Basic Biology Course

12 Case Studies in Genetics

BASIC BIOLOGY COURSE
UNIT 5
ASPECTS OF HEREDITY

BOOK 12

Case Studies in Genetics

MICHAEL A. TRIBE, IRWIN TALLAN &
MICHAEL R. ERAUT
University of Sussex.

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Foreword

This is the last book in the Basic Biology Course, and like others in the series it is written in a carefully programmed style to facilitate individual learning.

Our aim is to teach you some fundamental concepts and terms used in eukaryote genetics, by using a case study approach. In doing so, the book assumes that most readers will start with a basic interest in problems related to man. For this reason, most, but not all of the ten case studies revolve around aspects of human genetics, or subjects in which man has been indirectly involved.

As with most of the other subjects covered in the course, we have laid considerable emphasis upon experimental design and evaluation of data to improve understanding of the subject. Also, by the very nature of genetics, with its strong dependence on notation and symbolism, we have had to assume a knowledge of simple algebra and probability theory as a prerequisite to study.

Finally, we also wish to draw your attention in the book to a few problems (some contentious), which have considerable bearing upon, and a significance in, contemporary society.

Brighton, Sussex, 1977

M.A. Tribe
I. Tallan
M.R. Eraut

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Contents

Introduction

Discussion

Some important aspects of genetics (the study of heredity) were discussed in earlier books within this Basic Biology Course, particularly mitosis and meiosis in Book 3 and the molecular basis of heredity in Book 9.

In Book 3 (*Dynamic Aspects of Cells*) we spent some time looking at the processes of mitosis and meiosis. Mitotic cell division is associated with growth. It is essentially a process which ensures that the daughter cells of such a division have the *same* chromosomal (and hence genetic) complement as the parent cell. This form of cell division occurs many times in the life cycle of multicellular organisms and is an asexual method of reproduction. A characteristic feature of asexual reproduction is that compared with sexual reproduction it leads to a reduction in genetic variability within a population.

In contrast, meiotic division is a rarer event, confined either to germ line cells of the gonads, i.e. those cells which give rise to haploid gametes (oocytes and spermatozoa), or to the zygotes formed as a result of the fusion of haploid cells in lower organisms (e.g. fungi). In all instances, meiosis is an essential feature of sexual reproduction in eukaryotes (see Glossary), and through its agency the necessary variation of genetic material, upon which natural selection can operate in the process of evolution, is maintained within a population. The theory of evolution by natural selection, as proposed by Darwin, revolutionized not only our understanding of biological order, but also brought into perspective man's own position in the hierarchy. It was debatably the greatest scientific idea of the nineteenth century.

In Book 9 (*Protein Synthesis*), the molecular basis of heredity was dealt with in some detail. One of the main aims of the book was to examine critically the evidence that DNA is the basic material of heredity, and that 'DNA makes RNA makes protein' – the 'Central Dogma' of biology as postulated by Crick. These findings in molecular biology have probably made the greatest contribution to the advancement of biological sciences in the twentieth century.

Another great discovery, and the cornerstone of present-day genetics, is the laws of inheritance as enunciated by Gregor Mendel.* Those of you who have studied Book 3, will have already encountered examples of these laws in operation, although at that time we did not mention Mendel specifically by name. From his experiments with the garden pea (*Pisum sativum*), Mendel proposed three fundamental ideas:

(1) the concept of dominance;
(2) the principle of segregation;
(3) the law of independent assortment.

Although some of these ideas have had to be modified since to accommodate phenomena such as penetrance and linkage, Mendel's laws still remain essentially true, despite the fact that their significance was not appreciated until some 35 years after their original publication in 1865, when they were 'rediscovered' independently by Correns, von Tschermak and der Vries.

*Those of you who wish to go deeper into the fascinating historical development of genetics, and perhaps examine a translation of Mendel's original paper, should consult the Recommended Reading at the back of this book.

