphic mutation

The mutant allele has novel function that produces a mutant phenotype in homozygous and heterozygous organisms, and may be more severe in homozygous organisms.
Single gene inheritance: dominant and recessive

The purple inheritance factor is dominant and the factor governing “white” is recessive.

Complete dominance: homozygous dominant cannot be distinguished from heterozygous, $A/A = A/a$.

$A/A →$ active enzyme $→$ purple pigment
$A/a →$ active enzyme $→$ purple pigment
$a/a →$ no active enzyme $→$ white

Genotype: $1 A/A : 2 A/a : 1 a/a$

Phenotype: $3 : 1$
Incomplete dominance, partial dominance

The occurrence of intermediate phenotypes:
1 : 2 : 1 segregation in the F2
Classical example for flower colors:
In the heterozygous the amount of anthocyan is half – pink, dosage effect

Codominance

Codominance is defined as the expression in a heterozygote of both the phenotypes normally shown by the two alleles.
**Overdominance**

Phenotype of heterozygous outperforms phenotype of homozygous parental lines.

Overdominance is a genetic model for heterosis; it posits that increased heterosis is the result of positive interactions between two functional alleles that leads to a phenotypic value beyond the range of both homozygous parents.

![Overdominance vs Dominance](image)

**Heterosis, hybrid vigor**

Heterosis is a phenomenon whereby the phenotype of F1 hybrids is superior to that of their parents.

Other hypothesis: dominance, epistasis

**Underdominance** is the opposite of overdominance
Recessive lethal alleles

Mutant alleles of essential genes capable of causing death are called lethal alleles.

Mostly recessive, dominant lethal alleles are rarely maintained in populations.

Expected monohybride segregation ratio from 1:2:1 would just be found in zygotes. But zygotes with the homozygous lethal alleles do not survive and can not be counted -> 2:1 ratio.

Recessive sublethal allele: Letalität varies from 0 to 100 %, depending from the gene, rest of the genome and environment.
### Summary: alleles of one gene

<table>
<thead>
<tr>
<th>Type of dominance</th>
<th>$A^1/A^1$</th>
<th>$A^2/A^2$</th>
<th>$A^1/A^2$ hybrids</th>
<th>$F_2$</th>
<th>$F_\infty$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3:1</td>
</tr>
<tr>
<td>Complete</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3:1</td>
</tr>
<tr>
<td>Incomplete</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1:2:1</td>
</tr>
<tr>
<td>Codominant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1:2:1</td>
</tr>
<tr>
<td>Overdominance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1:2:1</td>
</tr>
<tr>
<td>Lethal allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1:2</td>
</tr>
</tbody>
</table>

- $A^1$ is dominant to $A^2$ and $A^2$ is recessive to $A^1$.
- $A^2$ is dominant to $A^1$ and $A^1$ is recessive to $A^2$.
- $A^1$ and $A^2$ are incompletely dominant relative to each other.
- $A^1$ and $A^2$ are codominant relative to each other.
- $A^1$ and $A^2$ are together overdominant.
Summary: Interactions of alleles

The leaves of clover plants show several variations on the dominance theme. The different chevron forms (and the absence of chevrons) are determined by a series of alleles of one gene. The figure shows the many different types of interactions that are possible, even for one allele.

A gene can have several different states or forms — called *multiple alleles*. The alleles are said to constitute an allelic series, and the members of a series can show various degrees of dominance to one another.
Two genes inheritance

The F2 genotypes of 2 independently assorting genes with complete dominance result in a 9 : 3 : 3 : 1 ratio of phenotypes, provided there is no interaction between the genes. If there is interaction that renders two or more of the phenotypes indistinguishable, then the F2 ratio is modified. Epistasis is the interaction of alleles at different loci. The value of an allele or genotype at one locus depends on the genotype at other epistatically interacting loci, complicating the picture of gene action.

**no interaction:** unmodified ratio 2 dominant genes

<table>
<thead>
<tr>
<th></th>
<th>F2</th>
<th>F∞</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant epistasis</td>
<td>12:3:1</td>
<td>2:1:1</td>
</tr>
<tr>
<td>Dominant-inhibitory epistasis</td>
<td>13:3</td>
<td>1:3</td>
</tr>
<tr>
<td>Duplicate dominant epistasis</td>
<td>15:1</td>
<td>3:1</td>
</tr>
<tr>
<td>Duplicate recessive epistasis</td>
<td>9:7</td>
<td>1:3</td>
</tr>
<tr>
<td>Recessive epistasis</td>
<td>9:3:4</td>
<td>1:1:2</td>
</tr>
<tr>
<td>Polymeric gene interaction</td>
<td>9:6:1</td>
<td>1:2:1</td>
</tr>
<tr>
<td><strong>no interaction:</strong> Additive genes</td>
<td>1:4:6:4:1</td>
<td>1:2:1</td>
</tr>
</tbody>
</table>
Dominant epistasis

Also known as **masking action** and simple epistasis. A dominant allele at one locus masks the expression of both alleles (dominant and recessive) at another locus.

