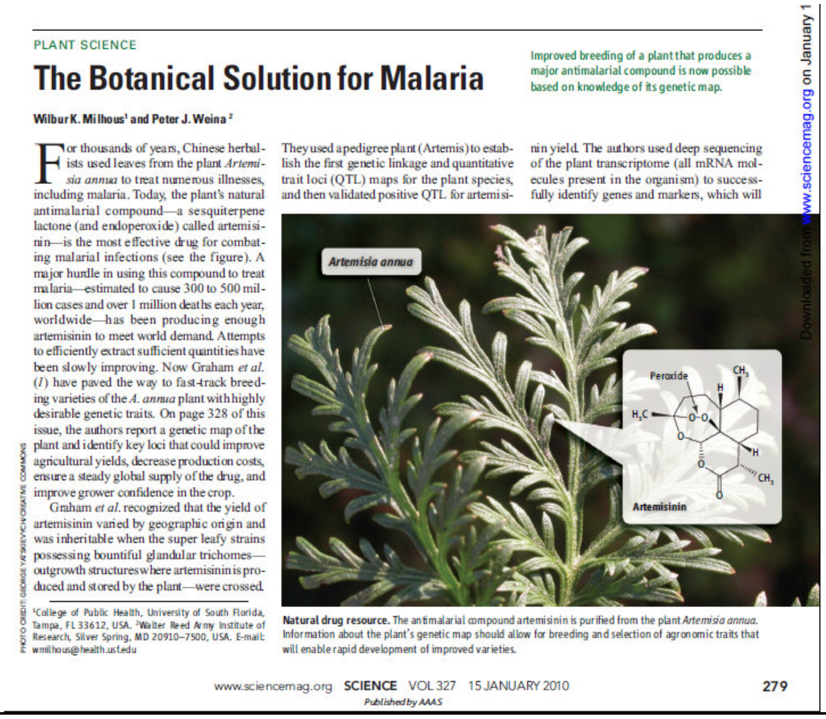
Why study genetics ??

human health (human genome project)

agriculture (crop, livestock improvement)

conservation biology



PLANT SCIENCE

The Botanical Solution for Malaria

Improved breeding of a plant that produces a major antimalarial compound is now possible based on knowledge of its genetic map.

Wilbur K. Milhous[1] and Peter J. Weina[2]

For thousands of years, Chinese herbalists used leaves from the plant *Artemisia annua* to treat numerous illnesses, including malaria. Today, the plant's natural antimalarial compound—a sesquiterpene lactone (and endoperoxide) called artemisinin—is the most effective drug for combating malarial infections (see the figure). A major hurdle in using this compound to treat malaria—estimated to cause 300 to 500 million cases and over 1 million deaths each year, worldwide—has been producing enough artemisinin to meet world demand. Attempts to efficiently extract sufficient quantities have been slowly improving. Now Graham *et al.* (*1*) have paved the way to fast-track breeding varieties of the *A. annua* plant with highly desirable genetic traits. On page 328 of this issue, the authors report a genetic map of the plant and identify key loci that could improve agricultural yields, decrease production costs, ensure a steady global supply of the drug, and improve grower confidence in the crop.

Graham *et al.* recognized that the yield of artemisinin varied by geographic origin and was inheritable when the super leafy strains possessing bountiful glandular trichomes—outgrowth structures where artemisinin is produced and stored by the plant—were crossed. They used a pedigree plant (Artemis) to establish the first genetic linkage and quantitative trait loci (QTL) maps for the plant species, and then validated positive QTL for artemisinin yield. The authors used deep sequencing of the plant transcriptome (all mRNA molecules present in the organism) to successfully identify genes and markers, which will

Natural drug resource. The antimalarial compound artemisinin is purified from the plant *Artemisia annua*. Information about the plant's genetic map should allow for breeding and selection of agronomic traits that will enable rapid development of improved varieties.

PHOTO CREDIT: GEORGE YATSKIEVYCH/CREATIVE COMMONS

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- Parkinson's, Cholesterol, Type 2 Diabetes and BMI
- Parkinson's Disease, Back Pain and Joint Replacement

reports

Thiopurine Methyltransferase Deficiency

Thiopurines are a family of drugs used to treat acute lymphoblastic leukemia, inflammatory bowel disease and autoimmune diseases, and to prevent the rejection of transplanted organs. Thiopurine methyltranserase (TPMT) is one of the enzymes that converts these drugs into...