In the case studies chosen for this book we have given prominence to examples from human genetics, because of the natural interest shown in man and society. Human beings, however, are not the ideal subjects for genetic research when seeking basic principles. Consequently, where no suitable case study exists to exemplify certain concepts, we have not hesitated to take examples from other organisms. In fact to establish some of the basic genetic principles we have decided, perhaps rather surprisingly, to look at the red bread mould, *Neurospora crassa*, which you first encountered in Book 3. The choice of *Neurospora* for the first case study is particularly suitable because it can be used to illustrate clearly a considerable number of important points, which we shall be applying to more complex situations later.

Some of the terminology and concepts that we have used in this introduction may be unfamiliar to you, but we hope that you will have a much better understanding of them after you have worked through the case studies.

The main aims of this book are twofold:

(1) To teach many of the fundamental concepts and principles of genetics through the agency of some well researched case studies.
(2) To show how the application of genetic principles can further our understanding of biological and social problems and thus facilitate better decisions about them.

Preknowledge requirements

An understanding of the mechanisms of cell division by MITOSIS and MEIOSIS in the context of the cell cycle, and of the genetic consequences of these two processes (Book 3).

Knowledge:

(1) that the basic material of heredity is DNA and that DNA is contained within the chromosomes of eukaryotic cells;
(2) that changes in DNA give rise to mutations;
(3) that DNA is responsible for making the various RNAs, which in turn make polypeptides or protein at the ribosomal sites in the cell (Book 9).

Knowledge of elementary probability theory, in particular that the probability of a number of independent events occurring simultaneously is the product of their individual probabilities. If you wish to refresh your memory on these points, consult one of the statistical textbooks given in the Recommended Reading at the back of this book.

It would also be helpful to know the following terms which were first introduced in Books 3 and 9:

haploid, diploid, dominance, recessive, homozygous, heterozygous, segregation, independent assortment, linkage, gene, allele, mutation, autosome, X chromosome, Y chromosome, karyotype.

If you are not familiar with them, be prepared to make good use of the Glossary.

Objectives

At the end of this book you should be able to:

(1) Define all the terms in the Glossary and use them in their correct context.

(2) Use pedigree data to calculate simple probabilities concerning the likelihood of genetic traits appearing in offspring.
(3) Use the Hardy–Weinberg equilibrium to calculate the frequency of alleles in a population.
(4) Construct genetic maps from data involving three linked genes.
(5) Correctly interpret data to deduce some of the selective factors involved in the evolution and maintenance of polymorphic types.
(6) Correctly apply the principles and knowledge derived from the case studies to the solution of unfamiliar, yet related problems.

Instructions on working through programmed text

The book is arranged into 10 case studies, each programmed into questions and answers arranged sequentially down the page. You are provided with a masking card with which you should cover each page in turn and then move the masking card down to reveal two thin lines

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This marks the end of the first question on that page. Record your answer to the question in a notebook under the section heading or case study. Then *check* your answer with the answer given. If your answer is correct, move the masking card down the page to the next double line, and so on. If any of your answers is incorrect retrace your steps and try to find out why you answered incorrectly. If you are still unable to understand the point of a given question, make a note of it and consult your tutor. The single thick line

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is a demarcation between one frame and the next. Summaries in the book are convenient stopping and starting points, since it is unlikely that you will have time to read through the whole book in one session. Always read the appropriate summary before going on to a new section.

Genetic symbols used in the case studies

(It is most important that you read this section before starting)
Geneticists write down the genotype (the actual genetic make-up) of an individual organism by using a shorthand notation of symbols, usually in the form of letters and numbers to represent the gene(s) in which they are interested. However, since there are usually alternative forms of a gene (ALLELES), it is necessary to use symbols which make it clear to which alleles they are referring. For example, a hypothetical gene A may have two alleles a and a'; they are all alleles of the same gene.

Although this may appear to be quite straightforward, we find that during the development of genetics notation has got rather out of hand, because slightly different forms of notation have been employed by geneticists working with different organisms. With this in mind, we have tried from the outset to rationalize the symbolism used in this book, and you should refer back to this section if you are puzzled by notation as you work through the case studies.

