CHAPTER 2
TEACHING BASIC GENETICS IN HIGHER ORGANISMS

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I. INTRODUCTION

The science of genetics was founded on the basis of the laws of inheritance proposed by Mendel. These laws, although they are in some ways an over-simplification, are still fundamental to genetics. Nevertheless they can be difficult to understand since they can be described in highly abstract terms, unfamiliar to many biologists. There is therefore a strong argument for teaching elementary genetics through a practical medium: the laws of genetics have their basis in real events and operate in all higher organisms.

There are three aims in teaching basic practical genetics. Firstly, the principles of inheritance in higher organisms must be explained. Too often a student can quote the 'magical' ratios of 3 : 1 or 9 : 3 : 3 : 1, but he has no understanding of how they are generated. Secondly, these principles must be demonstrated to illustrate their ubiquitous nature. Thirdly, basic genetics should be related to other aspects of the subject, such as biochemical, population or biometrical genetics, and so to wider areas of biology.

Basic genetics nowadays involves more than just the Mendelian Laws, for, as genetics has progressed so the scope of elementary genetics has increased. An understanding of basic genetics would not be complete without some mention of multiple alleles, linkage, gene interaction, and phenomena relating to gene expression, such as pleiotropy, penetrance and expressivity. Moreover, any comprehensive practical course in genetics cannot ignore the statistical problems associated with either sampling errors or viability disturbance.

Fundamental genetics can be taught in many organisms, but to avoid the presentation of a miscellaneous list of higher organisms showing the basic genetic phenomena (for the number of examples is almost infinite!), no attempt will be made to be exhaustive.

This chapter has been divided into two sections. The first section outlines types of investigation and includes several examples. The second section contains course suggestions for teaching basic genetics at various levels. Clearly the two sections are complementary, and should be considered together. This chapter should also be considered in relation to the other parts of this book so that a fully comprehensive, interesting course can be produced geared to the needs of the student.

II. TYPES OF INVESTIGATION

1. CROSSES

Experiments in genetics are limited to a large extent by the generation time of the organisms involved. Genetics courses usually last a relatively short time, and even a year's course is only adequate for one generation in most higher organisms. Nevertheless, several organisms with a relatively short generation time are suitable, and although they may not appear to be as ideal as *Drosophila*, they each have certain advantages. They all have the advantage of showing that genetics isn't something that just happens in *Drosophila*! One of the simplest ways of teaching elementary genetics is to have the students perform crosses using not living organisms but coloured beads or counters, where these represent either genes or chromosomes (see also p. 296).

(a) Beads

Beads, counters or children's building units form very useful vehicles for teaching basic genetics. One and two gene models are possible, and if interlocking beads are available, more sophisticated models involving linkage can be developed.

The sequence of operations for a simple one gene case would be as follows:

1. Sort out the beads into two colour groups of about 20 each, one group representing genes of one homozygous parent, the male, the other group genes of the other homozygous parent, the female.
2. Pair up the beads within each colour group to indicate the diploid state.
3. Separate the beads to indicate the process of meiosis, and place the single beads (representing gametes) into the appropriate bag (one labelled male, the other female).
4. Pick out one bead (gamete) from each bag at random and pair the two together to represent fertilization. Record the genotype of this offspring (combination of beads) and then return each bead to its appropriate bag. Repeat till a sufficiently large family has been produced. The resulting list indicates the genotypes of the F_1.

5. Now pair up the beads in the F_1 combination and let one half of the pairs represent males (or male cells) and the other half females (or female cells).
6. Now repeat operation 3. Separate the beads to indicate meiosis and place the single beads from the male into one bag, those from the female into another bag.
7. Now repeat operation 4. That is to say, pick out one bead from each bag at random and pair the two together (fertilization). Record the genotype and return the beads to their correct bags. Repeat the process till an array of genotypes representing the F_2 is obtained.
8. Sort out the different classes of genotype to indicate the frequency with which each occurs in the F_2.

These classes will not necessarily occur in the precise expected frequency, since the selection of beads at stage 7 (fertilization) was at random. The F_2 phenotypes are assessed on the assumption of a certain type of gene expression (e.g. dominance of one colour class).

The sequence for the two gene case is similar, and is best done using interlocking beads.

1. First define which colours represent which alleles, e.g. gene 1: allele *A*, allele *a*; gene 2: allele *B*, allele *b*, where *A*, *a*, *B*, *b* represent beads of different colours (or beads of two colours with *A* distinguished from *a* and *B* from *b* by a distinguishing mark such as a razor notch or ink spot).
2. Match up the beads to represent the appropriate parental type, e.g. *AB*/*AB* and *ab*/*ab*.
3. Now separate the beads to indicate meiosis. Note that there is separation of homologous chromosomes, i.e. the resulting gametes must be of the types *AB* and *ab*, not *AA* or *BB*, etc.

Now interlock *A* with *B* and *a* with *b* to indicate that they are part of one gamete, and place those from one parent into one bag (labelled male), those from another into another bag (labelled female).

4. Pick out one set (gamete) from each bag at random and pair the two sets together (fertilization). Record the genotype and return the sets to their appropriate bag. Repeat until enough F_1 genotypes have been obtained.
5. Let half of these represent males, half females.
6. The next step is meiosis and gamete formation, and during this there is recombination. This is achieved by pairing up sets of interlocked beads and exchanging partners between a required proportion (depending on recombination rate) of them. Thus if there is 50 per cent (maximum) recombination in both male and female, half the paired sets are selected from each bag and one pair of beads is exchanged between sets. Any other proportion may be chosen to represent different levels of linkage. Now separate the sets and place the sets from the males into one bag and the sets from the females into another bag.

7. These sets are then sampled in the usual way from each and joined in pairs at random to give the F_2.
8. The different types are recorded to get the frequency with which each genotype occurs in the F_2.
9. The F_2 phenotypes can now be assessed based on certain assumptions of gene interaction and expression.

Again the results do not necessarily fit the theoretical expectation because of random effects. The results of individual students can also be pooled into class data, which can then be examined by everyone.

This 'quick' way of performing 'crosses' emphasizes the role of chance in producing genetic ratios: the student can actually perform random union of gametes, and not just imagine it happening on a chequerboard diagram. As the student is dealing with 'genes' he will also realize, particularly in the two genes case, that the genotypic ratio is always the same, and that the phenotypic ratios that are produced depend entirely on the nature of expression and interaction of the genes. He can postulate different interactions (e.g. that gene *A* required gene *B* for its expression) which will lead him into developmental and biochemical considerations. Or he can assign quantitative values to the different genes, and so lead into quantitative genetics. Since beads are in common use for demonstrating principles of population genetics, Mendelian and population genetics can be related (p. 296). The main disadvantage of this method of teaching is that it is still highly abstract, but when combined with more conventional crossing it does provide a background that is less theoretical than mere paper work.

Sources of material

Poppet beads from T. Gerrard & Co Ltd, East Preston, Littlehampton, Sussex; available in two colours (red and yellow).
Lego single brick building units, from almost any toy shop; available in red, yellow, blue and white.

Reference

NUFFIELD BIOLOGY TEACHERS' GUIDE V (1967) *The Perpetuation of Life*, pp. 26–27. Longmans/Penguin.

(b) Mice

Of the commonly used genetic organisms, mice are the most closely related to man and therefore of intrinsic interest. They are particularly valuable for teaching genetics to children who can keep them as pets at the same time. An additional advantage of mice is that their size and individuality makes them ideal for studying the nature of variable gene expression.

Rearing, handling and breeding mice are relatively easy and adequate methods for keeping, feeding and handling mice, together with accounts of how to detect mating and how to preserve the offspring by deep-freezing them are given in Nuffield Biology Teachers Guide V, pp. 31–35.

Useful mutants for teaching the genetics of mice are given in the following list.

Monohybrid ratios with dominance

Coat colour: agouti (white tip to coat hairs), *A*, dominant to non-agouti, *a*;
black, *B*, dominant to brown, *b*;
intense, *D*, dominant to Maltese dilution, *d*.

Eye and coat colour: black-eyed intense, *P*, dominant to pink-eyed dilution, *p*.

Ear size: normal long ears, *Se*, dominant to short ears, *se*.

Hair type: wavy hair, *Ca* (caracul) or *Re* (rex), dominant to normal straight hair, *ca* or *re*.

Monohybrid ratio with no dominance

Coat colour: agouti, *A*, crossed with tan belly, a^t, gives a heterozygote that is agouti and has a tan belly.

Dihybrid ratio with dominance

Ear size and eye-colour: short eared, black-eyed mice (*se P*) crossed with long eared pink-eyed mice (*Se p*) will produce a 9 : 3 : 3 : 1 ratio in the F_2.

Linkage

Danforth's short tail (*Sd*) wellhaarig (*we*, wavy hair) and non-agouti (*a*) are on chromosome V in the following order: *Sd*—40 per cent—*we*—14 per cent—*a*

Pleiotropy (one gene affecting several characters)

Eye and coat colour: the gene, albinism, *c*, produces a pink eye and white coat, while its allele extreme chinchilla, c^e produces a black eye and a pale coffee coloured coat. Crossing $c^e c^e \times c\ c$ gives in the F_2 black-eyed coffee coloured, black-eyed white and pink-eyed white mice in the ratio 1 : 2 : 1. With regard to eye colour, pink eye is recessive to black eye, while with regard to coat colour white coat is dominant to coffee. This is an excellent illustration that dominance is a property of the character and not the gene.

Coat colour and behaviour: the varitint waddler, *Va*, produces both a coat streaked with black, grey, and white, and mice which tend to waddle and shake.

Ear size and kidney defect: the short ear mutant, *se*, causes both a short ear and hydronephrosis of the kidney. The kidney appears enlarged, watery and balloon-like when the mouse is dissected. However, only a proportion of short ear mice show this enlarged kidney because of variable penetrance (only a certain proportion of individuals show the character, even though all have the gene), sex limited penetrance (one sex shows the character more than another) and variable expressivity (character expressed to different degrees in different individuals). The hydronephrosis characteristic of short eared mice, *se*, illustrates all three phenomena. The balloon-like kidney is not seen in all mice, it is more common in males than females, and sometimes it is obvious while in other mice hardly noticeable. The short ear character on all these mice confirms the presence of the same gene.

Modifying genes

Number of digits: the effect of modifying genes is best seen in the mutant polydactyly, *py*, where the feet have from 5–8 toes and different numbers of bones to the extra toes. Stocks showing high and low incidence of this character are available and illustrate modifying genes either enhancing or repressing a character.

Coat colour: an example of a specific modifier is yellow coat, A^y, which reduces the size of the white areas in mice carrying genes for spotting (e.g. dominant pied, *W*).

Variable dominance

The gene Danforth's short tail, *Sd*, results in a short tail length, and the heterozygote varies in its tail length depending on its genetic background: stocks where this character is recessive, dominant or shows no dominance are available. A very illuminating class study would be possible if one group in the class were given mice where the character was dominant, and another group where the character was recessive. The value of this particular example is that it demonstrates variable expression, modifiers, and can lead into a discussion of the evolution of dominance and other types of gene expression.

Sex linkage

The gene Tabby, *Ta*, produces a striped appearance in the coat (cf. tabby cats) when present in the heterozygous state (*Ta ta*) in the female. *Ta Ta* females and *Ta Y* males do not show the tabby coat: instead the coat is greasy and absent from the tail or behind the ears. The greasy phenotype is controlled

by a sex linked gene, the striped tabby mice being the female carriers of the gene for greasy coat.

Multiple linkage and three point test crosses

The multiple mutant stocks supplied by Harris Biological Supplies (see Sources of Materials) can be used to set up test crosses involving several genes.

Sources of materials

Stocks are available from the following sources:

Department of Education and Science Laboratories, Ivy Farm, Knockholt, Sevenoaks, Kent. They supply: *AA; aa; BB; bb; DD; dd; PP; pp; SeSe; sese; CaCa; caca; ReRe; rere;* $a^t a^t$.

Harris Biological Supplies, Oldmixon, Weston-super-Mare, Somerset. They supply *AA;* $\bar{a}a$*;* $a^t a^t$*;* Aa^t*; AAy; bbaa; ddaa; ccaa;* $c^e c^e aa$*;* $cc^e aa$*;* (*aa* indicates that colour types are non-agouti); *pp; PP; dd pp bb aa* (dilute, pink eye dilution, brown, non-agouti, together=silver champagne); *sese; seSe; Tata*(X), *TaY; pypy unun wewe* $a^t a^t$ *papa* (multiple mutant); *wa-2wa-2 vtvt* $c^{ch} c^{ch}$ (multiple mutant).

Dr M.E.Wallace, Department of Genetics, Cambridge University, will supply *Sd* lines showing variable dominance on request, although supplies cannot be guaranteed.

T. Gerrard & Co Ltd, Worthing Road, East Preston, Littlehampton, Sussex, supply a range of hair colour mutants.

References

FALCONER D.S. (1963) The use of mice in teaching genetics. In Darlington, C.D. & Bradshaw A.D. (Eds) *Teaching Genetics in School and University*, pp. 44–49. Oliver and Boyd, Edinburgh. (The use of mice, particularly in developmental genetics.)

NUFFIELD BIOLOGY TEACHERS' GUIDE V (1967) *The Perpetuation of Life*, pp. 30–35. Longmans/Penguin, London. (Best account of basic mouse technology.)

WALLACE M.E. (1965) Using mice for teaching genetics, I. *School Science Review, No.* 160, pp. 646–658. Using mice for teaching genetics, II. *School Science Review, No.* 161, pp. 39–52. (Excellent range of examples for teaching elementary and advanced concepts in genetics. Copies available from Association for Science Education, 52 Bateman Street, Cambridge, or Harris Biological Supplies, Oldmixon, Weston-super-Mare.)

WALLACE M.E., GIBSON J.B. & KELLY P.J. (1968) Teaching genetics: the practical problems of breeding investigations. *Journal of Biological Education*, **2**, 273–303.

(c) Arabidopsis

Once hailed as the *Drosophila* of the plant world, this plant has not been accepted as readily as was originally anticipated, because its short generation time of one month can only be achieved under ideal conditions of light and

temperature, and because it is difficult to cross. The ideal conditions for growing *Arabidopsis* are 20°–25°C and daylight supplemented with anywhere between 3·5–5·5 klx to give continuous illumination. A life cycle of about two months can however be produced if the plants are grown at a reasonably warm temperature and with spring or autumn day length. Any fine sieved soil is suitable, but preferably it should be light and sandy. The soil should be sterilized by heating or steaming. Seed trays or 75 mm pots are suitable. Seeds may be collected about 14 days after pollination: these are usually still unripe and will germinate in a few days. The seeds ripen at about three weeks and when first collected may be dormant, and remain dormant for about two months. This dormancy can be broken by soaking the seeds in water for about an hour and then keeping them at between 0°C and 5°C for four days. Germination then occurs in 2–3 days. Seeds older than two months germinate without pretreatment in about three days at 20–25°C. The seeds should not be allowed to dry out after sowing and are best sown directly on to the soil.