Example: color of the hull in oat seeds
Black-hull phenotype -> dominant allele A
Gray-hull phenotype -> another dominant allele B
this effect is apparent only in **aa** genotypes.

F1 genotype Aa Bb (black)

F2
9/16 A B   black hull
3/16 A  bb  black hull
3/16 aa  B   gray hull
1/16 aa  bb  white hull
Dominant-inhibitory epistasis

Also known as inhibitory gene interaction, or dominant-recessive interaction.
A dominant allele (A) either homo- or heterozygous of one gene and a homozygous recessive allele (bb) of another gene produce the same phenotype.

The green colour of plants is governed by the gene A which is dominant over purple colour. The purple colour $\rightarrow$ dominant B.
Two genes inheritance

**Duplicate dominant epistasis**

Also known as **duplicate action**.
Two genes control a trait and only one dominant is necessary to express the trait. Just the double recessive shows the mutation.

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**Example: bean flower color**

- **PpRr** (Purple) \( \times \) **Pprr** (Purple)

**F2**

<table>
<thead>
<tr>
<th>Gamete</th>
<th>ABB</th>
<th>ABb</th>
<th>AaBb</th>
<th>AAbb</th>
<th>aabb</th>
</tr>
</thead>
<tbody>
<tr>
<td>aB</td>
<td>ABB</td>
<td>Abb</td>
<td>AbBb</td>
<td>ABBb</td>
<td>aBbb</td>
</tr>
<tr>
<td>Ab</td>
<td>Abb</td>
<td>ABB</td>
<td>AAbb</td>
<td>AaBb</td>
<td>abbb</td>
</tr>
<tr>
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<td>AAb</td>
<td>AaBb</td>
<td>Aabb</td>
<td>AaBB</td>
<td>aBb</td>
</tr>
<tr>
<td>Ab</td>
<td>ABB</td>
<td>Abb</td>
<td>AbBb</td>
<td>AAbb</td>
<td>aBBb</td>
</tr>
<tr>
<td>aB</td>
<td>ABB</td>
<td>Abb</td>
<td>AbBb</td>
<td>AAbb</td>
<td>aBbb</td>
</tr>
</tbody>
</table>

**Example:**

- **9/16** (P-R-) Precursor I \( \rightarrow \) Anthocyanin \( \rightarrow \)** Purple
- **3/16** (P-rr) Precursor I \( \rightarrow \) Anthocyanin \( \rightarrow \)** Purple
- **3/16** (ppR-) Precursor I \( \rightarrow \) Anthocyanin \( \rightarrow \)** Purple
- **1/16** (pprr) Precursor I \( \rightarrow \) No pigment \( \rightarrow \)** White

Quelle: Sanders
Two genes inheritance

**Duplicate recessive epistasis**

Also known as **complementary epistasis**. When recessive alleles at either of the two loci can mask the expression of dominant alleles at the two loci, it is called duplicate recessive epistasis.

At least one dominant allele from each of the two genes needed for the phenotype.

Example: flower color in sweet pea
The purple color is governed by two dominant genes A and B. When these genes are in separate individuals (AAbb or aaBB) or recessive (aabb) they produce white flower.
Also known as **modifying action**. Wild-type alleles of two genes (\(w^+\) and \(m^+\)) encode enzymes catalyzing successive steps in the synthesis of a blue petal pigment. Homozygous \(m/m\) plants produce magenta flowers and homozygous \(w/w\) plants produce white flowers. The double mutant \(w/w; m/m\) also produces white flowers.

**Recessive epistasis**

F2

<table>
<thead>
<tr>
<th>Gametes</th>
<th>AB</th>
<th>aB</th>
<th>aB</th>
<th>AB</th>
<th>AB</th>
</tr>
</thead>
<tbody>
<tr>
<td>aB</td>
<td>AAbb</td>
<td>AAbb</td>
<td>AAbb</td>
<td>AAbb</td>
<td>AAbb</td>
</tr>
<tr>
<td>ab</td>
<td>aAbb</td>
<td>aAbb</td>
<td>aAbb</td>
<td>aAbb</td>
<td>aAbb</td>
</tr>
<tr>
<td></td>
<td>aABb</td>
<td>aABb</td>
<td>aABb</td>
<td>aABb</td>
<td>aABb</td>
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<tr>
<td></td>
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<td>aABB</td>
<td>aABB</td>
<td>aABB</td>
<td>aABB</td>
</tr>
<tr>
<td>Gameten</td>
<td>Ab</td>
<td>Ab</td>
<td>Ab</td>
<td>Ab</td>
<td>Ab</td>
</tr>
</tbody>
</table>

9:3:4

Two genes inheritance
Two dominant alleles have similar effect when they are separate, but produce enhanced effect when they come together. The joint effect of two alleles appears to be additive or cumulative, but each of the two genes show complete dominance, hence they cannot be considered as additive genes. In case of additive effect, genes show lack of dominance.
2 genes contribute to a trait in a quantitative fashion, each ‘positive’ allele increases the amount of gene product: sum of positive alleles determine level of the trait.