Genetics is concerned with *differences* between individuals, so that genes are usually given names based on the description of the phenotype of the mutant (altered) form of a gene. The most common form of any gene in a population is referred to as the wild-type allele. The usual nomenclature is to use '+' for wild-type and a letter for describing the mutant in abbreviated form. For example, in Book 3 we came across a mutant of the fruit-fly *Drosophila melanogaster* whose phenotype showed a black body colour rather than the wild-type grey colour. The italicized letter *b* is used to symbolize the gene for black body and its wild-type allele is designated as $+^b$ or b^+ (both are used), the letter signifying that we are considering the wild-type allele of black body and not of any other gene. *Drosophila*, however, is a diploid organism and thus carries two copies of each gene – one copy from the mother and one copy from the father. For black body to appear in the phenotype, we found (Book 3) that the gene must be present in the homozygous condition (i.e. $b//b$). In other words, *b* is recessive to $+^b$ or, conversely, $+^b$ is dominant to *b*. The homozygous recessive genotype is, therefore, $b//b$; the homozygous wild-type genotype at the black body gene locus is $+^b//+^b$; and the genotype of the heterozygous wild-type (i.e. phenotypically wild-type) is $+^b//b$ or $b//+^b$.

You will notice that in all cases above we have used the notation of a double bar, // (or =) to represent the two homologous chromosomes on which the gene locus for black body is to be found. A single bar is often used to indicate the situation in a gamete after meiosis, i.e. $b/$ or $+^b/$.

When we consider two different gene loci, say ebony body and vestigial wing, on two different chromosomes in a diploid, the geneticist frequently represents the situation as follows $\frac{e}{e}, \frac{vg}{vg}$, where *e* represents the gene ebony body colour and *vg* vestigial wing. But when the two gene loci under consideration are located on the *same* chromosome (i.e. they are linked), the situation is represented as follows $\frac{b\ vg}{b\ vg}$.

So far, we have only considered mutants that are recessive. Bar eye, however, is a dominant mutation to the wild-type in *Drosophila melanogaster*. Consequently (as with all dominant mutants) it is symbolized by a capital italicized letter, *B*, and the wild-type allele is represented by $+^B$ or B^+.

Geneticists sometimes want to refer to certain genes carried on the X chromosome (one of the sex-determining chromosomes). For example, bar eye is known to be carried on the X chromosome in *Drosophila melanogaster* and this can be written X_B in order to draw attention to the fact that a specific gene on the X chromosome is being considered. For example, in one of the case studies you will come across the notation X_{ClB}. This signifies that the X chromosome carries three linked genes: *C* the dominant cross-over suppressor; *l* a recessive lethal mutation; and *B* the dominant gene bar eye. You will see why it is necessary to use this rather complex notation later.

Up until now we have described the situation in a diploid organism, but the organism that you will be looking at in Case Study 1 is *Neurospora crassa*, which is a haploid organism. Now, as you will have realized, with a diploid organism it is not always possible to determine the genotype (i.e. the actual genetic constitution) for a particular character from observation of the phenotype (i.e. the outward appearance of that individual), because of the phenomenon of dominance – a feature first recognized by Mendel. With a haploid organism the situation is different. Since each haploid individual has a single genome (*one* copy of each gene) present, all genes will be expressed in the phenotype.

Below are summarized most of the genetic notations which are encountered in this book, and their meanings.