The seedlings must be watered gently. They can be watered using a very

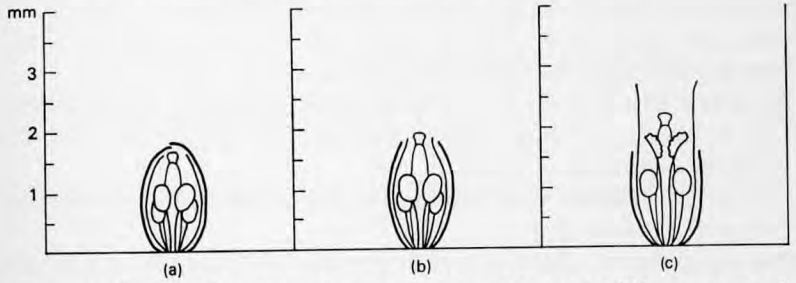


Fig. 2.1. Cross-section through a flower of *Arabidopsis* at different stages of development. Stages (a) and (b) are the preferred ones for emasculation. (From Muller, 1961.)

fine spray or the pots can be irrigated from below by standing them in shallow trays of water; in this way the water is absorbed into the soil from below, and the seed and seedlings are not disturbed. Another way is to cover the pots or seed trays with Saran Wrap. This is a thin cellophane-like material, which prevents water loss yet permits the passage of carbon dioxide and oxygen. If the soil in which the seed is sown is moist, then pots covered with Saran Wrap need watering only very occasionally.

Arabidopsis is normally completely self-pollinated, but the flower is slightly protogynous and emasculation can be performed by removing the anthers with a very fine pair of forceps. The best stages for removal of anthers are shown in fig. 2.1.

Because the flowers are tiny, it is helpful to use a binocular microscope (mounted on an arm and adjustable to take up a wide range of heights and orientations), a watchmaker's lens (×5–10), or lens-glasses. The flowers can

be held still with the finger and thumb or a pair of forceps which have the tips wrapped in cotton wool or soft synthetic sponge. Success must not be expected to come easily and a few plants should be set aside for practising on. If the female parent carries a recessive gene, then this can be used as a marker: seed produced by selfing in the F_1 will show the recessive character and can be discarded. If the stigmas are smothered with pollen then it is not necessary to bag the flowers to prevent accidental crossing, as the chances of this happening are remote.

The recent discovery of a male sterile mutant, *ms*, with reduced anthers and only 0·3 per cent seed setting on self pollination, by A.J.Müller, should greatly increase the value of *Arabidopsis* in teaching and research. The male sterile mutant can be used as female parent without emasculation.

The following mutants have a high viability and easily recognizable characteristics.

Recessive

angulosa (a_1): leaves narrow, angular, serrated, pale green; stems slender.
apetala (*ap*): most flowers without petals, but variable number of petals on later flowers; only two sepals, large and persistent; pods erect.
axillaris (*ar*): two flowers growing from base of each pod; otherwise normal.
clavata–1 (clv_1): pods large, lumpy and club-like, rosette flat; flower buds visible at early stage of growth.
cordata (*cor*): smallish plant with heart shaped leaves, curled over; leaves pale green; rosette deep.
glabra (*g*): glabrous; vigorous growth; few hairs occasionally on leaf margins or stems.
longipetiola (*lp*): petioles long; rosette leaves elliptical; stem leaves linear.
maculosa–1 (m_1): rosette leaves lightly mottled, yellow green, paler at base.
nigra (*nig*): rosette leaves dark green and glossy; cotyledons small and dark; anthocyanin visible at base of stems; stems several, slender; height 175 mm.
pallida–1 (pd_1): rosette leaves yellow green, paler at base; stems pale green with anthocyanin; pods small.
serrata–1 (se_1): leaves serrated; cotyledons long; flower bud opening early; petals few; pods small.
variegata–2 (v_2): plant variegated white green; expression varying; pods small.
The mutants listed above also illustrate, to varying degrees, the phenomena of pleiotropy and variable expressivity.

No dominance

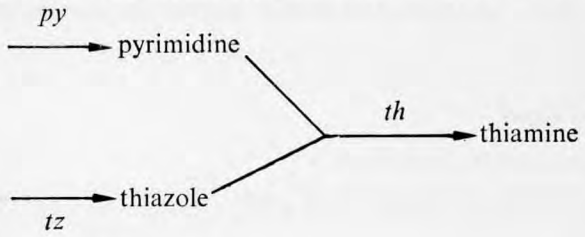
filicaulis–1 (fi_1): stems many, thin, crinkled; not exceeding 50 mm in height; pods long, thin with few seeds. Heterozygote intermediate.

Linkage

late (gi_1): late flowering (two months); large rosette.
patula (*pa*): dwarf; leaves dark green and curled; plant spreading and straggly; pods small.
chlorophyll-free (ch_1): no chlorophyll–*b;* yellow green plants.
These three linked in the order *gi–pa–ch* and separated by 25 and 8 map units respectively.

Nutritional mutants

pyrimidine requiring (*py*): requires 2:5 dimethyl–4 amino pyrimidine.
thiazole requiring (*tz*): requires 4–methyl–5 thiazole.
thiamine requiring (*th*): requires vitamin B_1.
These three mutants control the biochemical pathway shown below



The techniques for growing the mutants in aseptic culture are given by Redei (1968).

Sources of materials

Professor A.D.McKelvie, Agricultural Botany Department, College of Agriculture, 581 King Street, Aberdeen, Scotland, supplies: a_1, *ap*, *ar*, clv_1, *cor*, *g*, *lp*, m_1, *nig*, pd_1, se_1, v_2, fi_1.
Professor G.P. Redei, Department of Genetics, University of Missouri, Columbia, Missouri 65201, U.S.A., supplies: gi_1, *pa*, ch_1, *py*, *tz*, *th*.
Dr A.J.Müller, Deutsche Akademie der Wissenschaften Zu Berlin, Institut für Kulturpflanzenforschung, Gatersleben, Berlin, Germany, supplies: male sterile mutant (*ms*).

References

McKelvie A.D. (1962) A list of mutant genes in *Arabidopsis thaliana* (L.) Heynh. *Radiation Botany*, 1 233–241. (A list of mutants.)
Nuffield Biology Teachers' Guide V (1967) *The Perpetuation of Life*, pp. 81–82. Longmans/Penguin, London. (Good account of basic techniques.)
Redei G.P. (1968) *Arabidopsis* for the classroom. *Arabidopsis Information Service*, **5**, 5–7. (An account of a wide range of fairly advanced investigations, including nutritional mutants and aseptic culture.)

MÜLLER A.J. (1961) Zur charakterisierung der Blüten und Infloreszenzen von *Arabidopsis thaliana* (L.) Heynh. *Kulturpflanze*, **9**, 364–393.

MÜLLER A.J. (1968) Genic male sterility in *Arabidopsis*. *Arabidopsis Information Service*, **5**, pp. 54–55.

Arabidopsis Information Service, published by the Institut für Pflanzenbau und Pflanzenzüchtung, Universität Göttingen, Göttingen, Germany, is a useful source of information and further references.

(d) Tribolium

The flour beetles (*Tribolium confusum and T. castaneum*) have for many years been used in the study of population dynamics, and only recently have their advantages as a genetic organism become appreciated. The techniques used for culturing them can be obtained from *Tribolium Information Bulletin*, **1–3**, pp. 3–13.

Useful mutants for teaching *Tribolium* genetics are given in the following list.

Tribolium castaneum

Monohybrid ratios with dominance

Body colour: sooty, *s*, (black body gene) recessive to wild type (reddish brown);
jet, *j*, (black body gene) recessive to wild type (reddish brown).
Eye colour: pearl eye, *p*, (whitish eye) recessive to wild type (black eye).
Head size: microcephalic, *mc*, (small narrow head) recessive to wild type (large head).
Antenna shape: antennapedia, *ap*, (antennae larger than normal and foot like; segments elongated) recessive to wild type (with short segmented antennae).

Monohybrid ratio with no dominance

Black *B*, body colour semi-dominant to reddish brown wild type body colour. Heterozygote has bronze colour.

Dihybrid ratio with no linkage

Pearl eye and sooty body, are on separate chromosomes and will give a dihybrid ratio.

Linkage

Jet, *j*, body colour and microcephalic, *mc*, are both on chromosome *V*. The cross over value is 25 per cent.

Dominance and penetrance influenced by temperature

The scar mutant, *sc*, has an engraved transverse groove on the lower body segment (metasternum) just anterior to base of leg (coxa), shows temperature dependent dominance and penetrance. The percentage of population of *sc*/*sc* genotypes showing scar phenotype increases between 33°C and 35°C (relative humidity 70 per cent) from 70–80 per cent to 90–100 per cent, and dominance (i.e., percentage of population of genotype *Sc*/*sc* showing *sc* phenotype) from 0–21 per cent to 12–77 per cent.

Sex-linkage

Red, *r* (light red eye colour) sex linked and recessive to wild type black eye colour.

Recessive lethals

Short antenna, *Sa*, and fused tarsi and antennae (*Fta*) are both homozygous lethals.

Gene interaction

The genes *Sa* and *Fta* are at different loci but when *Fta* and *Sa* occur in same genotype, that individual dies.

Sex difference in recombination values

Jet, *j*, and ruby, *rb*, eye colour show different recombination rates in backcrosses depending on whether the heterozygote is male (33 per cent) or female (21 per cent).

Tribolium confusum

Monohybrid ratios with dominance

Pearl, *p*, (white compound eye) recessive to wild type (black eye).
Ebony, e_2, (black body colour) recessive to wild type (reddish brown body colour).

Linkage

Pearl, *p*, eye and ebony, e_2, body colour are both on the same chromosome and have a recombination value of 2·5 per cent.

Source of materials

Stocks can be obtained from:
Tribolium castaneum; wild type, *s*, *j*, *p*, *mc*, *ap*, *B; Tribolium confusum; p*,

e_2 from T. Gerrard & Co Ltd, Worthing Road, East Preston, Littlehampton, Sussex.

Tribolium castaneum, sc, From Dr A.E.Bell, Population Genetics Research Institute, Purdue University, Lafayette, Indiana.

Tribolium castaneum, r, Sa, Fta, from Professor A.Sokoloff, Department of Genetics, University of California, Berkeley, California.

Tribolium castaneum, rb, from Dr A.A.Dewees, Population Genetics Research Institute, Purdue University, Lafayette, Indiana.

Food packs available from T. Gerrard & Co Ltd, Worthing Road, East Preston, Littlehampton, Sussex.

References

Nuffield Biology 'O' Level Five Year Course catalogue available from T. GERRARD & CO LTD, Worthing Road, East Preston, Littlehampton, Sussex. (Good brief description of basic techniques and mutants.)

DEWEES A.A. (1967) Sex differences in recombination values for linkage group V of *T. castaneum. Tribolium Information Bulletin*, **10**, 89–90.

SOKOLOFF A. (1963) Two exercises demonstrating factor interaction in *Tribolium castaneum* Herbst. *Tribolium Information Bulletin*, **6**, 69–71.

BELL A.E., SHIDELER D.M. & EDDLEMAN H.L. (1964) Dominance and penetrance of the scar (*sc*) mutant in *Tribolium castaneum* as influenced by temperature. *Tribolium Information Bulletin*, **7**, 46–48.

SOKOLOFF A. (1966) *The Genetics of Tribolium and Related Species*. Academic Press, New York. (Advanced research text describing findings rather than techniques.)

HOSTE R. (1968) The use of *Tribolium* beetles for class practical work in genetics. *Journal of Biological Education*, **2**, 365–372.

HASKINS K.P. (1969) *Using Tribolium for Practical Genetics*. Harris Biological Supplies Ltd, Weston-super-Mare, England.

(e) Other organisms

Very few other organisms are in common use for crossing experiments carried over several generations. Either the generation time is too long or there is a shortage of mutants. Where the generation time is too long, an investigation could be initiated in one year and carried on by a set of students in the following year, but in many ways this is unsatisfactory since the first generation of a cross is relatively uninformative. It is far better for the teacher to perform the initial cross himself and report the results when the students look at the F_2 generation.

The species described in the following section can be used for long-term investigations. Such investigations may be done as a class research project covering several years and aimed at investigating a complex of major gene characters such as are found in coat colours of mice, hamsters or guinea-pigs, or flower pigments and other characteristics of garden plants.

2. SEGREGATION DEMONSTRATED IN LIVING MATERIAL

The most potent effects of the laws of inheritance are seen in the second generation, and the demonstration of segregation in this generation is therefore one of the best ways of showing these laws in action. The parental lines and the F_1 can be indicated theoretically, kept from previous generations or demonstrated by using the appropriate phenotypes selected from the F_2.

When an F_2 generation is required, it is not normally necessary to start the whole sequence of crosses *de novo*. There are several methods whereby the F_2 can be obtained in a far shorter time.

(i) If the dominance of one gene is incomplete such that the F_1 genotype can be recognized amongst the F_2 classes, this genotype can be maintained for production of another 'F_2' in the following season.

(ii) The F_1 can be maintained vegetatively and crossed to give an F_2 when required.

(iii) The F_2 can be maintained as a collection of seeds which are sown and scored by the student as required. Where juvenile or seedling characters can be studied an F_2 can be obtained in a matter of a few weeks.

The latter two techniques are more or less restricted to plant material, which is therefore particularly suitable for this category of investigation.

(iv) An alternative scheme, which can be combined with the above, is to use backcrosses, since even with dominance all the progeny of a backcross are of known constitution. The sequence of crosses is as follows: $AA \times aa \rightarrow Aa$; $Aa \times aa \rightarrow Aa$ and aa, which can then be intercrossed to give the same ratio again. This scheme can be used for two gene cases as well since

$$\frac{A}{A}\frac{B}{B} \times \frac{a}{a}\frac{b}{b} \rightarrow \frac{A}{a}\frac{B}{b}. \quad \frac{A}{a}\frac{B}{b} \times \frac{a}{a}\frac{b}{b} \rightarrow \left[\frac{A}{a}\frac{B}{b}\right], \frac{a}{a}\frac{B}{b}, \frac{A}{a}\frac{b}{b} \;\&\; \left[\frac{a}{a}\frac{b}{b}\right].$$

The types in brackets can be picked out and used again. In this way, after the initial F_1 has been obtained, only 'one generation' is necessary for the production of a segregating backcross progeny.

(a) Recognition of F_1 amongst the F_2 classes

(i) Groundsel: *Senecio vulgaris* L.