=> this is the transition to **Quantitative Genetics**

Discontinuous binomial distribution becomes a continuous normal distribution.

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**Additive gene effects**

2 genes contribute to a trait in a quantitative fashion, each 'positive' allele increases the amount of gene product: sum of positive alleles determine level of the trait.

Example seed color in wheat *(Nilsson-Ehle, 1909)*
Penetrance and Expressivity

Penetrance: percentage of individuals having a particular genotype that express the expected phenotype

Expressivity is a related concept that describes the degree to which a character is expressed

Incomplete penetrance and variable expressivity are due to effects of other genes or environmental factors. Make genetic analysis difficult!
Calculation of Chi-square ($\chi^2$) test for deviation from Mendelian ratios

“goodness-of-fit” test
Breeders wonder if data support or fit a particular hypothesis and therefore help to explain the results.

A **null hypothesis** is formed that states there is no real difference between the observed and expected data. If differences are due to chance, then the hypothesis can be accepted, but if not, the null hypothesis is rejected and the breeder can modify the hypothesis in favor of a better one.

The equation used to calculate the ($\chi^2$) statistics is as follows:

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

for all classes

in which
- $E =$ expected number in a class
- $O =$ observed number in a class
- $\sum$ means “sum of:”
Example 1: CHI-SQUARE (2) TEST to test for Mendelian segregation ratios

Mendel’s results when phenotyping traits in his pea experiments

<table>
<thead>
<tr>
<th>Parental phenotype</th>
<th>F₁</th>
<th>F₂</th>
<th>F₂ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Round × wrinkled seeds</td>
<td>All round</td>
<td>5474 round; 1850 wrinkled</td>
<td>2.96:1</td>
</tr>
<tr>
<td>2. Yellow × green seeds</td>
<td>All yellow</td>
<td>6022 yellow; 2001 green</td>
<td>3.01:1</td>
</tr>
<tr>
<td>3. Purple × white petals</td>
<td>All purple</td>
<td>705 purple; 224 white</td>
<td>3.15:1</td>
</tr>
<tr>
<td>4. Inflate × pinched pods</td>
<td>All inflated</td>
<td>882 inflated; 299 pinched</td>
<td>2.85:1</td>
</tr>
<tr>
<td>5. Green × yellow pods</td>
<td>All green</td>
<td>428 green; 152 yellow</td>
<td>2.82:1</td>
</tr>
<tr>
<td>6. Axial × terminal flowers</td>
<td>All axial</td>
<td>651 axial; 207 terminal</td>
<td>3.14:1</td>
</tr>
<tr>
<td>7. Long × short stems</td>
<td>All long</td>
<td>787 long; 277 short</td>
<td>2.84:1</td>
</tr>
</tbody>
</table>

F2 ratio close to 3:1 ratio.

BUT do these traits really segregate in the predicted ratio?

-> use Excel file example 1
Example 1: CHI-SQUARE (2) TEST
to test for Mendelian segregation ratios

<table>
<thead>
<tr>
<th>Classes</th>
<th>Expected ratio</th>
<th>observed</th>
<th>expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round seed</td>
<td>3</td>
<td>5475</td>
<td></td>
</tr>
<tr>
<td>Wrinkled seed</td>
<td>1</td>
<td>1850</td>
<td></td>
</tr>
<tr>
<td>Σ</td>
<td></td>
<td>7325</td>
<td></td>
</tr>
</tbody>
</table>

1) Determine the expected frequencies
2) Calculate the test statistics
3) Test for significance

\[ \chi^2 = \sum \frac{(O - E)^2}{E} \]

\[ x^2 = \frac{(O_1 - E_1)^2}{E_1} + \frac{(O_2 - E_2)^2}{E_2} \]

used for all cases
E = expected number in a class
O = observed number in a class
Critical Chi-square values for different degrees of freedom and $p$-values