Symbol	Meaning
Neurospora	
ad	A mutant requiring adenine before it will grow.
$+^{ad}$ or ad^+	The wild-type allele of *ad*, able to grow on minimal medium without addition of adenine.
met	A methionine-requiring mutant.
$+^{met}$ or met^+	The wild-type allele of *met*.
ylo	A mutant with yellow-coloured conidia.
$+^{ylo}$ or ylo^+	The wild-type allele of *ylo* with orange-red conidia.
A and *a*	Alleles determining the two different mating types in *Neurospora* that are necessary before sexual reproduction will occur.
Man	
T and *t*	The dominant gene (*T*) determining the ability to taste phenylthiocarbamide (PTC) and its recessive allele (*t*), producing an inability to taste PTC.
A^h and A^H	The recessive, sex-linked gene (A^h) responsible for haemophilia A and its 'normal' wild-type dominant allele (A^H).
I^A, I^B, I^O	A multiple allelic system determining one of the phenotypes of the ABO blood groups in man. I^A and I^B are codominant, but both are dominant to I^O, which is recessive. (The letter '*I*' has been selected for its immunological implications since it actually stands for isoagglutinogen, a normally occurring antigen.
L^M, L^N	Alleles (codominant) determining the MN blood group antigens in man. The gene locus *L* is used to honour the discoverers of the trait, Landsteiner & Levine.
Hb^A	The gene for normal (wild-type) haemoglobin in man; *Hb* is an abbreviation for *H*aemoglo*b*in.
Hb^S	The recessive allele causing sickling of red blood cells.
Rh^+ and Rh^-	A multiple allelic series of the Rhesus blood group antigens, Rh^+ symbolizing that the individual produces the Rhesus blood antigen and Rh^- the inability to produce the Rhesus factor.
c and *C*	The recessive, sex-linked gene for colour-blindness and its dominant (wild-type) allele for normal colour vision. (These symbols have also been used in a different context in self-assessment question 3, where *C* represents the gene for coloured aleurone in maize and *c* its recessive allele conferring colourless aleurone.)
Drosophila	
B	The dominant, sex-linked (i.e. on X chromosome) gene for bar eye shape; its (recessive) wild-type allele is $+^B$.
car	The recessive, sex-linked gene for carnation eye colour; its dominant wild-type allele is $+^{car}$.

y	The recessive sex-linked gene for yellow body colour; its wild-type allele conferring normal body colour is $+^{y}$.
v	The recessive sex-linked gene for vermilion eye colour; the normal wild-type allele (red eye) is $+^{v}$.
m	The recessive sex-linked gene for miniature wing; the normal wild-type allele (long wing) is $+^{m}$.
sn	The recessive sex-linked gene for singed bristles; the normal wild-type allele is $+^{sn}$.
X_{ClB}	Symbolizes an X chromosome carrying the dominant cross-over suppressor (*C*), a lethal gene (*l*) and the dominant gene bar eye (*B*).

Case Study 1 The red bread mould and the experiments of Beadle & Tatum

1 The fungus *Neurospora crassa* is an orangey-red mould which is commonly found on bread. Typically it grows on a suitable food medium by forming a mass of tangled threads, collectively known as the mycelium (see fig. 1.1 opposite). The filaments or hyphae of the branching mycelium are made up of many syncytial cells (i.e. with several nuclei in a common cytoplasm), each cell being about 15 μm by 5 μm in size. The fungus obtains its food by secreting enzymes which break down the large organic macromolecules in the medium into smaller ones, and these can then be imbibed by the fungal mycelium and used for further growth. When used for genetic experiments in the