A cross between groundsel without ray florets (normal form) and groundsel with long ray florets results in an F_1 with short ray florets which are intermediate in length. The long ray florets are longer than broad whereas the short ray florets are square shaped. The F_2 segregates in a 1 : 2 : 1 ratio for the three classes, and since groundsel is almost entirely self-pollinated the seeds from plants with short ray florets again produce the same ratio. The seeds are viable for several years. Groundsel will flower in about 6–8 weeks,

in any soil, provided it is kept reasonably warm (i.e. greenhouse in winter). Groundsel readily sheds its seeds but this problem can be eliminated by digging up the appropriate genotypes and letting them ripen off in a vase, beaker or jam jar containing water. This can be placed indoors in a convenient draught-free position and seeds collected every morning.

Sources of material

Department of Education and Science Laboratories, Ivy Farm, Knockholt, Sevenoaks, Kent.

References

The only published works on this subject are some 50 years old, and include accounts of other characters in groundsel.

TROW A.H. (1912) On the inheritance of certain characters in the common groundsel—*Senecio vulgaris* Linn—and its segregates. *J. Genet., Cambridge*, **2**, 239–276.

TROW A.H. (1916) On the number of nodes and their distribution along the main axis in *Senecio vulgaris* and its segregates and on albinism in *Senecio vulgaris* L. *J. Genet., Cambridge*, **6**, 1–74.

A brief account of this work is given in Turrill W.B. (1958) *British Plant Life* 2nd Ed. Collins New Naturalist Series, p. 219.

(ii) Seedling characters

The following seedling characters show intermediate expression in the heterozygote (see section (c)(ii) for further details and sources of materials).

Tomato: normal/lanceolate cotyledons
white/green cotyledons
Radish: red/white hypocotyl
round/long hypocotyl
Geranium (*Pelargonium zonale*): green/white seedling
Marrow Stem Kale: red/green hypocotyl
Soybeans: green/white leaves on seedlings.

(b) F_1 maintained vegetatively

(i) White clover (*Trifolium repens*)

White clover is of special interest, since its populations are polymorphic for several sets of major gene characters. Clover can be easily grown from cuttings (a few internodes long) pushed into ordinary garden soil or potting compost. It thrives best in cool bright conditions, and the leaf mark characters in particular are seen most clearly outdoors in spring, or in a cool greenhouse with artificial light. These conditions also reduce the chances of infection

from aphids or red spider—an important consideration since greenhouse sprays and insecticides can easily damage clover leaves. Clover grows quickly and has to be replanted at least once a year, or cut back to prevent stolons trespassing into adjacent pots. Unwanted seed heads should also be removed before they are ripe, to prevent foreign seed germinating and 'contaminating' the existing plants.

Clover seed germinates slowly, and it is best *scarified* (weakening of seed coat) by immersing dry seed in *conc.* H_2SO_4 for 15 min, draining off the acid, and washing with plenty of water. Alternatively the seed can be rubbed with sandpaper. The seed is then sown directly or germinated on a moist filter pad and then sown as a small seedling.

The seedlings will flower if they are given summer (long) day length, but plants which have flowered once or adult plants collected in the field need short days and/or cold treatment (outdoors in winter) for about two months, followed by long days for flowering to occur. In natural conditions the plants normally flower between May and July. Clover is to all intents and purposes self-sterile so emasculation is unnecessary. Pollen is best transferred using the pointed tip of a cardboard triangle, which has been folded down the middle (see fig. 2.2). The keel is pulled away from the head with a pair of fine

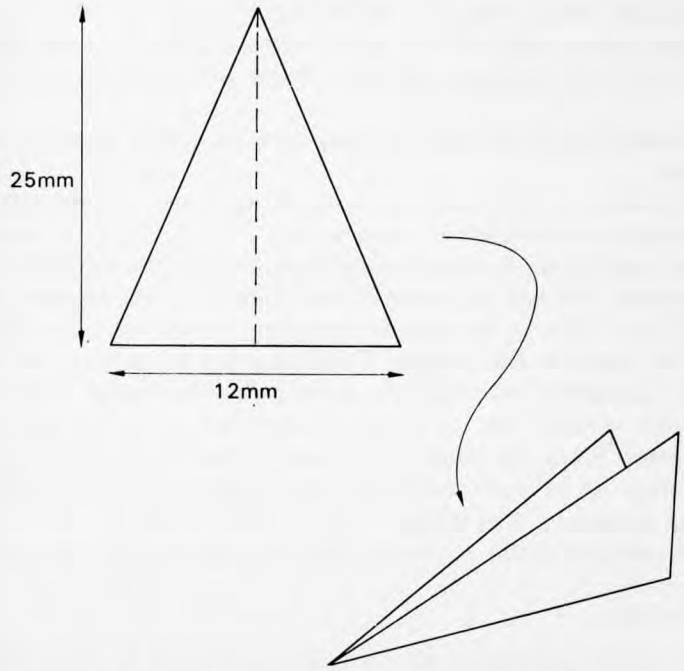


Fig. 2.2. Cardboard pollinator for clover plants.

forceps and the stigma is extruded for pollination. The plants or flowerheads can be covered in muslin to prevent stray pollination, or an insect-free greenhouse should be used. About four seeds per floret are produced after 4–6 weeks. Pollination is made easier if the number of florets per head is reduced to about 20 which are at an equivalent stage with their wing petals about to open.

The following sets of characters are of particular interest:

V-shaped white leaf markings

The V-shaped leaf marks are determined by an allelic series, where absence of leaf mark is recessive to its presence. The different leaf marks show no dominance with respect to each other, so that where the marks are in different positions on the leaf, the heterozygotes can be distinguished (fig. 2.3) and can be propagated vegetatively.

The following leaf marks are known (their percentage frequency in wild populations in Britain is indicated):

vv: absence of leaf mark (23·4 per cent)
V^l: V mark in lower half of leaflet, size and position variable (72·2 per cent)
V^h: V mark extending into upper half of leaflet (0·4 per cent)
V^f: full leaf mark, area enclosed by V is all white (0·2 per cent)
V^b: broken leaf mark, point of V is absent (very rare)
V^{by}: broken yellow, point of V is yellow, arms are white (1·1 per cent)
V^{ba}: full leaf mark, but basal, narrower, longer and fainter than V^f (0·4 per cent).

The remaining few per cent are types showing double marks of one sort or another.

The incidence of leaf mark types other than *v*, and V^l is therefore rare and a whole day's collection is necessary to pick up some of the rarer types from wild populations. In mixed populations of *v* and V^l types, heterozygotes (phenotypically V^l) can be recognized by looking at the products of seed collected from plants in the wild: heterozygotes should show an appreciable number of *v* types in the progeny. These can then be collected as a group which is maintained by vegetative propagation for future intercrossing. Alternatively crosses can be made between the types to generate the heterozygotes. Since the phenotypes closely resemble the genotypes other types of cross can be made to illustrate the consequence of say, crossing two genotypes containing four different alleles. Used purely for demonstration purposes, such leaf marks are an excellent illustration of an allelic series.

Red leaf marks

Red anthocyanin patterns on the leaves are also known in clover. The most common type are the *R*-series of three alleles.

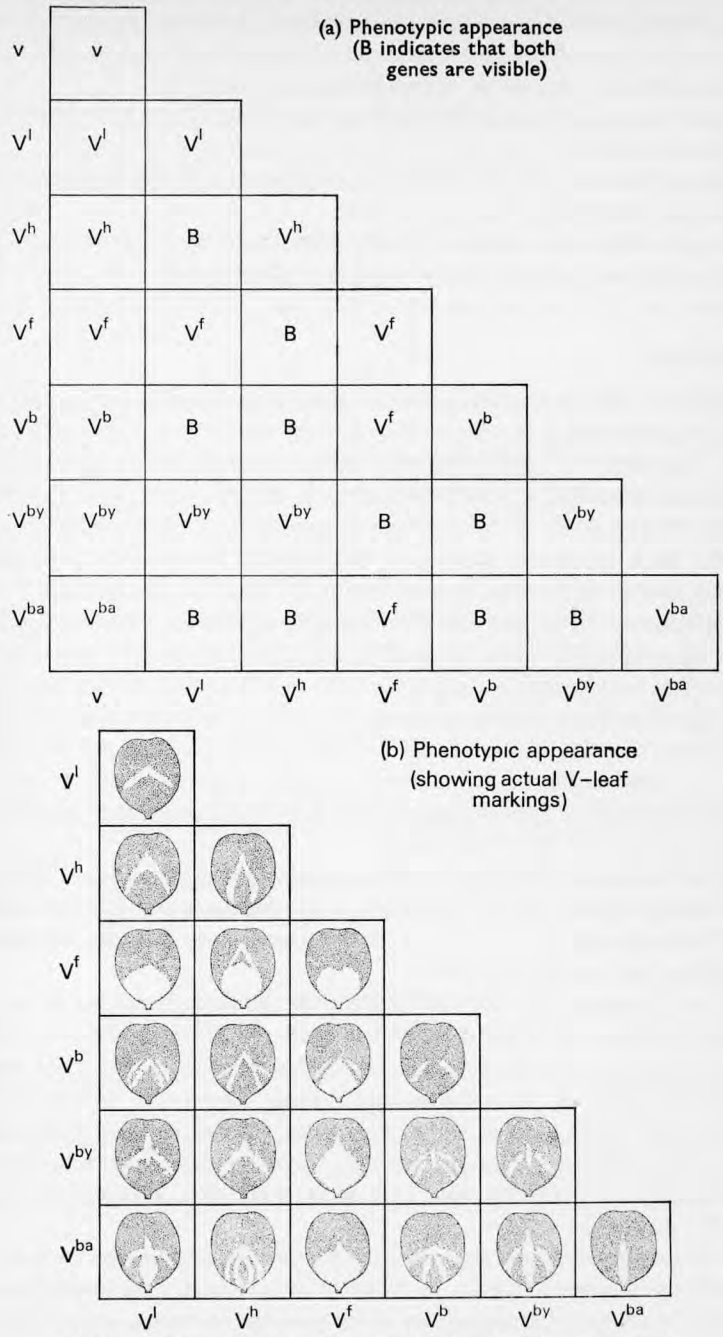


Fig. 2.3. V-shaped leaf marks in white clover (*Trifolium repens*).

R^f: red flecks or streaks of varying sizes, tending to be elongated parallel to lateral veins, also somewhat concentrated around the midrib. This is fairly common, but the expression of the gene is very variable.
R^m: here the anthocyanin is restricted to just either side of the midrib. This is rarely found wild.
R^l: here all the leaf is red. This too is rarely found wild but is often preserved in Botanical Gardens as var. *purpureum*.

All these alleles are dominant, and unlinked to the *V*-series. Again, cool bright conditions enhance the expression of these genes.

Cyanogenesis

The ability to release cyanide when the plant is damaged is determined by two unlinked genes, and populations of clover are normally polymorphic for both genes. One gene, *Ac*, determines the ability to produce the glucosides, linamarin and lotaustralin, which will liberate HCN when acted upon by an enzyme. The presence of this enzyme, linamarase, is determined by another gene *Li*. Both genes are dominant (to inability to produce glucoside and enzyme), and both have to be present for full cyanide production.

The normal test for cyanide production is as follows. Crush and macerate two or three leaves in a few drops of water in the bottom of a small 50 mm × 15 mm glass tube (using a glass rod). Add two drops of toluene and insert a short slip of sodium picrate paper by jamming it between the cork and the side of the tube. The paper should be clear of the liquid at the bottom. The tubes are then incubated for 2 hours at 40°C, and if cyanide is released it will turn the sodium picrate papers from yellow to a reddish brown. This will only be positive for the *Ac Li* plants.

However, *Ac Li* plants can be distinguished from *Ac li* because spontaneous (non-enzymatic) hydrolysis of the glucosides also takes place but more slowly. If the tubes are left for at least another 24 hours, *Ac li* plants will also turn the sodium picrate paper brown.

The *ac Li* plants can only be detected by adding glucosides to see if the enzyme is present to break them down. Both *Ac li*/*li* and *Ac Li*/- plants can be used, because if the leaves are autoclaved (110°C for 25 min) then the enzyme but not the glucoside is inactivated. About 100 leaves should be macerated in about 25 ml of water, the extract filtered, and two drops (instead of two drops of water) used to macerate the cyanogenic leaves to test for *Li*. The preparation should be made just prior to the tests, as it keeps its activity for a few days only.

Once recognized by the appropriate tests, the genotypes can be propagated vegetatively. Crosses between completely acyanogenic and cyanogenic types will serve to identify the genotypes of the cyanogenic forms, and can serve as a backcross. Once these genotypes have been recognized, F_2 ratios can be

generated by crossing genotypes heterozygous for either one (to give a 3 : 1 ratio) or both genes. If no attempt is made to distinguish the non-cyanogenic types a 9 : 7 ratio is obtained, but if *Ac li*, *ac Li* and *ac li* plants are distinguished a 9 : 3 : 3 : 1 ratio will be obtained. This is a good example of evidence of complementary gene action since both genes are necessary for the biochemical sequence to take place, and therefore for the character to be expressed.

The value of these various investigations in clover is that they provide a link between elementary genetics, population genetics and natural selection. The cyanogenesis character is also a clear demonstration of the way in which genes act in metabolism.

Source of materials

Homozygotes and heterozygotes can be obtained from Dr W.E.Davies, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth. However a supply of every possible type cannot be guaranteed. Many of the types can be obtained by collection from natural populations.

Sodium picrate paper is made by neutralizing a nearly saturated solution (0·05M) of picric acid with sodium bicarbonate $NaHCO_3$, filtering, and soaking filter paper in the solution. When dried the papers can be kept in a stoppered jar and used as required.

References

DAVIES W.E. (1963) Leaf markings in *Trifolium repens*. In DARLINGTON C.D. & BRADSHAW A.D. (Eds) *Teaching Genetics in School and University*, pp. 94–98. Oliver & Boyd, Edinburgh.

PUSEY J.G. (1963) Cyanogenesis in *Trifolium repens*. In DARLINGTON C.D. & BRADSHAW A.D. (Eds) *Teaching Genetics in School and University*, pp. 99–104. Oliver & Boyd, Edinburgh.

(c) F_2 maintained as seed

(i) Peas

Mendel's experiments can be repeated or mimicked with different varieties of peas showing round/wrinkled or green/yellow seeds. Suitable varieties (most of which are available commercially) are shown in table 2.1 (pp. 44–47). This table also distinguishes tall and dwarf, and blunt pod and pointed pod varieties but these characters do not appear till later in the life cycle.