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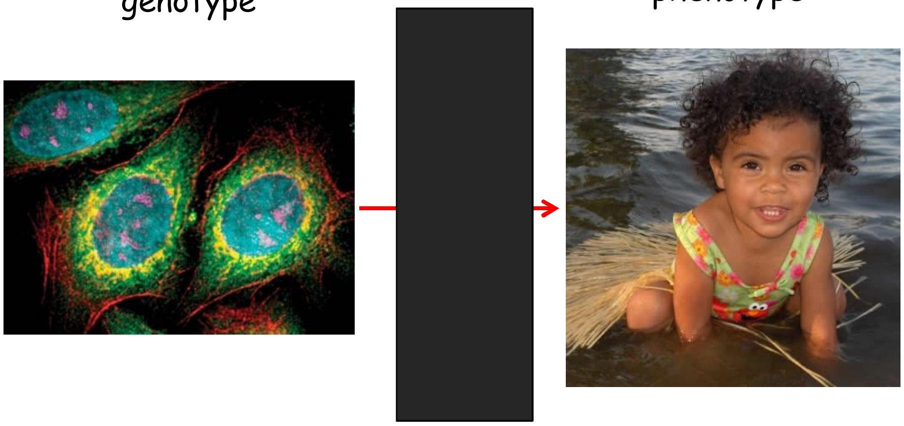
“Thanks @23andMe for the fascinating results! There were both good and bad surprises, with clear steps for the future for me. Very useful.



environment

genotype

phenotype

Heredity, Variation

Forward genetics

choose a phenotype of interest

↓

find variants of that phenotype

↓

check variants for single gene inheritance

↓

identify time and place of action of genes

↓

molecular nature of gene by genomic (DNA) analysis

Outline

- Mendel and transmission genetic theory
- segregation of alleles in single trait crosses
- independent assortment of alleles
- using probability to predict outcomes
- statistical analysis of hypotheses

Gregor Mendel 1866

observations ↓

theory ↓ *explanation*

hypothesis ↓ *prediction*

experiment ↓ *genetic cross*

statistics *phenotypic ratios*

Genetics Terminology

gene/locus: DNA sequence which affects a trait [*locus A*]

allele: variant form of a gene 'allele for purple, allele for white'
[*A, a* or A_1, A_2]

phenotype: an individual's 'apperance' for a particular trait [*purple, white*]

genotype: which pair of alleles an individual carries at a particular locus
[*AA, Aa, aa*]

phenotype ≠ genotype!

homozygous: a genotype where both alleles are the same [*AA, aa*]

heterozygous: a genotype where the alleles are different [*Aa*]

dominant: one copy of the allele is sufficient to express the phenotype
[*AA* = *Aa* → '*A*' *phenotype*]

recessive: two copies of the allele are necessary to express the phenotype
[*aa* → '*a*' *phenotype*]

Mendel's Theory of Particulate Inheritance

- --- genes determine the phenotype
- --- genes exist as pairs of alleles at a locus
- --- each gamete carries a single allele at a locus (i.e., *A* or a)
- --- gametes unite independent of which alleles they are carrying [*different alleles, same locus*]
- --- alleles at different loci assort independently into the gametes* [*different loci*]

-inheritance of parental characteristics by offspring

-variation among offspring;
differences between offspring and parents

P_0 purple ♂ x white ♀ white ♂ x purple ♀

F_1 all purple all purple

F_2 705 purple, 224 white 781 purple, 263 white

F_3 all purple 3/4 purple 1/4 white all white

P_o purple ♂ x white ♀
PP pp

F_1 all purple
Pp

F_2 705 purple, 224 white
PP Pp pp

F_3 all purple | 3/4 purple 1/4 white | all white

P_0 is homozygous

F_1 is heterozygous

phenotype of F_1 is dominant, rare phenotype in F_2 is recessive

↓

the allele for purple is dominant to the allele for white

Pp x Pp

	P	p
P	PP	Pp
p	Pp	pp

1 PP: 2 Pp: 1 pp

3 purple :1 white

P_o purple ♂ × white ♀

PP **pp**

↓

F_1 all purple

Pp

↓

F_2 705 purple, 224 white

PP **Pp** **pp**

F_3 all purple | 3/4 purple 1/4 white | all white

test cross:

F_1 × recessive parent

↓

$\frac{1}{2}$ purple, $\frac{1}{2}$ white

---

single-gene phenotypic ratios

phenotype dist^n	parent genotypes
1 "A" :0 "a"	AA × aa AA × AA AA × Aa
3 "A" :1 "a"	Aa × Aa
1 "A" :1 "a"	Aa × aa

Mendel's law of independent assortment:

two independent traits (dihybrid cross):

P_0 purple tall × white short
P/P T/T p/p t/t

F_1 purple tall
P/p T/t

F_2	purple tall	purple short	white tall	white short
	315	108	101	32
	P/- T/-	P/- t/t	p/p T/-	p/p t/t

---

two independent traits (dihybrid cross):

P_0 purple tall × white short
P/P T/T p/p t/t

F_1 purple tall
P/p T/t

F_2	purple tall	purple short	white tall	white short
	315	108	101	32
	P/- T/-	P/- t/t	p/p T/-	p/p t/t

*look at traits individually in the F_2:

purple: white	423:133	3:1
tall: short	416:140	3:1

Outline

- Mendel and transmission genetic theory
- segregation of alleles in single trait crosses
- independent assortment of alleles
- using probability to predict outcomes
- statistical analysis of goodness of fit: X^2 test

Using probability to predict genetic transmission ratios

when outcomes are independent, multiply the individual probabilities

$$\boxed{\text{pr(a and b)} = \text{pr(a)} \times \text{pr(b)}}$$

when outcomes are equivalent, add the individual probabilities

$$\boxed{\text{pr(a or b)} = \text{pr(a)} + \text{pr(b)}}$$

conditional probability: only a subset of outcomes are relevant

$$\text{if } \boxed{\text{pr(a)} + \text{pr(b)} + \text{pr(c)}} + \text{pr(d)} = 1$$

$$\text{then pr(a or b)} = [\text{pr(a)} + \text{pr(b)}]/[1 - \text{pr(d)}]$$

F_2	purple tall	purple short	white tall	white short
	315	108	101	32
	P/- T/-	P/- t/t	p/p T/-	p/p t/t

*look at traits individually in the F_2:

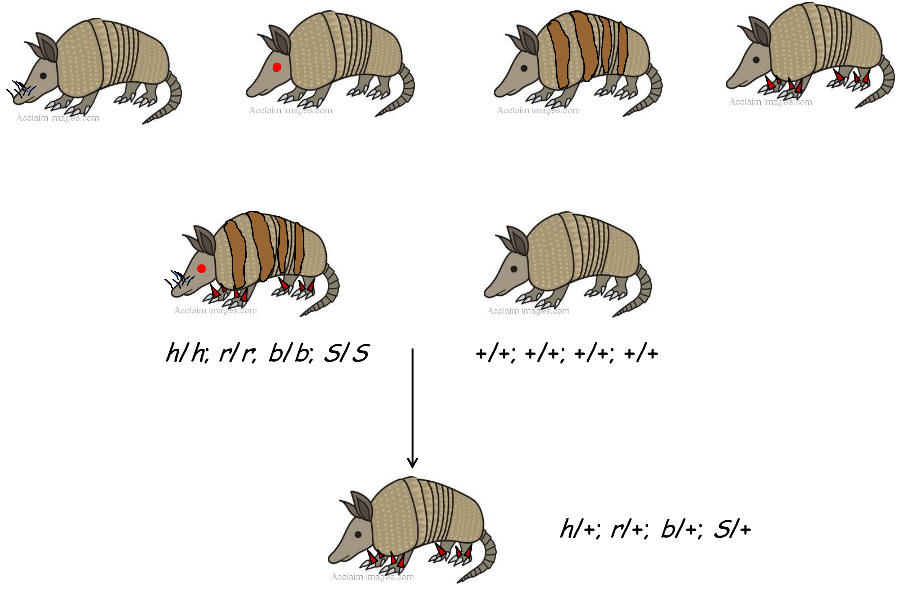
purple: white	423:133	3:1
tall: short	416:140	3:1

two separate traits = independent outcomes

ex: pr (PP, TT) = pr (PP) x pr (TT) = $\frac{1}{4} \times \frac{1}{4}$ = 1/16

pr (PP, T/t) = pr (PP) x pr (tT or Tt)
= pr (PP) x [pr (tT) + pr (Tt)]
= $\frac{1}{4} \times [\frac{1}{4} + \frac{1}{4}] = \frac{1}{4} \times \frac{1}{2}$ = 1/8

pr (purple, tall) = pr (purple) x pr (tall)
= pr (P/-) x pr (T/-)
= $\frac{3}{4} \times \frac{3}{4}$ = 9/16



hairy snout (*h*)

red eyes (*r*)