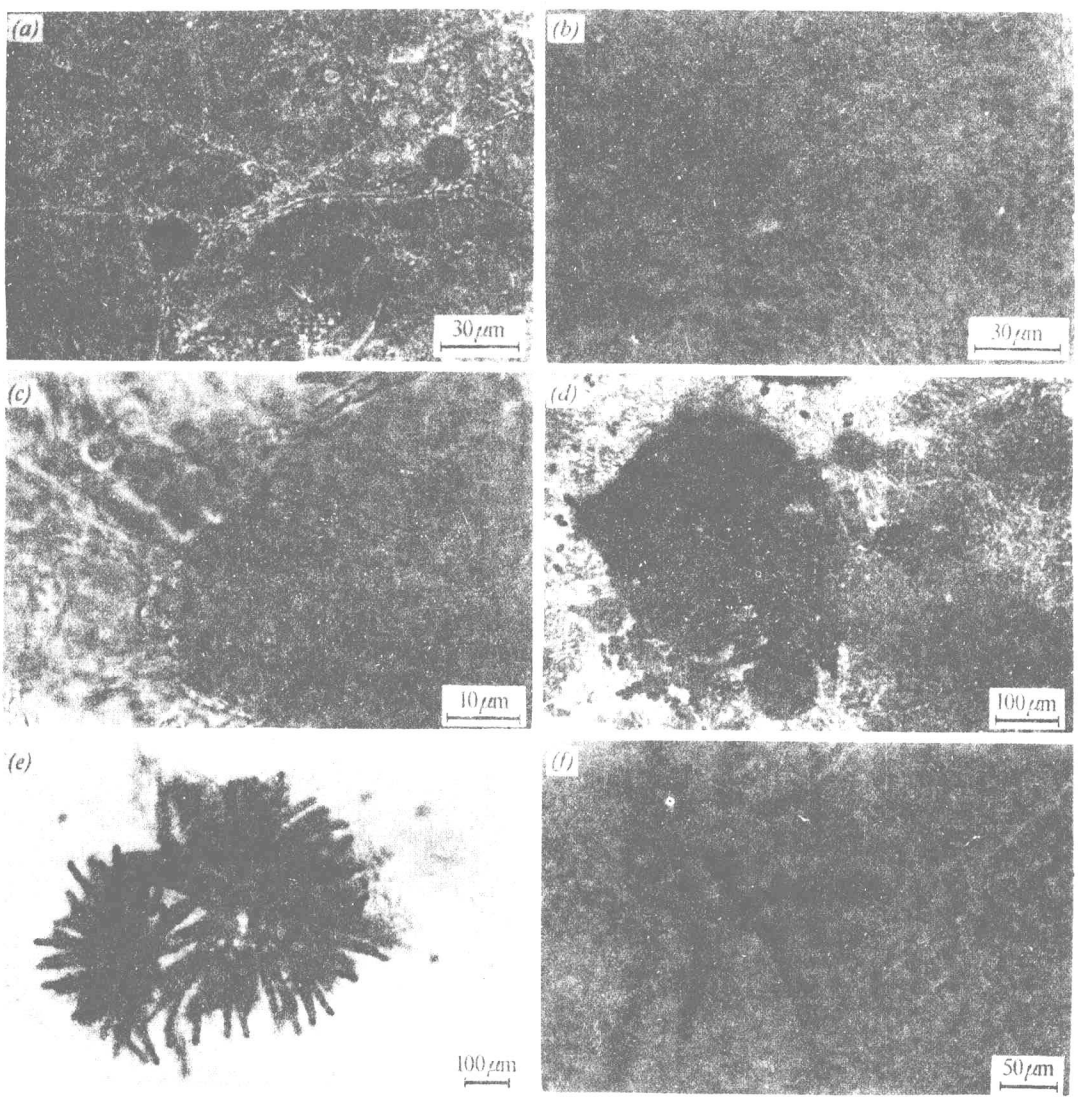

Fig. 1.1. Stages in the life cycle of *Sordaria fimicola*. (*a*) Mycelium of *Sordaria* showing hyphae and developing perithecia. (*b*) Hyphae, showing clamp cells and a single, young protoperithecium. (*c*) More highly magnified view of a protoperithecium. (*d*) Mature perithecium disrupted to show ascospore contents: developing perithecia shown nearby. (*e*) Asci with ascospores inside from a single perithecium. (*f*) High-power view of asci, each ascus with eight ascospores.

laboratory, *Neurospora* is often grown in Petri dishes containing minimal agar medium. This is a jelly-like base containing the simplest chemically defined medium (usually inorganic salts and a sugar) on which the normal (wild-type) fungus will grow. Fungi which will grow on this medium are said to be PROTOTROPHIC. Stages in the life cycle of the prototrophic form of *Sordaria fimicola*, a closely related fungus to *Neurospora crassa*, are shown in fig. 1.1.

Neurospora is a haploid organism for most of its life cycle (i.e. it has a single set of chromosomes, like the gametes of man and *Drosophila*). Periodically, however, the fungus produces spores of different mating types, each of which is self-sterile. Fusion of these different haploid spores from different parents takes place in the sexual stage of the life cycle, forming a diploid zygote. The zygote then undergoes meiosis to give four haploid cells; these in turn each undergo one mitotic division forming eight ascospores. They are called ascospores because they are housed as a group of eight spores in a sac-like structure called an ascus. In turn, several asci are housed within a cup-shaped structure called a perithecium. The essential points are summarized in figs. 1.1 and 1.2.