Table 2.1 Basis of classification

Pod type	Seed	Cotyledons	Pod shape	Stem	Group
Normal. *Pisum sativum* sub sp. *hortense*	Round	Yellow	Pointed	Under 450 mm.	I
				450–750 mm.	II
				750–1100 mm.	III
				Above 1100 mm.	IV
			Blunt	Under 450 mm.	V
				450–750 mm.	VI
				750–1100 mm.	VII
				Above 1100 mm.	VIII
		Green	Pointed	Under 450 mm.	IX
				450–750 mm.	X
				750–1100 mm.	XI
				Above 1100 mm.	XII
			Blunt	Under 450 mm.	XIII
				450–750 mm.	XIV
				750–1100 mm.	XV
				Above 1100 mm.	XVI
	Wrinkled	Yellow	Pointed	Under 450 mm.	XVII
				450–750 mm.	XVIII
				750–1100 mm.	XIX
				Above 1100 mm.	XX
			Blunt	Under 450 mm.	XXI
				450–750 mm.	XXII
				750–1100 mm.	XXIII
				Above 1100 mm.	XXIV
		Green	Pointed	Under 450 mm.	XXV
				450–750 mm.	XXVI
				750–1100 mm.	XXVII
				Above 1100 mm.	XXVIII
			Blunt	Under 450 mm.	XXIX
				450–750 mm.	XXX
				750–1100 mm.	XXXI
				Above 1100 mm.	XXXII
Edible Podded. *Pisum sativum* sub sp. *saccharatum*	Round	Yellow	Pointed	450–750 mm.	XXXIII
			Blunt	Above 1100 mm.	XXXIV
	Wrinkled	Green	Pointed	Above 1100 mm.	XXXV
Purple. *Pisum sativum* sub sp. *arvense*				Over 1100 mm.	XXXVI

Against each name is the Roman number of the group in which it falls.

Abundance	XVIII	Cropper	XVIII
Abundant	XXVII	Cropwell	XXVI
Achievement	XXVIII		
Admiral Beatty	XXVIII	Daisy	XXV
Advance	XXVI	Daffodil	XVIII
Advance Guard	XXVII	Deg Grace	XXXIII
Alaska	XV	Delicatesse	XXVII
Alderman	XXVIII	Doux Provence	IX
Allotment Holder	XVII	Dreadnought	XXXI
American Wonder	XXX	Dr. Kitchen	XXVI
American Wonder Improved	XXX	Droitwich Victory	XXVI
Annonay	V	Droitwich Wonder	XXVIII
Aristocrat	XXVII	Duchess	XXVIII
Autocrat	XXXI	Duke of Albany	XXVIII
Aviator	XII	Duke of York	XXVIII
		Duplex	XXVI
Banqueter	XXVIII	Dwarf Alderman	XXVI
Bantam, The	XVIII	Dwarf Champion	XXVI
Beaconsfield	XXVII	Dwarf Defiance	XXVI
Bedford Champion	XXVIII	Dwarf Exhibition	XXVI
Benefactor	III	Dwarf Prolific	XVIII
Best of All	XXVII	Dwarf Quite Content	XXVI
Better Times	XXVI	Dwarf Sugar	XXXIII
Big Ben	XIV	Dwarf Wonder	XVIII
Borderer	XXXI	Earl Grey	IV
Boston Unrivalled	XXVIII	Earl Haig	XVII
Bountiful	XI	Earle King	XXX
Breck, The	XXVII	Earliest of All	XV
British Lion	XII	Early Bird	VII
		Early Custom	XXXI
Cambria	XXVI	Early Dawn	XI
Canners Perfection	XXXI	Early Dwarf	IX
Caractacus	VI	Early Giant	XX
Carrington Gem	XXVII	Early Giant Marrowfat	XXVIII
Carrington Perfection	XXXI	Early June	XXVI
Carrington Wonder	XXVI	Early Market	XXIV
Certain Satisfaction	XVII	Early Marrowfat	XVII
Challenger	XII	Early Morn	XX
Champion Marrowfat	XXVII	Early Onward	XXX
Chancelot	XXVII	Early Prolific	XXX
Charles Ist	XXVII	Early Superb	X
Cheltonian	XXVII	Early Wonder	XXVI
Chemin Long	VI	Eclipse	XV
Clipper	VII	Eight Weeks	I
Clucas, The	XV	Emerald	XXVIII
Comet	XXVI	Emigrant	XIV
Commonwealth	XXVI	English Wonder	XXX
Contentment	XXVII	Essex Star	IV
Continuity	XXXI	Essex Wonder	XXVIII
Coronation	XXVIII	Eureka	XXVI

Everbearing	XXVI
Everest	XXII
Evergreen	XXVIII
Evergreen Gem	XXVIII
Evesham No. I	IX
Exceptional	XXVI
Exhibition	XXVII
Exquisite	XXIV
Extra Early	III
Favourite	XXVI
Feltham Advance	XIV
Feltham First	IX
Feltham Forward	XV
Fenland Wonder	XXVI
Field Marshall	XXVI
Fillbasket	X
Fin des Gourmets	IX
First Early	VI
First of All	III
Foremost	XI
Forerunner	XI
French Canners	III
Giant Laxtonian	XVIII
Giant Stride	XXVI
Giant Sugar Pod	XXXIV
Gilt Edge	XXVII
Gladiator	X
Gladstone	XXVII
Gradus	XX
Greenfeast	XVIII
Greenmantle	II
Gregory's Surprise	XXXI
Half-a-League	XXXI
Harbinger Improved	XXII
Harrison's Glory	XIV
Harvestman	XXVIII
Highland Laddie	XXVI
Holdfast	XXXII
Hundredfold	XVIII
Hurst's 40	IX
Ideal Dwarf	XXVI
Improved Harbinger	XXII
Impudence	XVII
International	XX
Invicta	XXVII
Invincible Marrowfat	XVIII
Jap, The	IV
June Wonder	XX
Kelvedon Champion	XXV
Kelvedon Hurricane	XXVI
Kelvedon Maincrop	XXVI
Kelvedon Monarch	XXXI
Kelvedon Perfection	XXVI
Kelvedon Spitfire	XXV
Kelvedon Standby	XXVI
Kelvedon Triumph	XXVI
Kelvedon Wonder	XXVI
King Edward	XXVI
King George	XXVIII
King Marrowfat	XX
King of Dwarfs	XXXI
Lancashire Lad	XXVI
Lancashire Lady	XXVI
Lancashire Pride	IX
Last of All	XXVII
Late Duke	XX
Late Queen	XXXI
Lathom Wonder	XXVI
Laxtonian	XVIII
Laxton's Progress	XVII
Laxton's Superb	X
Leader	XXVI
Liberty	XXVII
Lincoln, The	XVIII
Little Giant	XXVI
Little Hero	IX
Little Marvel	XXX
Local Lady	IV
Loyalty	XVIII
Lord Chancellor	XXVII
Magnum Bonum	XXVII
Maid of Kent	III
Maincrop (Suttons)	XXVII
Maincrop Marrowfat	XXVIII
Manchester Man	XXVI
Manifold	XVIII
Market Gem	VI
Market Pride	X
Market Wonder	XXX
Marvellous	XXX
Masterpiece	XVIII
Matchless Marrowfat	XXVIII
Meteor	IX
Mid-Century	XVI
Miracle, The	XXVIII
Morse's Market	XXVI
Munnings	XXVII
My Own Marrowfat	X
New Surrey Star	XXVIII
No. 457	XXVI
Northumbria	XXVI

Olympic	XII
Onward	XXXI
Ormskirkian	XXVIII
Osmaston Pride	XXVI
Osmaston Surprise	XXVIII
Perfected Freezer	XXXI
Perfection Marrowfat	XXIV
Peter Pan	XVIII
Petit Provençal	IX
Phenomenal	XXVI
Phenomenon	XXVI
Pilot	III
Pioneer	XVIII
Poulwell	XVII
P.P.	XXVII
Premier	XXVI
Primo	III
Prince Edward	XXVIII
Prince of Wales	XXIII
Priority	XVI
Prize	XXVIII
Profusion	XXIII
Progress No. 9	XXV
Provost	XXVIII
Purple Podded	XXXVI
Queen	XXVII
Queen of The Marrowfats	XXVIII
Recorder	XXVII
Referendum	XVIII
Rentpayer	XXVI
Right Royal	XXVI
Rival	XVIII
Rivenhall Wonder	XXVI
Robust	XXVII
Roi des Conserves	XI
Royal Favour	XXVII
Royal Salute	XXVII
Royal Standard	XXVIII
Senator	XXVII
Sensation	XVII
Serpette d'Auvergne	III
Shasta	XXXI
Sherwood, The	XXVI
Speed	VII
Springtide	VII
Standard	XXVIII
Steadfast	XXXI
Stella	IV
St. George	XXVI
Stourbridge Marrow	XXVIII
Stratagem	XXVI
Success	XVIII
Succession	XXVI
Successional	XI
Sugar Paramount	XXXIV
Sugar Tall	XXXV
Superlative	XXVII
Super Telegraph	XII
Supremacy	III
Supreme	XXXI
Tall Sugar	XXXV
Taxpayer	XXVI
Telegraph	XII
The Bantam	XVIII
The Breck	XXVII
The Clucas	XV
The Jap	IV
The Lincoln	XVIII
The Miracle	XXVIII
The Sherwood	XXVI
Thos. Laxton	XXIV
Timperley Wonder	XVII
Tip Top	VII
Tom Clucas	XI
Tomorrow	XI
Tremendous	XX
Trophy	XXXI
Union Jack	XXVII
Unique	I
Universal	XXVI
Veitch's Perfection	XXXI
Victoria	VIII
Victoria (Toogood)	XXXI
Victory Freezer	XXXI
Volunteer	XI
What's Wanted	XI
William Hurst	XXVI
William The First	III
Witham Wonder	XXVI
Wireless	II
Wonder	XXVI
Wonder Marrowfat	XVIII
World's Record	XX
Yielder	XXVI
Yorkshire Hero	XXXI
Zelka	XIV

(ii) Maize

Few plants have been more closely investigated from the genetic point of view than maize. Many genes affecting the 'seeds' (kernels) of maize are known and maize has the advantage that these kernels are all held together in a cob. These dried cobs can be kept for several years.

Procedure for controlled pollination of maize

Materials required

An exercise book for recording pollination details
300–400 150 mm × 100 mm transparent paper bags
200 400 mm × 250 mm 1000 grade polythene bags (these can be washed and used again each year)
A sharp pocket knife
Stakes each bearing a maize family number
Paper clips
A chinagraph pencil
Support stakes and string

Planting

Seed is sown during the last week of April or the first week of May. A single maize seed is planted 350 mm apart from the next in rows which are 650 mm apart. Place a stake with the appropriate family number at the beginning of each family in the rows. Each family is limited to contain 20 plants.

Covering of female flowers

The female flowers appear about mid-July as a flat green sheath in the leaf axil (A1 fig. 2.4). Select only the female nearest the top of the plant (usually the least forward on the plant). Cut a slit on each side of the leaf base and, after ensuring that no stigmas or 'silks' have protruded, trim off the top half inch of the female flower. Immediately pull a transparent paper bag down over the female so that it is completely covered and the open end of the bag is securely tucked down in the slit between leaf base and stem (A2 fig. 2.4).

Check the plants at two or three day intervals to cover the topmost females as they appear and trim off any excessive sheath growth on those previously trimmed. Make sure that all are properly covered by the bags.

Pollen collection

The male flowers are at the top of the plant and are carried on a number of smaller stems. When pollen shedding begins on the required male, enclose as many of the flowers as can be easily contained in a transparent paper bag, fold over the excess opening, and secure with a paper clip (B fig. 2.4).

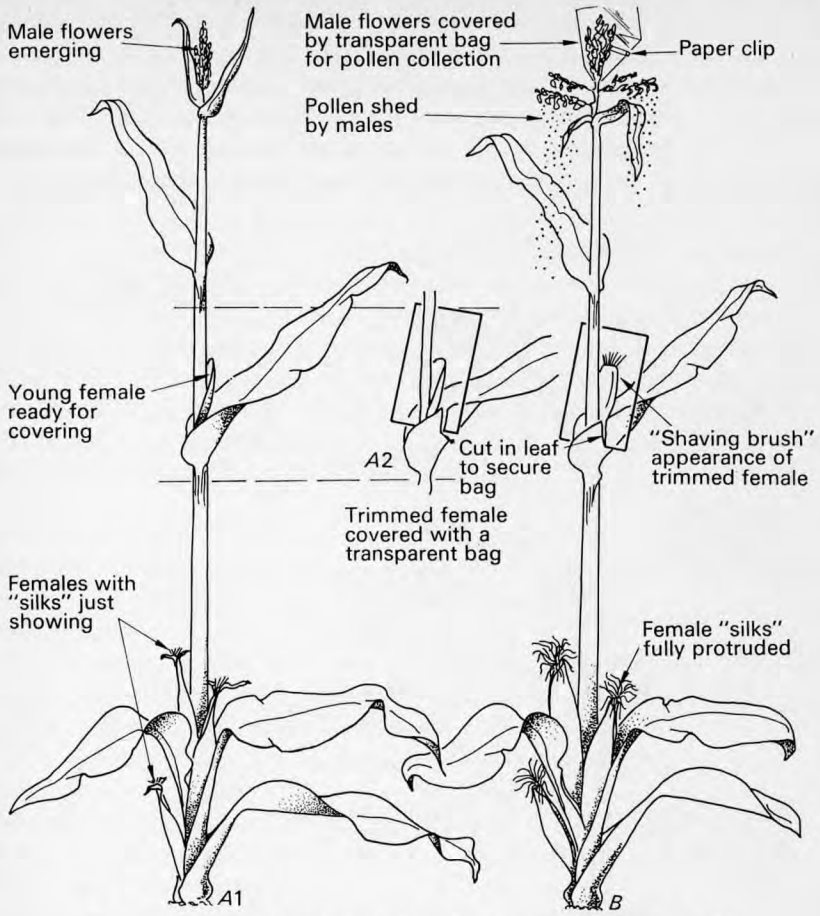


Fig. 2.4. Pollination techniques in maize (for explanation see text).

To collect a supply of pollen, cover the males (provided they are dry and shedding pollen freely) as described above during mid-morning and let the pollen accumulate in the bag until the afternoon.

Pollination

From the pollinating programme check the details of the pollination about to be made. Collect the pollen from the male flower by *carefully* bending the plant over (C fig. 2.5) and gently tap the bag so that a small heap of pollen is gathered. Keeping the bag in the same horizontal position, very carefully withdraw the inflorescence, leaving the pollen behind. Carry the bag of pollen (still horizontally) to the required female, tuck the top half of the plant behind

the free (usually left) arm, and when 'foreign' pollen disturbed by the movement has cleared, rip off the top portion of the bag covering the female (C). Quickly dust the collected pollen over the exposed female (C) and immediately cover with a large polythene bag and secure with two paper clips (D fig. 2.5).