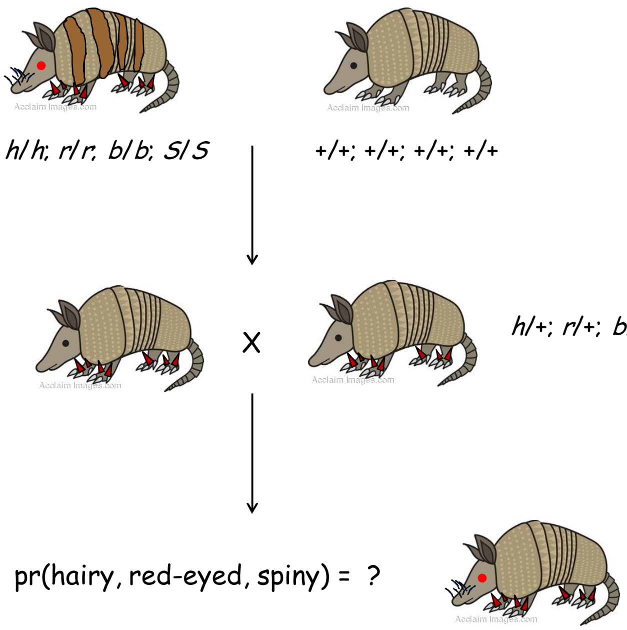
banded body (*b*)

spiny paws (*S*)

h/*h*; *r*/*r*; *b*/*b*; *S*/*S*

+/+; +/+; +/+; +/+

h/+; *r*/+; *b*/+; *S*/+



h/h; r/r; b/b; S/S

+/+; +/+; +/+; +/+

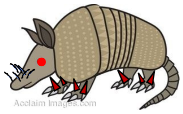
X

h/+; r/+; b/+; S/+

pr(hairy, red-eyed, spiny) = ?

---

pr(hairy, red-eyed, spiny) = ?



= pr(h/h; r/r; S/-)
= pr(h/h) x pr(r/r) x pr(S/-)
= $\frac{1}{4} \times \frac{1}{4} \times \frac{3}{4}$

= 3/64

Outline

- Mendel and transmission genetic theory
- segregation of alleles in single trait crosses
- independent assortment of alleles
- using probability to predict outcomes
- statistical analysis of hypotheses

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when do the data meet mendelian expectations?

if cross two heterozygotes,
expect 3:1 phenotypic ratio in offspring

-how closely do the numbers need to match???

-importance of statistical analysis

Chi-square tests: goodness-of-fit to mendelian ratios

See also pp. 47 - 50 in text

Some examples:

observed		expected	
short	long	short	long
118	42	120	40
28	9	27.75	9.25
46	16	45.75	15.25

all are approximately 3:1, but none is <u>exact</u>

for the sample size, how unlikely is result?

Compare observed and expected numbers with a goodness of fit chi-square test with n-1 degrees of freedom

$$\chi^2_{\text{dof}} = \sum \frac{(\text{observed number} - \text{expected number})^2}{(\text{expected number})}$$

dof = degrees of freedom
= number of independent classes
= number of classes - 1

observed		expected	
short	long	short	long
118	42	120	40
28	9	27.75	9.25
46	16	45.75	15.25

$$\chi^2_1 = \frac{(118 - 120)^2}{120} + \frac{(42 - 40)^2}{40}$$

$$= 0.033 + 0.1$$

$$= 0.133$$

---

scientific method -- reject null hypothesis

H_0: data fit a 3:1 ratio consistent with heterozygous parents

statistics: test how well data fit hypothesis

two possible outcomes:

- correct inference
- incorrect inference
  - --reject H_0 that is true
  - --accept H_0 that is false