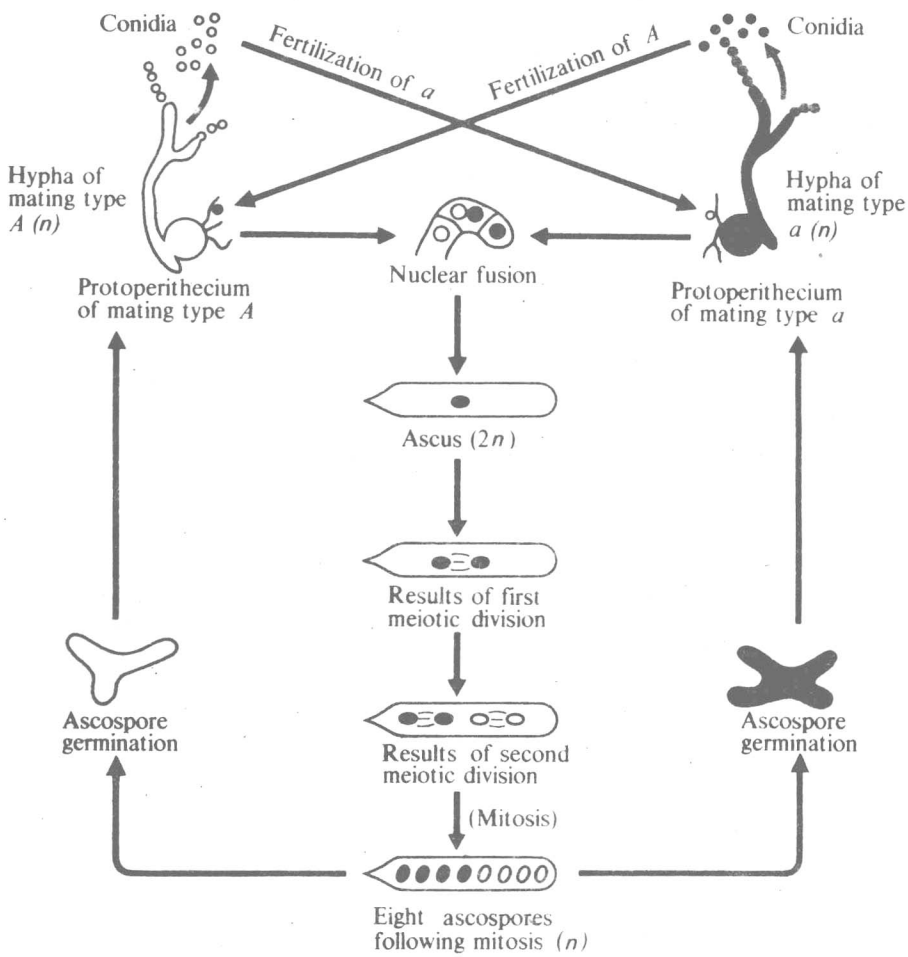

Fig. 1.2. The life cycle of *Neurospora crassa*.

Each spore can then, if desired, be plated onto agar medium and characterized as to its physical traits, such as nutritional requirements. The observed traits are the individual's PHENOTYPE, as distinct from the individual's genetic constitution or GENOTYPE.
In the case of a haploid individual such as *Neurospora* can we tell the spore's genotype by the phenotype of the resultant colony?

Normally yes, because each spore is haploid (i.e. it possesses only one set of genes). However, since the phenotype can also be affected by the environment there could be exceptions, although this is rare in *Neurospora*.

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2 One can carry out genetic studies with *Neurospora* by taking advantage of the mutations that occur occasionally, but the frequency of spontaneous mutations is very low.
How could the frequency of mutations be increased?

By the use of physical and chemical agents, known as MUTAGENS, which increase the frequency of mutations.
Such agents have been known since 1927, when H.J. Muller used *Drosophila* to show that X-rays are mutagenic. Since then the list of mutagens has proliferated greatly.

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3 In 1941 Beadle & Tatum used X-ray treatment of *Neurospora* spores to increase the frequency of mutation.
After X-ray treatment it was found that some spores would only grow on minimal medium when it was supplemented in some way (e.g. by the addition of certain amino acids or certain purines or pyrimidines). These strains are referred to as AUXOTROPHIC or nutritional mutants.
How could you test whether a mutation of this kind was stable and heritable?