Table 2-2 Critical Values of the $\chi^2$ Distribution

<table>
<thead>
<tr>
<th>df</th>
<th>0.995</th>
<th>0.975</th>
<th>0.9</th>
<th>0.5</th>
<th>0.1</th>
<th>0.05</th>
<th>0.025</th>
<th>0.01</th>
<th>0.005</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.000</td>
<td>0.000</td>
<td>0.016</td>
<td>0.455</td>
<td>2.706</td>
<td>3.841</td>
<td>5.024</td>
<td>6.635</td>
<td>7.879</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0.010</td>
<td>0.051</td>
<td>0.211</td>
<td>1.386</td>
<td>4.605</td>
<td>5.991</td>
<td>7.378</td>
<td>9.210</td>
<td>10.597</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>0.072</td>
<td>0.216</td>
<td>0.584</td>
<td>2.366</td>
<td>6.251</td>
<td>7.815</td>
<td>9.348</td>
<td>11.345</td>
<td>12.838</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>0.207</td>
<td>0.484</td>
<td>1.064</td>
<td>3.357</td>
<td>7.779</td>
<td>9.488</td>
<td>11.143</td>
<td>13.277</td>
<td>14.860</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>0.412</td>
<td>0.831</td>
<td>1.610</td>
<td>4.351</td>
<td>9.236</td>
<td>11.070</td>
<td>12.832</td>
<td>15.086</td>
<td>16.750</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>0.676</td>
<td>1.237</td>
<td>2.204</td>
<td>5.348</td>
<td>10.645</td>
<td>12.592</td>
<td>14.449</td>
<td>16.812</td>
<td>18.548</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>0.989</td>
<td>1.690</td>
<td>2.833</td>
<td>6.346</td>
<td>12.017</td>
<td>14.067</td>
<td>16.013</td>
<td>18.475</td>
<td>20.278</td>
<td>7</td>
</tr>
</tbody>
</table>

df ... degree of freedom  
= phenotypic classes - 1

accept hypothesis 
reject hypothesis

Table returns the probability ($P$) of the hypothesis being true for the observed distribution. Usually probabilities smaller than 5% ($p<0.05$) are chosen to define a cut-off when to reject a hypothesis.
Example 2: Analyze segregation results and predict the genetic basis of a trait

What hypothesis can we invent to explain the results?

F1

F2

77 182 61 Σ 320

-> use Excel file example 2
Examples 3 and 4: Analyze segregation of breeding populations and predict the genetic basis of traits

Data from current research projects conducted at IFA-Tulln:
Resistance breeding for Fusarium head blight (FHB, *Fusarium* spp.) and bunt (*Tilletia* spp.)

Example 3:
111 F7 durum wheat lines of a cross DBC480 * Karur
Evaluated for FHB resistance, plant height, date of anthesis

Example 4:
126 F7 hexaploid wheat lines of a cross PI119333 * Rainer
Evaluated for bunt resistance and plant height

Illustrate segregation for the traits by histograms and correlations of the traits by scatter plots (e.g. using excel). Speculate about number of involved genes and possible dependencies of the traits.

-> use Excel file example 3 and 4
duplicate dominant epistasis

2 dominant genes unmodified ratio

dominant epistasis

dominant (inhibitory) epistasis

duplicate recessive epistasis

recessive epistasis

polymeric gene interaction

Additive genes

1:4:6:4:1
### Summary - Segregation ratios for single and 2 genes inheritance in the F2 and F∞

<table>
<thead>
<tr>
<th>Phenotypic Class</th>
<th>F2</th>
<th>F∞</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td><strong>Single gene inheritance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>interaction of alleles</td>
<td>AA</td>
<td>aa</td>
</tr>
<tr>
<td>complete dominance</td>
<td>3 1</td>
<td>1</td>
</tr>
<tr>
<td>incomplete dominance</td>
<td>1 2 1</td>
<td>1</td>
</tr>
<tr>
<td>codominance</td>
<td>1 2 1</td>
<td>1</td>
</tr>
<tr>
<td>overdominance, under dominance</td>
<td>1 2 1</td>
<td>1</td>
</tr>
<tr>
<td>lethal alleles</td>
<td>1 2</td>
<td></td>
</tr>
<tr>
<td><strong>2 genes inheritance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>unmodified ratio 2 dominant genes</td>
<td>9 3 3 1 1</td>
<td>1 1 1 1</td>
</tr>
<tr>
<td>dominant epistasis</td>
<td>12 3 1 2</td>
<td>1 1 1</td>
</tr>
<tr>
<td>recessive epistasis</td>
<td>9 3 4 1</td>
<td>1 1 2</td>
</tr>
<tr>
<td>duplicate dominant epistasis</td>
<td>15 1 3 1</td>
<td>3 1</td>
</tr>
<tr>
<td>duplicate recessive epistasis</td>
<td>9 7 1 3</td>
<td>1 3</td>
</tr>
<tr>
<td>polymeric gene interaction</td>
<td>9 6 1 2</td>
<td>1 2 1</td>
</tr>
<tr>
<td>dominant (inhibitory) epistasis</td>
<td>13 3 3 1</td>
<td>3 1</td>
</tr>
<tr>
<td>additive genes</td>
<td>1 4 6 4 1</td>
<td>1 2 1</td>
</tr>
</tbody>
</table>