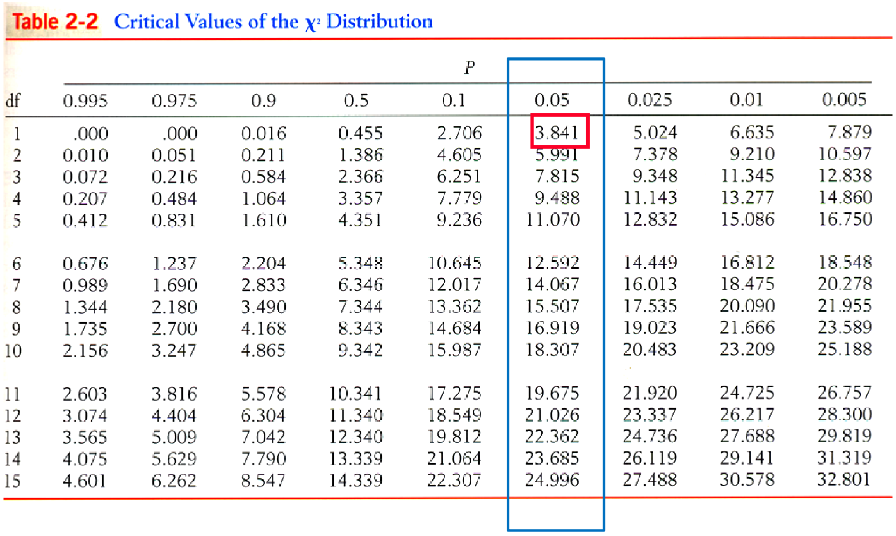


Table 2-2 Critical Values of the χ^2 Distribution

	P								
df	0.995	0.975	0.9	0.5	0.1	0.05	0.025	0.01	0.005
1	.000	.000	0.016	0.455	2.706	3.841	5.024	6.635	7.879
2	0.010	0.051	0.211	1.386	4.605	5.991	7.378	9.210	10.597
3	0.072	0.216	0.584	2.366	6.251	7.815	9.348	11.345	12.838
4	0.207	0.484	1.064	3.357	7.779	9.488	11.143	13.277	14.860
5	0.412	0.831	1.610	4.351	9.236	11.070	12.832	15.086	16.750
6	0.676	1.237	2.204	5.348	10.645	12.592	14.449	16.812	18.548
7	0.989	1.690	2.833	6.346	12.017	14.067	16.013	18.475	20.278
8	1.344	2.180	3.490	7.344	13.362	15.507	17.535	20.090	21.955
9	1.735	2.700	4.168	8.343	14.684	16.919	19.023	21.666	23.589
10	2.156	3.247	4.865	9.342	15.987	18.307	20.483	23.209	25.188
11	2.603	3.816	5.578	10.341	17.275	19.675	21.920	24.725	26.757
12	3.074	4.404	6.304	11.340	18.549	21.026	23.337	26.217	28.300
13	3.565	5.009	7.042	12.340	19.812	22.362	24.736	27.688	29.819
14	4.075	5.629	7.790	13.339	21.064	23.685	26.119	29.141	31.319
15	4.601	6.262	8.547	14.339	22.307	24.996	27.488	30.578	32.801

observed		expected	
short	long	short	long
118	42	120	40
28	9	27.75	9.25
46	16	45.75	15.25