Write the pollination details on the polythene bag with a chinagraph pencil (female × male) and also note the same details in the record book.

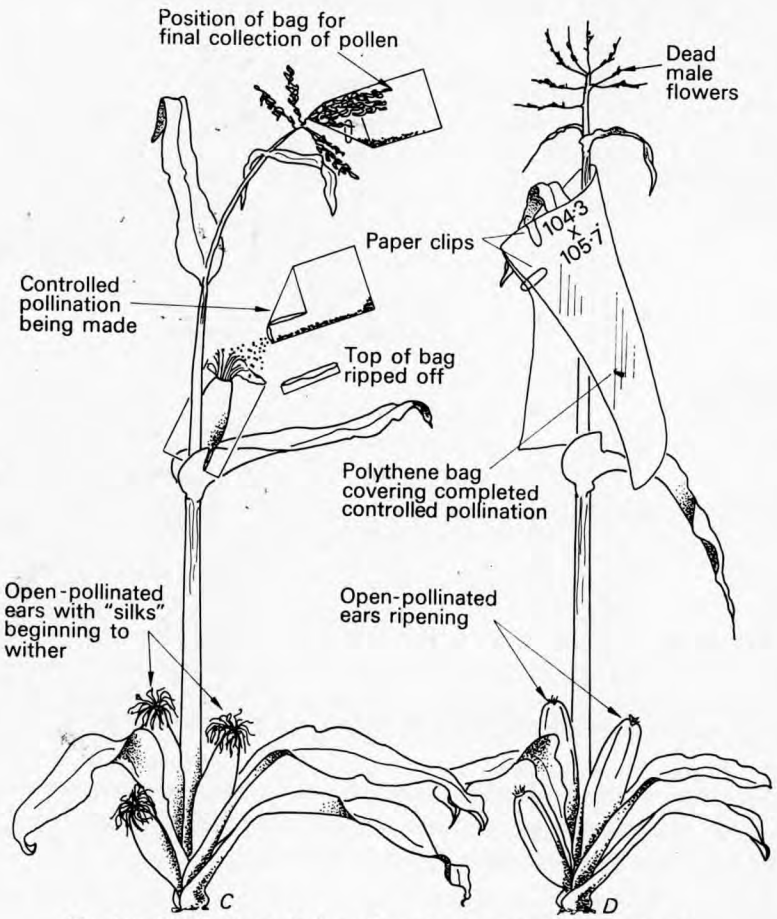


Fig. 2.5. Pollination techniques in maize (for explanation see text).

Ripening and harvest

Ripening time varies and is dependent on the weather conditions. All pollinations should be completed by mid-August and covered ears left to ripen. Stake and tie any plants that have been blown over by the wind. Harvesting can begin in mid-October if the late summer and autumn have been warm and sunny, but in bad conditions the ears may not be gathered until early November.

Detach the ear from the plant, strip off the sheath leaves and put back into its polythene bag for transport to the laboratory.

Storage

In the laboratory, remove the ear from its bag and attach a label bearing the pollination details. Lay the ears out on a suitable rack or bench (near a radiator if possible) and leave to dry and harden. Check the ears from time to time in case any fungal growth starts (smaller patches of contamination can be eradicated by dipping the ear into 2 per cent lysol solution and putting out to dry again). The ears should be dried and hard after about six weeks and must be stored in dry, moth-proof containers (plastic bags sealed at the top or tins). Care should be taken to see that the ears are really dry before storage.

It should be noted that the maize plant tends to be rather brittle in all its parts so that all handling must be done with care.

Source of materials

A very wide variety of corn cobs is available from Carolina Biological Supply Company, Burlington, N. Carolina 27215, U.S.A.

Purple: yellow, 3 : 1 and 1 : 1 (aleurone colour)
Also available from: Faust Scientific Supply Co, Madison, Wisconsin 53713; and Genetic Products and Services, Goshen, Indiana 46526.

Smooth: shrunken, 3 : 1 and 1 : 1 (seed texture)
Also available from: Faust Scientific Supply Co.

Starchy: sweet, 3 : 1 and 1 : 1 (endosperm texture)

White: purple, 3 : 1 (dominant colour inhibitor)

Starchy: waxy, 3 : 1 (endosperm character, stains with iodine)

Purple: yellow/starchy: sweet, 9 : 3 : 3 : 1

Purple: white/smooth: shrunken, 9 : 3 : 3 : 1, 1 : 1 : 1 : 1
From Faust Scientific Supply Co; Genetic Products and Services; and Connecticut Valley Biological Supply Co Inc, Southampton, Massachusetts 01073.

Yellow: white/starchy: sweet, 9 : 3 : 3 : 1

Purple: red/yellow: white, 9 : 3 : 3 : 1

A range of cobs illustrating various types of gene interaction giving ratios of 12 : 3 : 1, 9 : 3 : 4, 9 : 7, 13 : 3.

Two point linkage: anthocyanin and shrunken endosperm on chromosome III

Three point linkage: purple, shrunken, waxy on chromosome IX

Four point linkage: as with three point linkage, but addition of yellow/green seedling factor.

Large numbers of these cobs are fairly expensive to buy, but they can be obtained by crossing and then easily preserved.

The following mutants are available on request from Dr H.L.K.Whitehouse, Botany School, Cambridge.

Tu: tunicate (bracts surrounding grains)

P: various alleles for pericarp colour (purple anthocyanin pigmentation of pericarp)

A, C, R: genes for aleurone pigmentation

sh: shrunken endosperm

y: white endosperm

wx: waxy endosperm and pollen

su: sugary endosperm and wrinkled seed

Dt: dotted (causes mutation of gene *a* to *A* for aleurone pigmentation)

C and *sh* are on the same chromosome with 7 per cent crossover between them.

A wide range of mutants including those mentioned above are available from: Professor R.J.Lambert, Plant Breeding, College of Agriculture, University of Illinois, Urbana, Illinois 61801; and Mr Clarion Henderson, Illinois Foundation Seeds, Champaign, Illinois.

F_2 maintained as seed (seedling characters)

A wide range of seedling characters are known which are suitable for demonstrating genetic segregation (table 2.2). Seeds of all the types mentioned germinate readily at room temperature or in the greenhouse and the seedlings are ready for inspection in 2–3 weeks. The seed can be sown in soil, on moist filter paper or on agar.

For simple one gene segregation all the mutants can be mixed in the appropriate ratio, and for some other seedling characters progeny segregating for a two-gene difference are available on the market.

It does not seem the place here to mention the crossing techniques for this wide range of plants, but if further information is wanted on this it can be had by writing to the sources of the material, the people mentioned in the references, or to horticultural and agricultural breeding stations (addresses available from local agricultural advisory services).

Table 2.2(a) Seedling characters

Species	Character pair (* indicates dominant)	Other comments	Source or trade name	References
Tomato (*Lycopersicum esculentum*)	(a) (i) Red*—green hypocotyl		L.K.Crowe	(1) (2) (3) (4) (5)
	(ii) Purple*—green (*A, a*) hypocotyl	Chromosome II	P.G.L.† 5	
	(iii) Purple*—green (*Bls, bls*)	Chromosome III	P.G.L. 2, P.G.L. 6, P.G.L. 9	
	(iv) Purple*—green (*Ah, ah*) hypocotyl	Chromosome IX	P.G.L. 6	

Table 2.2(a).—*continued*

Species	Character pair (* indicates dominant)	Other comments	Source or trade name	References
	(b) (i) Normal cut*—potato leaf	(leaflets entire in potato leaf type)	L.K.Crowe	
	(ii) Normal cut*—potato (*C,c*) leaf	Chromosome VI	P.G.L. 5	
	(iii) Normal cut*—potato (*Sf, sf*) leaf	Chromosome III	P.G.L. 9	
	(c) (i) White—green (*Xa*-2, *xa*-2) cotyledons (het: yellow green)	White seedlings die soon after germination Chromosome X	P.G.L. 4	
	(ii) White—green (*Xa*-3, *xa*-3) cotyledons (het: yellow green)	Chromosome III	P.G.L. 8	
	(d) Normal*—yellow (*Sy, sy*) cotyledons	*sy sy* has yellow leaf bases Chromosome III	P.G.L. 9	
	(e) Hairy*—hairless (*Hl, hl*) stem and hypocotyl	Chromosome XI	P.G.L. 10 L.K.Crowe	
	(f) Normal—lanceolate cotyledons (het: intermediate)	Lanceolate lethal after germination	L.K.Crowe	
Radish (*Raphanus sativus*)	(a) Red—white hypocotyl (F_1: purple)	Gives 9 : 3 : 4 ratio in F_2 not 1 : 2 : 1 as often stated	'Scarlet globe' and 'Icicle'	(1) (6)
	(b) Round—long hypocotyl (F_1: intermediate)		'Scarlet globe' and 'Icicle'	(1)
Wallflower (*Cheiranthus cheiri*)	Red*—white hypocotyl	Correlated with red and yellow flowers	'Vulcan', 'Cloth of Gold'	(1) (7)
Tobacco (*Nicotiana tabacum*)	Green*—white seedlings		Harris Biological Supplies Ltd T.Gerrard & Co Ltd	

Table 2.2(a)—*continued*

Species	Character pair (* indicates dominant)	Other comments	Source or trade name	References
Barley (*Hordeum vulgare*)	Green*—white seedlings.		Harris Biological Supplies Ltd Department of Education and Science Laboratories	
Blackberry (*Rubus idaeus*)	Glandular*—eglandular cotyledons	Tetraploid *GG gg* giving approximately 21 : 1, correlated with thorny/thornless stems	John Innes	(1) (8)
Stock (*Matthiola incana*)	Dark*—light green cotyledons	Linkage with (i) pollen lethal gives 1 : 1 (ii) alleles for single and double flowers, maximum expression 9°C.	Hansen's varieties	(6) (12)
Snapdragon (*Antirrhinum majus*)	(a) Straight*—twisted 50 mm stems		L.K.Crowe	
	(b) Green*—yellow first leaf		L.K.Crowe	
	(c) Round*—narrow first leaf		L.K.Crowe	
Nasturtium (*Tropaeolum majus*)	Green*—variegated first leaf		L.K.Crowe	(6)
Wintercress (*Barbarea vulgaris*)	Green*—variegated first leaf		L.K.Crowe	(9)
Pelargonium zonale	Green—white seedlings. Het: gold	White sub-lethal	'Golden Crampel'	(7)
Marrow Stem Kale (*Brassica oleracea* var. *acephalia*)	Red—green hypocotyl. Het: purple	Linked with incompatibility gene. Maximum expression 19°C	L.K.Crowe	(10)

Table 2.2(a)—*continued*

Species	Character pair (* indicates dominant)	Other comments	Source or trade name	Reference
Maize (*Zea mays*)	Lethal seedling traits: albinos, lemon whites, leuteous, Necrotic non-lethals: dwarfs, virescents, yellow greens, pale greens	(Temperature sensitive)	R.J.Lambert	(11)

(1) Crowe L.K. (1963) Seedling characters. In Darlington C.D. & Bradshaw A.D. (Eds) *Teaching Genetics in School and University*, pp. 90–93. Oliver & Boyd.

(2) *Practical Plant Genetics*. Software accompanying tomato seed. 18 Harsfold Road, Rustington, Sussex.

(3) Rick C.M. & Butler L. (1956) Cytogenetics of the tomato. *Adv. Genet.*, **8**, 267–382. (List of genes affecting seedling characters p. 278.)

(4) Clayberg C.D., Butler L., Rick C.M. & Young P.A. (1960) Second list of known genes in tomato. *J. Hered.*, **51**, 167–174.

(5) *Tomato Genetics Cooperative Reports*. Chairman of coordinating committee: Rick C.M., Department of Vegetable Crops, University of California, Davis, California.

(6) Crane M.B. & Lawrence W.J.C. (1954). *The Genetics of Garden Plants*, 4th Ed. Macmillan, London.

(7) Bateman A.J. (1956) Cryptic self-incompatability in the wallflower: *Cheiranthus cheiri* L. *Heredity*, **10**, 257–261.

(8) Crane M.B. & Darlington C.D. (1932) Chromatid segregation in tetraploid *Rubus*. *Nature, Lond.*, **129**, 869.

(9) Thompson K.F. (1962) Breeding marrow-stem kale (*Brassica oleracea* var. *acephalia*). *Heredity*, **17**, 598.

(10) Tilney-Bassett R.A.E. (1963) Genetics and plastid physiology in *Pelargonium*. *Heredity*, **18**, 485–504.

(11) *Maize Genetics Cooperative Newsletter*.

(12) Fisher R.A. (1933) Selection in the production of the eversporting stocks. *Ann. Bot.*, **47**, 727–733.

Table 2.2(b) Ready made seedling crosses

Tomato		
P.G.L.† 2—(a) (iii)	P_1, P_2, F_1, F_2, B_1	F_2 ratio 3 : 1
P.G.L. 10—(e)	P_1, P_2, F_1, F_2, B_1	F_2 ratio 3 : 1
P.G.L. 4—(c) (i)	P_1, P_2, F_1, F_2, B_1	F_2 ratio 1 : 2 : 1
P.G.L. 5—(a) (ii) and (b) (ii)	P_1, P_2, F_1, F_2, B_1	F_2 ratio 9 : 3 : 3 : 1
P.G.L. 6—(a) (iii) and (a) (iv)	$P_1, P_2, F_1, F_2, B_1, B_2$	F_2 ratio 9 : 7
P.G.L. 8—(c) (ii)	P_1, P_2, F_1, F_2, B_1	F_2 ratio 3 : 6 : 3 : 1 : 2 : 1
P.G.L. 9—(a) (iii), (b) (iii) and (d)	P_1, P_2, F_1, F_2, B_1	F_2 ratio loose linkage

Table 2.2(b)—*continued*

Tobacco	
Harris Biological Supplies Ltd Faust Biological Supply Co Carolina Biological Supply Co	F_2 green and albino, segregates 3 : 1 at seedling stage
Maize	
Connecticut Valley Biological Supply Co Inc Genetic Products and Services, Indiana	F_2 ratio 3 : 1 green : albino
Faust Biological Supply Co Carolina Biological Supply Co	F_2 ratio 3 : 1 tall : dwarf (primary leaf rounded, wider and shorter)
Genetic Products and Services, Indiana	F_2 9 : 7 green : albino seedlings
Soybeans	
Faust Biological Supply Co Carolina Biological Supply Co	F_2 ratio for green/yellow cotyledons 3 : 1, leaf colour 1 : 2 : 1 green light-green yellow.
Connecticut Valley Biological Supply Co Inc Genetic Products and Services	

† P.G.L. stands for Plant Genetics Laboratory (see *Practical Plant Genetics*, p. 54).