By seeing whether the mutant maintains its auxotrophy through prolonged growth and subculture

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4 One mutant isolated by Beadle & Tatum required the purine adenine. By convention this auxotrophic mutant can be referred to in genetic 'shorthand' as *ad* and its wild-type or prototrophic counterpart as ad^+ or $+^{ad}$. The prototrophic form does not need to be supplemented with adenine.
Since *ad* and $+^{ad}$ are alternative forms of the same gene (here concerned

with adenine metabolism) they are referred to as _____?_____.

ALLELES

5 If a cross (fusion) is made between an *ad* spore of one mating type and a $+^{ad}$ spore of the other mating type, what will be the genotypes of the eight ascospores in each ascus and in what proportions? Explain your answer.

Four *ad* spores and four $+^{ad}$ spores
A diploid zygote nucleus ($ad//+^{ad}$) is formed after fusion, which then undergoes meiosis. During meiosis, SEGREGATION occurs giving equal numbers of each type back again. Each spore, therefore, receives only *one* allele with respect to adenine requirement; i.e. either *ad* or $+^{ad}$, but never both and never neither.

6 Now let us consider another mutant, one that requires the amino acid methionine in the medium (*met*). Its wild-type allele is $+^{met}$.
A cross is made between the adenine-requiring mutant and the methionine-requiring mutant. The ascospores resulting from the cross are plated out onto minimal medium containing both adenine and methionine. Then from the colonies produced on this medium samples are tested from each colony on three media: one without methionine, one lacking adenine, and one lacking both methionine and adenine. Why is it first necessary to rear all the spores on a medium containing both adenine and methionine *before* testing them on a medium without methionine or adenine or both?

Without this step auxotrophs requiring adenine or methionine or both would not grow and we would not have a colony to sample and work with; nor could we be certain why the spore failed to grow.

7 In Table 1 below, you see the results actually obtained from a cross between *ad* $+^{met}$ and $+^{ad}$ *met*.

Table 1

Colony phenotype	Colony genotype	No. of colonies
Requires adenine	*ad* $+^{met}$	72
Requires methionine	$+^{ad}$ *met*	76
Wild-type (no requirements)	$+^{ad}$ $+^{met}$	68
Requires both adenine and methionine	*ad met*	64

What two explanations can you give for these results?

The most likely explanations (that actually correspond to two different types of genetic analysis predictions) are:

(*a*) that the four types or classes are present in equal (or near equal) numbers;

(*b*) that the two parental classes *ad* $+^{met}$ and $+^{ad}$ *met* are present in equal (or near equal) numbers and in excess of the other two classes (*ad met* and $+^{ad}$ $+^{met}$), which are also present in equal (or near equal) numbers.

8 Is there any way of making a rational decision as to whether the results in Table 1 are 'near enough' to a ratio of 1 : 1 : 1 : 1 to support answer (*a*) above?

Yes, by carrying out a statistical analysis of the data

(*Note*: As a further check, it is also possible to dissect out and analyse the eight ascospores in the order in which they occur in each ascus. This is a lengthy and somewhat tedious process but it provides the geneticist with very precise segregation patterns, since each ascus represents the product of a single meiosis. Also, since the thin shape of the ascus restricts the orientation of the spindles to the long axis at each division, then the order in which the spores are found within the ascus reflects the segregation of the chromosome centromeres (and hence alleles) during meiosis I and II. Self-assessment question 1 is concerned with this particular method of analysis.)

9 We can have more confidence that the results obtained do conform to expectation by carrying out a chi-square (χ^2) statistical test. This test gives us a measure of the probability of the observed figures deviating from the expected figures by chance alone. As a result we can assess how often random fluctuations as large as those seen in the data are expected to depart from an exact 1 : 1 : 1 : 1 ratio for a sample of this size. The expected number in each class is 70 (i.e. $\frac{280}{4}$).

χ^2 is given by:

the sum of (Σ) $\dfrac{(\text{Deviation of observed value from expected value})^2}{\text{Expected value}}$

for all four classes.

Work out the value of χ^2 from the data of Table 1.