$$\chi^2_1 = \frac{(118 - 120)^2}{120} + \frac{(42 - 40)^2}{40}$$

$$= 0.033 + 0.1$$

$$= 0.133 \qquad p > 0.5$$

reject only if $p < 0.05$

Forward genetics

choose a phenotype of interest

↓

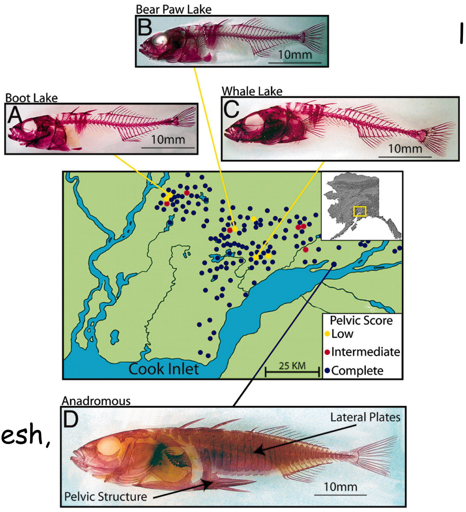
find variants of that phenotype

↓

check variants for single gene inheritance

Inheritance of lateral plate armour in sticklebacks

lacustrine =
lake-dwelling



anadromous =
born/breed in fresh,
marine juveniles

Cresko, William A. et al. (2004) Proc. Natl. Acad. Sci. USA 101, 6050-6055

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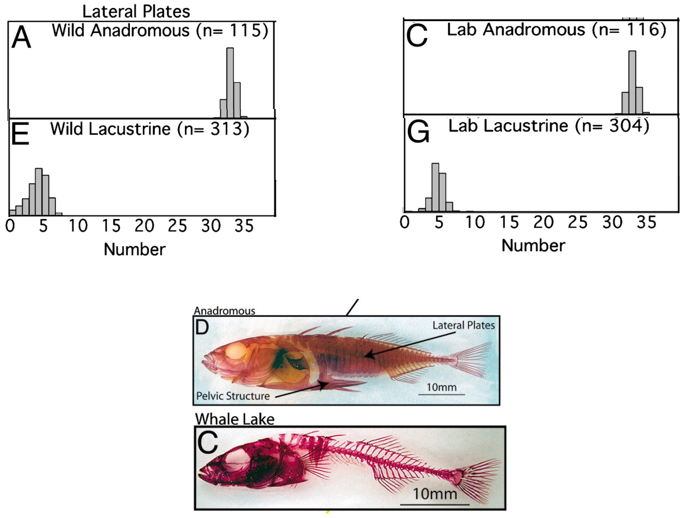

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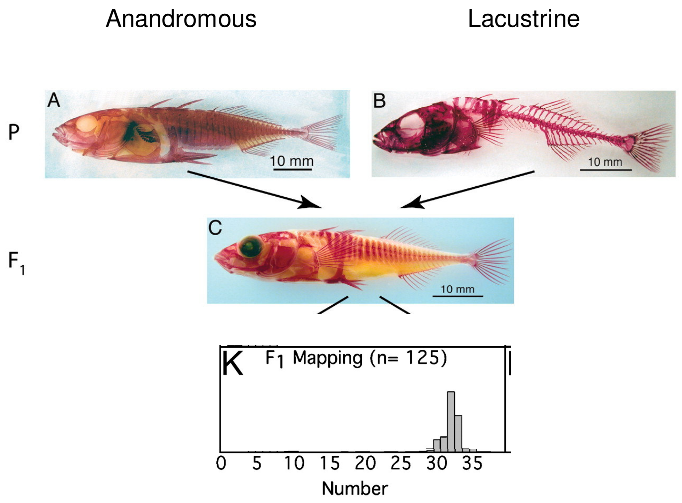


Cresko, William A. et al. (2004) Proc. Natl. Acad. Sci. USA 101, 6050-6055

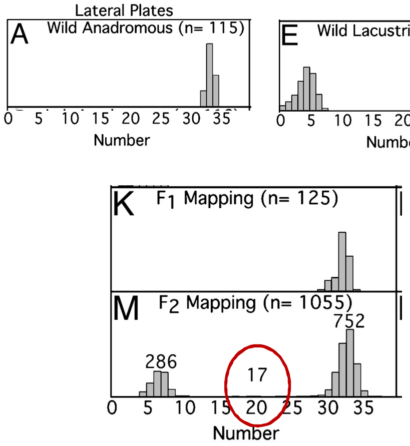
anadromous × lacustrine

↓

anandromous

↓

752 anandromous
286 lacustrine



Cresko, William A. et al. (2004) Proc. Natl. Acad. Sci. USA 101, 6050-6055

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observed		expected	
anandromous	lacustrine	anandromous	lacustrine
752	303	791.25	263.75

$$\chi^2_1 = \frac{(752 - 791.25)^2}{1055} + \frac{(303 - 263.75)^2}{1055}$$

$$= 1.4602 + 1.4602$$

$$= 2.92, \quad 0.1 > p > 0.05$$

Take home points

Mendel’s theory of particulate inheritance can explain both the inheritance of parental characteristics by offspring, and the differences between offspring and parents

heterozygous offspring display the dominant phenotype

a cross of heterozygous individuals will
produce a 3:1 ratio of the dominant : recessive phenotype

the pattern of inheritance of a trait can be determined by:
-reciprocal crosses of homozygous lines and their offspring
-analysis of many crosses with parents of unknown genotype*

probability can predict the frequency of a particular phenotype in the progeny of a cross