3. SEGREGATION DEMONSTRATED IN PRESERVED MATERIAL

The use of preserved material to demonstrate segregation over several generations of crossing is familiar to most teachers of genetics. Material may be preserved from crosses done in previous years or obtained from a supply agency. The importance of trying to retain as much material as possible, either in preserved form or as photographs, cannot be over-emphasized. Elementary genetics in higher organisms other than *Drosophila* is either time consuming or a long term study, and preserving material for future years helps to demonstrate how widespread the laws of genetics are, and provides further examples for students to analyse.

This approach has a grave danger of being extremely dull, particularly if it is the only one that is available, but several precautions and some small effort can make it only a little less rewarding than getting the students themselves to perform the crosses.

Firstly, the material must be kept in a scrupulously clean and new-looking condition. Fragmented herbarium specimens and dusty maize cobs are not the most inspiring objects. If necessary the material should be remounted and cleaned each year.

Secondly, students should be encouraged to ask questions about the material, to dissect it and to examine it microscopically. Is the difference between round and wrinkled peas just in the seed coat? Do they taste different? What does the agouti factor of mice look like when a few hairs are put under the miscroscope? The ruptured anther of a *Wx/wx* maize plant when stained in iodine in a watchglass, is an impressive sight when seen under a binocular microscope: most students will never have seen the 'release' of pollen 'laden with genes' demonstrated in such a dramatic fashion.

Thirdly, demonstrations are more attractive if the parental strains are available to show what they are like when alive. For most organisms this should provide no great difficulty and certainly adds interest to the 'musty demonstration sheet'.

(a) Seed characters

Characters which show themselves in the seed are easily preserved simply by keeping the seed in reasonably dry conditions, and using them when required. Suitable characters have already been described in the Pea (p. 43) and in Maize (p. 48).

(b) Pressed plants

Segregating progenies of plant material can be preserved on herbarium sheets as dried and pressed specimens. Distinct morphological differences are preferable but pigment differences are usually suitable. It is an advantage if the plants are small since a large sample can then be shown on only a few sheets: either seedlings (p. 56) or small adults such as *Arabidopsis* (p. 30) are suitable.

Plants are best pressed between sheets of blotting paper, either using weights (books or bricks) on top, or strapped between flat wire cake stands or wooden boards, with rope or two belts. The blotting paper is best changed after about two days, and if a less absorbent material like newspaper is used then changes should be made every two or three days for the first week. Two weeks is normally sufficient time for the specimens to dry.

(c) Mounted insects

Insects, provided they are not too small, such as *Drosophila* can be mounted and generally retain their colours for many years.

They may be pinned (when still fairly fresh) directly on to cork mounting, or pinned or stuck on a stage (thin card or Bristol board) which is then pinned to the cork. Alternatively, the insects may be stuck directly on to white card. The most suitable adhesive is made by dissolving celluloid pieces in amyl acetate to produce a viscous glue (just does not drip off a rod). This is sufficient to hold the insect in place, but dries slowly so permitting manipulation of the insect. Model aeroplane cement dries quickly, but otherwise is suitable, as is colourless nail varnish. Care should be taken not to use too much adhesive. If the insect becomes deformed (e.g. wings emerge from elytra) on etherization, then it can be frozen prior to etherization.

Suitable material

(i) Tribolium mutants (p. 34)

(ii) Ladybird (Ladybeetles) colour morphs

Ladybeetles show polymorphisms usually determined by multiple allelic series. *Adalia bipunctata* (two spot ladybird) has a wide range of types (see Creed 1966). The dominance order of this multiple allelic series is shown in fig. 2.6. The dark forms—mainly black with one, two, or three red spots per elytra are dominant to the red forms with one or two spots per elytra. One 'red' form is predominantly black with five red spots, but this form is extremely rare in Great Britain.

Adalia decempunctata (ten spot ladybird) has three main forms, determined by an allelic series with the dominance order as follows: *typica* dominant to 10-*pustulata*, dominant to *bimaculata*. *Typica* is the darkest form with only a small red area, 10-*pustulata* is the normal ten spot form, while *bimaculata* resembles the two spot ladybird. Ladybeetles are commonly found on lime trees (*Tilia*) and nettles (*Urtica*), the darker forms being commoner near town and industrial centres.

(iii) Lepidopteran polymorphisms

The butterflies and moths show a fairly wide range of polymorphisms, the best known being the melanic/non-melanic types of moths (e.g. *Biston betularia*). Melanism is usually dominant to non-melanism. Contrasting morphs can be collected and arranged to illustrate the inheritance of the character both as a demonstration of elementary genetics and as an introduction to population genetics.

Further information on genetics and evolution in Lepidoptera can be found in Ford (1953, 1967, 1971) and Robinson (1971).

Clearly the principles outlined here for a few insect groups are applicable to other insects and arthropods. If anyone has a particular interest in a polymorphic group then this can be turned to good account for demonstrating the genetics of that character.

(d) Preserved skins

Preserved skins are a useful way of demonstrating segregation for factors determining coat colour in mice and other organisms such as rats or guinea-pigs. These skins can either be given to the student as such, or pinned out on to a board. The preservation of interesting features of crosses (e.g. variable expression of genes for coat colour patterns) is therefore possible, as well as the more standard type of demonstration.

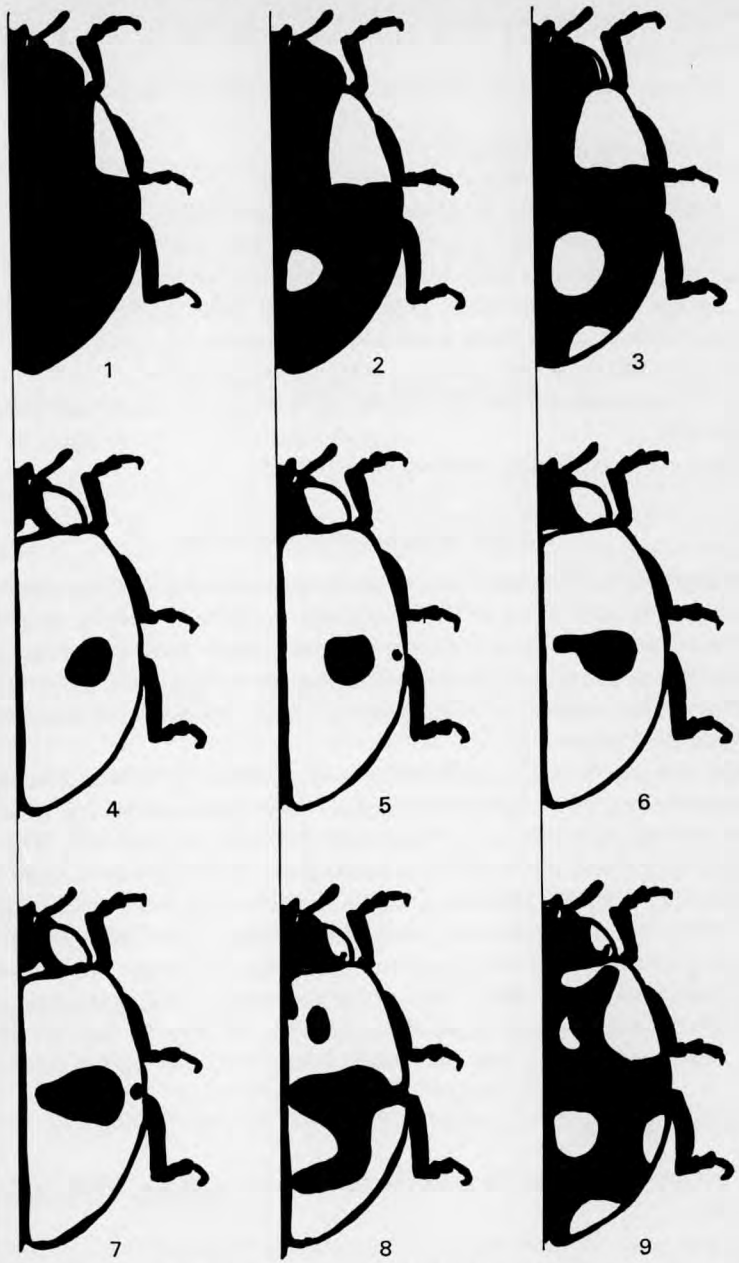


Fig. 2.6. Some varieties of the two spot ladybird, *Adalia bipunctata:* 1. *sublunata*, 2. *quadrimaculata*, 3. *sexpustulata*, 4. *typical*, 5. *stephensi*, 6. *rubiginosa*, 7, *unifasciata* 8. *annulata*, 9. 12-*pustulata*. (From Creed 1966.)

Several techniques are available, and the following account is taken from Mahoney (1966).

(i) Remove skin carefully with minimum amount of attached subcutaneous tissue.
(ii) Fix in 5 per cent formalin for one week.
(iii) Wash in running water for several hours.
(iv) Stretch out and pin to board with hair face down.
(v) While still wet, rub Lankroline FP4 (Lankro Chemicals, Eccles, Manchester) vigorously into skin. Lankroline does not affect human skin.
(vi) Scrape away (with blunt instrument, e.g. table knife) any fat or subcutaneous tissue, apply more Lankroline and repeat till clean.
(vii) Rinse skin in warm detergent (e.g. Teepol) solution.
(viii) Pin out on board, fur uppermost, allow to dry and brush hair thoroughly when dry.

Skins prepared by this method are very soft.

(e) Deep freezing and freeze drying

Small mannals such as mice can be easily preserved by putting them into a deep freeze (—20° to —30°C) in suitable containers (refrigerator boxes) between layers of cellulose wadding or tissue paper. After preservation they are dry thawed using silica gel crystals. Silica gel crystals are dried either in an oven for 3 hours at 60°C or in a warm cupboard overnight. They are ready to use when deep blue.

Mice are dry thawed as follows. The day before the demonstration, half the mice in a box are transferred to another box; a tray (made, e.g. of blotting paper) of silica gel crystals is placed on the mice in each box. The lid is replaced tightly and the boxes kept overnight in a refrigerator or cold room. The next day the silica gel (now pink) is replaced with fresh crystals, and an hour before the class the box is put at room temperature. As the mice reach room temperature, they are spread out on to blotting paper and if necessary dried further with a dry cloth. The mice should appear similar to freshly killed material and can be gently handled by the class. As soon as they are finished with, they should be put into the deep freeze in the same way as before.

Sources of materials

Silica gel crystals can be obtained from most biological or chemical supply agencies.

Reference

Nuffield Biology Teachers' Guide, V. *The Perpetuation of Life*, pp. 8–10.

Freeze drying is becoming increasingly used for drying birds, mammals and other soft-bodied animals which shrink if dried normally. The technique is a very useful one but quite elaborate apparatus is required and is best used if the apparatus is already available. (see Mahoney 1966).

(f) Photography

An alternative to making permanent specimens is to take photographs (preferably coloured) of groups of progeny as they become available: these can then be displayed in place of demonstration material. The main advantage of photographs is that they are the quickest and probably in the long run the cheapest form of preservation.

(g) Other methods and materials

(i) Starchy and waxy pollen grains of maize

A useful demonstration in maize is that of genes segregating to produce two types of pollen grains or 'gametes'. The waxy mutant of maize (*wx*) alters the chemical nature of the starch produced by the plant so that instead of staining the normal blue black it stains a reddish colour with iodine. (Crystals dissolved in 70 per cent alcohol to give light brown solution.) This gene not only expresses itself in the adult plant but also in the pollen grain, where the carbohydrate metabolism is determined by the genotype of the pollen grain (not by the parental anther). Plants heterozygous for this gene (*Wx*/*wx*) therefore segregate into two types of pollen grain, half the pollen grains staining red and the other half staining blue with iodine. There are a few inviable pollen grains without cytoplasmic contents and these appear colourless.

A very illuminating practical exercise involves giving the students the genotype of the parent plant, explaining the nature of the waxy gene and then asking them to explain what they see when a squash of the anther is made in dilute iodine solution. One anther per student is adequate.

Sources of materials

Seeds or anthers of *Wx*/*wx* plants from:
Dr H.K.L.Whitehouse, Botany School, Cambridge University.
Carolina Biological Supply Company, Burlington, North Carolina 27215.

(ii) Flower colour pigments

If flowers of several common garden species are dried rapidly (e.g. in a drying oven at 30°C), their pigment types are preserved so that they can be assessed either visually or by chromatography. One of the best examples is *Antirrhinum* the genetics of which has been studied for over 50 years.

Antirrhinum

The flower colours of *Antirrhinum* are analysed as follows:

(i) Grind upper lips of two corollas in a test tube with 1 ml of 1 per cent HCl in ethyl alcohol. One of the pigments, aureusidin, is invariably present as a spot on the lower lip, and only the upper lip shows genetic variation for its presence or absence.

(ii) Put four drops of the extract as a spot on some chromatography paper (e.g. Whatman No. 1), allowing one drop to dry before applying the next.

(iii) Run chromatogram with one of the following solvents

(1) butyl alcohol : acetic acid : water, in ratio 6 : 1 : 2

(2) *m*-cresol : acetic acid : water, in ratio 50 : 2 : 48.

Any method can be used (either ascending or descending) as long as the chromatogram is run in an airtight container. A good method is to spot a large sheet of paper with many different extracts, clip the edges to form a cylinder and stand in the solvent at the bottom of a suitable airtight container such as a large jar or saucepan with tight fitting lid.

(iv) From such chromatograms the pigments can be recognized by their colour. If the chromatogram is exposed to ammonia vapour the spots change to various colours and this helps their identification. In addition, the R_F value (distance from starting point to centre of spot divided by the distance from starting point to solvent front) can be used to identify a spot. The larger the R_F value the further the spot travels. Table 2.3 shows colours and R_F values.

Table 2.3

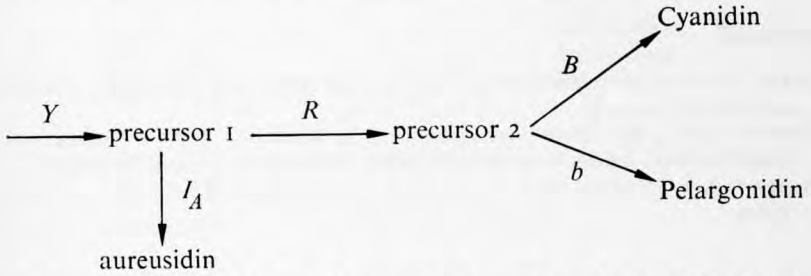
Pigment	Colour		R_F value	
	No treatment	NH_3 vapour	Butanol solvent	*m*-Cresol solvent
Anthocyanins				
Cyanidin	Magenta	Blue	0·32	0·28
Pelargonidin	Pink	Mauve	0·46	0·56
Flavonols				
Aureusidin (two spots)	Green	Orange	0·07 and 0·23	0·01 and 0·11
Luteolin	Brown	Yellow	0·48	0·19
Apigenin	Dark yellow	Yellow	0·64	0·36
		Green	0·29 and 0·41	0·21 and 0·43

The pigments with an asterisk in table 2·3 also appear on the chromatograms but are of secondary interest. Apigenin with R_F value in butanol of 0·64 is always found. Other apigenins are found always with pelargonidin. Occasionally there are traces of pigments not mentioned here.

The following genes are responsible for pigment production.

Y/-: pigment produced, depending on other genes.
yy: no pigment, irrespective of other genes.
R/-: cyanidin and pelargonidin formed.
rr: anthocyanins absent.
B/-: (with *R* present) cyanidin only.
bb: (with *R* present) pelargonidin only.
I_A*-:* aureusidin produced.
II: no aureusidin.

The following scheme for pigment synthesis in *Antirrhinum* can be suggested, although students can be invited to propose their own schemes.



The varieties in table 2.4 (obtainable from Carter's Seeds Ltd, Raynes Park, London) are suitable although clearly other varieties could be investigated.

Table 2.4

Variety	Genes	Pigments
White	*r B I*	None
Yellow	*r B* I_A	Aureusidin
Nelrose (glowing pink) and Royal Cerise (rich carmine)	*R b I*	Pelargonidin*
Scarlet Flame (fiery scarlet) and Guinea Gold (orange terracotta)	*R b* I_A	Aureusidin and pelargonidin*
Mauve Beauty	*R B I*	Cyanidin
Crimson	*R B* I_A	Aureusidin and cyanidin

* Where two colour varieties are grouped under one genotype there are differences in intensity of expression of the colours determined by modifier genes. Royal cerise has more pelargonidin than Nelrose, and Scarlet Flame has more pelargonidin but less aureusidin than Guinea Gold.

Another single gene colour mutant type is 'delila'—'delila' varieties have a colourless or yellow corolla tube due to the absence of anthocyanidins in this region.

Crosses between varieties of *Antirrhinum* are easily made, since the anthers are large and easily removed from flowers just before they open. Crossing is prevented by bagging with fine muslin to exclude insects. Pollen can be transferred with a brush and one pollination produces numerous seeds.

Suggested crosses:

1 Single factor—$R\,b\,I \times R\,B\,I$, $R\,b\,I \times R\,b\,I_A$, $r\,B\,I \times R\,B\,I$, $r\,B\,I_A \times R\,B\,I_A$ and pure white × coloured, delila (colourless corolla tube) × coloured corolla tube

2 Two factors—$R\,b\,I \times R\,B\,I_A$, $R\,b\,I_A \times R\,B\,I$

3 Two factors with epistasis—$r\,B\,I \times R\,b\,I$, $r\,B\,I_A \times R\,b\,I_A$

4 Modifier complex—Nelrose × Royal Cerise, or Scarlet Flame × Guinea Gold.

References

DAYTON T.O. (1956) The inheritance of flower colour pigments I. The genus *Antirrhinum*. *J. Genet.*, **54**, 249–260.

BRADSHAW A.D. (1963) Three teaching projects. In Darlington C.D. & Bradshaw A.D. (Eds) *Teaching Genetics in School and University*, pp 105–109. Oliver & Boyd.

WAGNER R.P. & MITCHELL H.K. (1964) *Genetics and Metabolism*, 2nd Ed, pp. 565–583. Wiley.

Sources of materials

Antirrhinum colour varieties are obtainable from practically all nurserymen. Varieties quoted available also from Carter's Tested Seeds Ltd, Raynes Park, London.

2. *Impatiens balsamina*

Three genes are responsible for colour variation of the flowers of Balsam, but they affect the sepals and the petals in different ways. Another gene *W* causes cream sepals in its recessive form (*w*), but this will not be dealt with in any detail.

The situation regarding the petal and sepal pigments is summarized in table 2.5. With regard to visible pigments, the following situation exists: in the petals when *L* is present, malvidin is formed. The gene *H* is responsible for the production of pelargonidin, but traces are also produced in $l\,h\,P^g$, and $l\,h\,P^r$ flowers. The gene *P* determines the amount of pigment, this increasing with $p \rightarrow P^g \rightarrow P^r$. P^r is dominant to P^g. In the sepals the situation is more complex. The gene *L* again leads to the production of malvidin, but *L* is

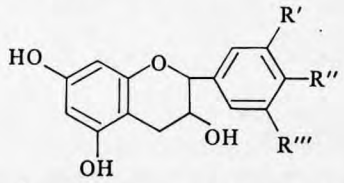
epistatic to *H* such that *H* produces pelargonidin only in the genotype *ll*. In the absence of *L*, P^g plants produce peonidin, while P^r plants produce peonidin and cyanidin.

Table 2.5 Anthocyanin pigments in the sepals and petals of *Impatiens*

	Sepals					Petals		
Genes	Phenotype	PEL	CYA	PEO	MAL	Phenotype	PEL	MAL
$l\,h\,p$	White	–	–	–	–	White	–	–
$l\,h\,P^g$	Pale pink	–	–	+	–	Pale pink	+	–
$l\,h\,P^r$	Pink	–	+	+	–	Pink	+	–
$l\,H\,p$	White	–	–	–	–	Pink	+	–
$l\,H\,P^g$	Pink	+	–	+	–	Rose	+	–
$l\,H\,P^r$	Red	+	+	+	–	Red	+	–
$L\,h\,p$	White	–	–	–	–	Pale lavender	–	+
$L\,h\,P^g$	Lavender	–	–	–	+	Lavender	–	+
$L\,h\,P^r$	Purple	–	–	–	+	Purple	–	+
$L\,H\,p$	White	–	–	–	–	Rose lavender	+	+
$L\,H\,P^g$	Pink lavender	–	–	–	+	Pink lavender	+	+
$L\,H\,P^r$	Magenta	–	–	–	+	Magenta	+	+

The above results are better understood if one looks at the chemistry of the anthocyanin compounds concerned.

Anthocyanins have the following basic structure:



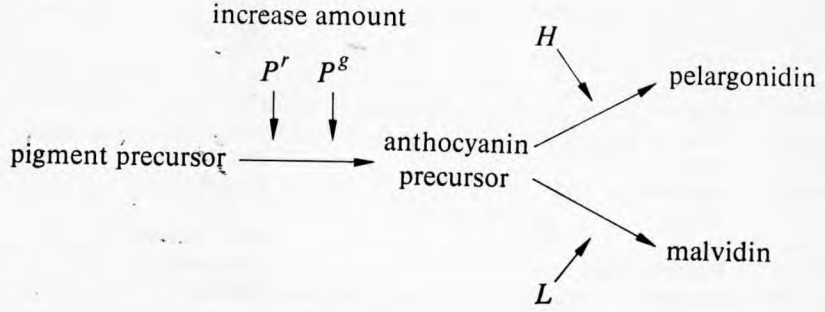
The different types of anthocyanidins are distinguished according to the nature of the groups at R' R'' R''' (table 2.6).

Table 2.6

Pigment	R'	R''	R'''	Phenotype
Pelargonidin	H	OH	H	Red
Cyanidin	H	OH	OH	Blue
Delphinidin	OH	OH	OH	Purple
Peonidin	OCH_3	OH	H	Rosy
Petunidin	OCH_3	OH	OH	Purple—not present in *Impatiens*
Malvidin	OCH_3	OH	OCH_3	Mauve

Because young buds and stems contain precursors of the anthocyanins cyanidin and delphinidin, these are probably the substances from which the other pigments are derived. We can therefore postulate the following schemes for pigment synthesis but students can be invited to formulate their own models. In actual fact the situation is probably more complex than the outline given here.

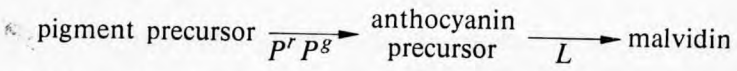
Petals



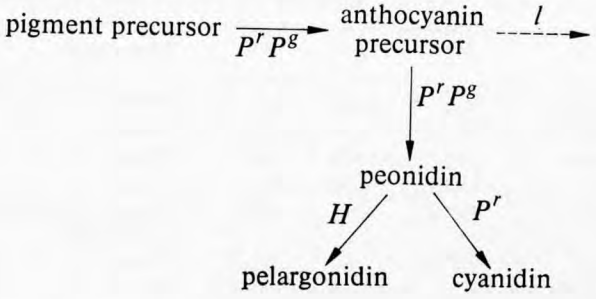
gene *L*—addition of groups at *R′ R‴*
gene *H*—addition of group at *R″*
If no *L* present, pathway diverted to producing some pelargonidin even though *H* not present.

Sepals

(a) Pathway if *L* present



(b) Pathway if *L* absent



Anthocyanin extraction

1. Boil 4–5 freshly opened flowers (petals or sepals) with 1 per cent HCl in 95 per cent ethanol until no more visible extraction. Alternatively material preserved by freezing or rapid drying at 30°C can be used.
2. Concentrate extracts over steam bath.
3. Apply to chromatograms as short streaks on Whatman No. 1 filter paper using one or more of the following solvent mixtures:
t-butanol : acetic acid : water (3 : 1 : 1)
m-cresol : acetic acid : water (55 : 7 : 6)
acetic acid : hydrochloric acid : water (30 : 3 : 10)

Two per cent ethanolic $AlCl_3$ sprayed on to chromatograms accentuates the colour differences—cyanidin, delphinidin and petunidin turn deeper blue. The R_F values of these pigments are given in table 2.7.

Table 2.7

	Solvent		
Pigment	*t*-butanol	*m*-cresol	HCl
Pelargonidin	0·64	0·63	0·72
Peonidin	0·74	0·75	0·73
Cyanidin	0·41	0·33	0·53
Malvidin	0·36	0·85	0·67
Petunidin	0·30	0·48	0·50
Delphinidin	0·18	0·12	0·32

Since R_F values do vary to some extent with temperature and conditions, pigments from species with standard anthocyanins can be used as controls:
Pelargonidin from *Pelargonium zonale* (but other pigments also present)
Pelargonidin from *Salvia splendens*
Cyanidin from *Centaurea cyanus* (blue)
Delphinidin from *Viola papilionacea* or *Delphinium ajacis* (blue)
Peonidin from *Paeonia lactiflora* (deep red)
Malvidin from *Parthenocissus quinquefolia* (fruits)
Petunidin with Malvidin from *Petunia hybrida* var *Alderman*.

References

ALSTON R.E. & HAGEN C.W. (1958) Chemical aspects of the inheritance of flower colour in *Impatiens balsamina* L. *Genetics*, **43**, 35–47.
CLEVENGER S. (1958) The flavonols of *Impatiens balsamina* L. *Arch. Biochem. Biophys.* **76**, 131–138.
CLEVENGER S. (1964) Flower pigments. *Scient. Am.*, June 1964, 84–92.

(c) Other flower pigments

The techniques described here for extracting flower pigments can be used to analyse anthocyanin pigments in a wide variety of species, particularly if known pigments are available as controls.

Sources of materials

Impatiens can be obtained from nearly all nurserymen, and colour charts, chemicals and chromatography paper from nearly all chemical or biological supply agencies.

4. HUMAN GENETICS

A practical approach to human genetics usually consists of a demonstration of single gene differences in human populations followed by case histories or pedigrees relating to the genes demonstrated in the class and to other features such as congenital abnormalities. Students may be asked to score their own families for certain characteristics. Most university students can only do this during the vacations. This type of practical may expose cases of illegitimacy or adoption. About 5–6 per cent of all children are illegitimate, about 1 per cent are legitimate but have been adopted, while an unknown number (estimated as high as 3 per cent) may be the children of men who are not their legal fathers. There is, therefore, a chance that three out of every 30 children will not be living with their real parents. This may not be known to some of the students concerned, and if the results of their genetic studies are inconsistent it could be the cause of considerable distress and suspicion. It is better, therefore, to avoid this approach to human genetics, although abnormal segregation is often due to abnormal expression or erroneous assessment.

(a) Ability to taste PTC

Individuals differ in their ability to taste the substance PTC (phenylthiocarbamide or phenylthiourea), this character being inherited as a simple dominant trait but with minor complications.

The PTC can be tasted as a solution, but the most convenient method is to impregnate filter paper with it. The following methods are suitable.

(i) Dissolve 0·08 g of PTC in boiling water and make up to 1 litre. Use as such.

(ii) Dissolve 1 g of PTC in 100 ml of acetone (1 per cent solution) and soak filter paper in this solution. Remove and allow to dry. Cut into strips of 15 mm × 30 mm. Store in jar or envelope.

(iii) An appropriate method for advanced classes is to detect the lowest concentration that can be tasted by each individual and to plot these values on a histogram. 1·3 g of phenylthiourea in a litre of tap water is concentration No. 1. Take half of this and make it up to 1 litre by adding tap water. Repeat this procedure with concentration No. 2 and continue until you have 12 serial dilutions. Individuals are tested by dipping a clean glass rod into solution No. 12 and then tasting the liquid on the rod. If there is no distinctive taste, solution No. 11 is tried and so on until the phenylthiourea is detected. The value of this final solution is entered on the histogram. The result, if enough people are tested, is a bimodal distribution with an antimode at tube 4 or 5. This strength is then the distinction between taster and non-taster. The method demonstrates to the student that there is variation in threshold within each taster class and also that the two main classes show some overlap in threshold.

Sources of materials

PTC is available from most chemical and biological supply agencies.

(b) Blood Groups

It is common knowledge that the blood of human beings can be classified into various groups, the most well known being the groups forming the ABO system. These blood groups reflect the nature of the 'proteins' on the red-blood cells, thus

A group contains 'protein' A
B group contains 'protein' B
AB group contains 'protein' A and B
O group contains 'protein' neither A nor B.

The liquid part of the blood, or plasma, contains antibodies which are the agents mainly responsible for combating foreign substances and organisms in the blood, whether they be infective bacteria, viruses or allergic substances from say pollen grains. Two such substances are 'anti-A' and 'anti-B' which cause clumping of the red-blood cells containing 'protein' A and B respectively. 'Anti-A' will, therefore, cause clumping (agglutination) of A and AB blood, but not B and O. A consequence of this is that blood of groups A and AB do not contain 'anti-A' otherwise the blood would clump and not flow freely. Nor does AB contain 'anti-B' since this too would cause clumping of AB. The ABO blood groups are determined by three alleles or forms of one gene, which have been termed I^A, I^B, and i. The situation is summarized in table 2.8.

The clumping of red-blood cells can be seen with the naked eye or under a binocular microscope, and the practical determination of blood groups,

Table 2.8

Blood group	Substance on wall of red cell	Antibody in plasma	Genotypes	Blood can be transfused to
A	A	Anti-B	I^AI^A, $I^A\,i$	A, AB
B	B	Anti-A	I^BI^B, $I^B\,i$	B, AB
AB	A and B	Neither anti-A nor anti-B	I^AI^B	AB
O	Neither A nor B	Anti-A and anti-B	*ii*	All groups

apart from certain precautions that have to be taken, is a straightforward process. Blood is obtained by piercing the thumb* with a sterile lancet in the following way.

(i) Sterilize a mounted needle by heating in a bunsen and cooling in sterile water. A far better alternative is to use *sterilized disposable lancets* for the purpose. Under no circumstances should a needle or lancet be passed from person to person since certain blood-borne diseases are very resistant to sterilization.

(ii) Shake the hand (which should have been washed in soapy water) vigorously downwards and apply tourniquet with a clean handkerchief round base of thumb so that blood is retained in the thumb (the end of the thumb becomes red). If the person is right handed, the left thumb should be used.

(iii) Wipe the thumb with cotton wool soaked in surgical spirit and allow to dry.

(iv) Make quick small jab through the skin; do not put needle on skin and press down.

(v) Squeeze the thumb and put one or two drops in two positions on a white tile, on separate microscope slides, or on blood testing cards (see below).

(vi) Wipe punctured area with surgical spirit and apply plaster. There should be no further bleeding but a plaster is a useful precaution against infection through contact with material used in the rest of the practical. Students should be told to remove the plaster afterwards as this hastens healing.

(vii) To one drop of blood add one drop of anti-A serum, and to the other anti-B, carefully noting which is which.

(viii) Mix with corner of slide, a glass rod, or sterile lancet (cleaning between mixing or using separate rods and lancets) and leave for about 10 min.

(ix) Observe clumping, using binocular if necessary. Classify blood groups accordingly (see table 2.9).

* Some people prefer to prick an ear lobe and then use sterile cotton wool not a plaster under (vi).—Ed.

Table 2.9

	Clumping reaction with	
Blood group	Anti-A	Anti-B
A	+	−
B	−	+
AB	+	+
O	−	−

An alternative to using anti-A and anti-B serum is to use blood group testing Eldon cards. These cards have four spots on them impregnated with different anti-sera, so that the ABO and Rhesus (either Rhesus positive or Rhesus negative) blood groups can be identified if the blood is dropped on to the four spots.

During such a practical it is interesting to demonstrate the importance of blood groups in blood transfusion and disease, as well as (if Eldon cards are used) the importance of the Rhesus factor during child birth and modern methods of preventing Rh haemolytic disease of the newborn (see Clarke *et al.* 1966). The ABO system therefore, demonstrates a clear-cut multiple allelic difference, and shows one of the most interesting human polymorphisms with considerable practical relevance.

Sources of materials

Blood group anti-sera can usually be obtained without difficulty by contacting the local hospitals. Blood group testing (Eldon) cards and disposable lancets can be obtained from most biological supply agencies.

(c) Secretor testing

The ABO antigens as well as being found on the red-blood cells are in some people also present in the body fluids and secretions: they are most easily detected in the saliva. The presence or absence of such antigens in the secretions is genetically determined: about 78 per cent of the population of the United Kingdom are secretors (with genotype *SS* or *Ss*) while 22 per cent are non-secretors (with genotype *ss*). The *S* gene is inherited independently of the genes controlling the ABO system.

Only the same antigens are present in the blood as are found in the body fluids except that all the secretor types, including group O, produce an antigen called H which reacts with anti-H serum. This antigen is also found on all red-blood cells.

The presence of A, B or H substances in the saliva is determined by an Inhibition Test. The principle behind this is that the saliva is mixed with the

appropriate anti-serum. If the antigen is present then it will inactivate the anti-serum which therefore won't cause clumping of the appropriate blood group type. A difficulty is that it is difficult to define the concentration of saliva that will precisely inactivate an often unknown concentration of anti-serum. The method adopted is therefore one of serial titration or dilution of the saliva.

A row of small (precipitin or analysis) tubes is required. To each tube except the first an equal volume of saline solution is added so that each tube is less than half full. This is best done using a pipette and teat with the appropriate volume marked off with a grease pencil. The empty first tube is filled with an equal volume of undiluted saliva. This is best collected in a test tube which is then placed in a beaker of boiling water for ten min. The saliva is then centrifuged for 4 min and the clear supernatant fluid used for the test. An equal volume of undiluted saliva is added to the second tube and the two are mixed by squeezing the liquid up and down the pipette. An equal volume is then removed from this tube and added to the third, mixed, and so on. When the last tube is reached half of the liquid (equal volume) is discarded. In this way are obtained equal volumes of saliva at successive dilutions of 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, etc.

A drop of the appropriate anti-serum is now added to each. Which anti-serum to use is determined from the person's blood group, or alternatively anti-H can be used for all types. The tubes are left for 15 min and then a drop of blood of the appropriate blood group is added. After an hour the suspensions are examined microscopically. If no agglutination occurs in several of the tubes (where the saliva is more concentrated) then the person has secreted antigen, if agglutination occurs in all the tubes then the person is a non-secretor.

Sources of materials

Blood, A and B anti-sera, and saline can all be obtained from local hospitals or blood-transfusion centres. For a class of 30 about 10 ml of each blood group type, a small vial of each anti-serum type and a litre of physiological saline should be sufficient. H anti-serum is obtainable from Baxter Laboratories Ltd, Caxton Way, Thetford, Norfolk. The anti-serum can also be made up by making an extract of the seeds of *Ulex europeaus* in physiological saline. This can be diluted appropriately and then can be frozen when not in use. Add O cells in preference to A or B when using anti-H.

(d) Other human characteristics

The characteristics described below all have a very strong genetic component but the precise details of their inheritance are complex because either several alleles are involved, or their expression is easily modified by other genes.

Their use in pedigree studies is, therefore, to be regarded with caution, although they still provide an extremely useful demonstration of human genetic variability. More advanced students can try to construct pedigrees with these characters and try to interpret them bearing in mind the uncertainties about their inheritance.

(i) Eye colour—blue eyes recessive to other colours, but expression very variable, inheritance complex.

(ii) Hair colour—blonde hair recessive to dark hair, red hair recessive to darker colours, but expression very variable, inheritance complex.

(iii) Freckles—dominant to non-freckling, expression enhanced by exposure to sunlight.

(iv) Hair whorl direction—hair at back of head whorling in clockwise direction dominant to whorl in anti-clockwise direction.

(v) Hair form—curly hair dominant to wavy hair dominant to straight hair, but expression variable.

(vi) Presence of hair in middle segment of fingers (other than index finger) dominant to absence of hair—but probably several genes involved.

(vii) Colour blindness can take quite a wide range of forms, but the commonest type (red-green colour blindness) is sex linked. Eight per cent of European men show this character but only 0·7 per cent of women. This can be tested by using the Ishihara Test Cards available from most biological supply agencies.

(viii) Premature or pattern baldness which is the best known sex-limited (as opposed to sex-linked) trait in human beings. The character occurs to some extent in more than 40 per cent of the male population over the age of 34, but is extremely rare in females. In males the character is inherited as a dominant, hairiness being recessive. The character is transmitted by females, but not phenotypically expressed in them.

(ix) Ear lobes. The ear lobes may be either free and pendulous, or adherent. The adherent type is recessive. Again the expression is variable.

(x) Double-jointed thumbs. The loose ligaments in the thumb, permitting it to be bent sharply backwards, is a character dominant to the normal condition of tight ligaments.

(xi) Ability to taste thiourea. Ability to taste this substance (tastes bitter) is dominant to inability to taste it, but not linked to ability to taste PTC.

(xii) Taste of sodium benzoate. Paper impregnated with this chemical tastes either sweet, salty, bitter or not at all. Its inheritance is not understood. Note: Some older practical manuals recommend brucine as another substance which shows a tasting/non-tasting difference. This substance is not recommended because it is highly poisonous if swallowed.

(xiii) Dimpled cheeks dominant to plain cheeks.

(xiv) Widow's peak—a point of the hair line which extends down in the centre of the forehead—dominant to straight hairline.

(xv) Tongue rolling—ability to roll the tongue so that the sides form a U is dominant to the inability to roll it.

An interesting addition to a practical on basic human genetics is to have students score themselves for a range of characters and then plot the results on an 'individuality chart' (fig. 2.7). This is done by shading in the section of the chart referring to the student's particular character, starting from the centre and working outwards. Two or more charts can be used to incorporate all the features listed above.

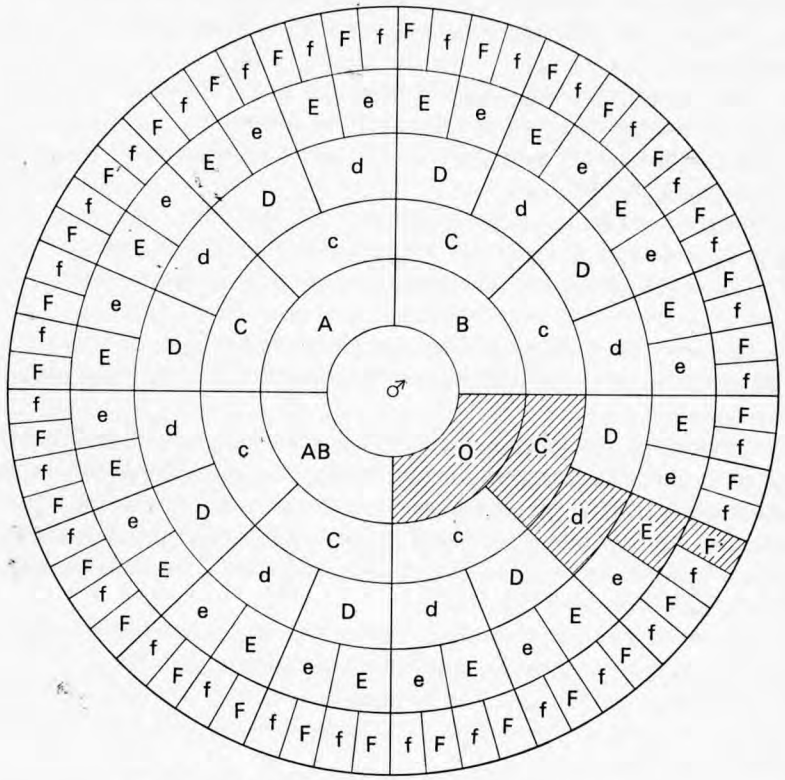


Fig. 2.7. Individuality chart (for explanation see text).

For example if
C/c refers to ability/inability to taste PTC,
D/d refers to ability/inability to roll tongue laterally,
E/e refers to dark/blue eyes,
F/f refers to free/attached ear-lobes,
then a male with blood group O, ability to taste PTC, inability to roll tongue, with dark eyes and free ear lobes would have a chart as in fig. 2.7. When all

individuality charts have been plotted, they can be compared to see if any two human beings are genetically identical (on the basis of the few clear-cut characters studied).

5. STATISTICS

For the teaching of elementary genetics to students with little mathematical knowledge it is fortunate that only one type of statistical test is absolutely necessary: this is the Chi-squared test (see also p. 11). There is very little that is really difficult about this test, provided that three things are accepted without explanation: the term 'chi-squared' the concept of 'degrees of freedom', and the conversion of the chi-square value into a probability value. The theory behind these is far too advanced to even be hinted at for most biologists without mathematical training.

From the teaching point of view, one of the best approaches is not to mention either the words 'chi-squared' or 'statistics' until the basis of the test has been made clear. One way of doing this is to get the student to do an almost trivial project involving different coloured beads. They should be asked to perform the following simple procedures: firstly, mix an equal number of beads of two colours (about 100) in a bag. Then pick at random samples of 20 beads (see p. 296) and note down the number of beads of each colour that occur in each sample. Ten samples should be adequate. The results of one of the students can then be chosen and written up on the blackboard.

A series of questions are then asked of the class. What do you notice about this data? You put in exactly equal quantities of the two colours, but what has happened? Is this what you expect? Would you not have expected equal quantity of each colour in each sample? How do you known that the differences are due to chance? How would you tell that the differences were due to chance *if you did not know* that you had put in equal quantities of the two colours? Let us for example look at the following set of data. (An extreme case is put up on the board where the ratios deviate very much from 1 : 1.) Would you not say that there was something wrong here with our presumption that the beads were thoroughly mixed in equal proportions? At this point the crucial question can be put, namely, where do you draw the line between a normal deviation by chance and a real deviation from the expected value.

The first step towards answering this question is to obtain some measure of how much the values deviate from the expected. This is done by calculating the following quantity for each colour class:

$$\frac{(\text{observed} - \text{expected number})^2}{\text{expected number}}.$$

The reason why this quantity is used can be explained by an appeal to common sense.

A value is obtained for each colour class in one sample and they are then added to give a chi-squared (χ^2) for that sample. This chi-squared is then assigned a number called 'degree of freedom' which equals the number of expected classes minus one (i.e. for two-colour classes as in the bead example the degrees of freedom $=2-1=1$ or say if it had been for the four classes in an F_2 segregation with dominance, the degrees of freedom $=4-1=3$). Once chi-squared and its degrees of freedom have been estimated, we have a reasonable measure of degree of divergence from expectation. How then can this measure be used?

This measure can be used to calculate the probability with which we expect the observed deviation to arise by chance. This probability does not have to be calculated every time we want an estimate but can be read off from tables of chi-squared. The resultant probability gives the probability of obtaining the observed deviation or a greater one from the expected value by chance: in other words if the deviation is large, the chi-squared will be large, but the probability of getting such a deviation by chance will be small.

The smaller the probability the more we are likely to say that the original hypothesis about the expected values is wrong, and that the deviations have not come about by chance but indicate that we have made wrong predictions. In the particular example using beads this might mean either that equal numbers were not put in originally or that they were not mixed properly or that they were not selected at random. However, there is still the problem of where to draw the line: when does one say that the probability is so small as to make the truth of the hypothesis unlikely. The level usually chosen is 5 per cent (and most tables only give chi-squared in the range of probabilities immediately below and immediately above 5 per cent). This value is essentially arbitrary but provides a useful working norm generally accepted among scientists. When the probability value falls on or below 5 per cent then the deviation from expected is deemed 'significant'. This is a technical term rather than an emotive expression, and is used in statistics in this specific context.

So far chi-squared has only been applied to several classes in one sample: it can also be used for looking at the situation over many samples, to see if there is an 'overall deviation' from expected value or heterogeneity between samples. These more complicated extensions of the chi-squared test are not normally required in teaching very elementary genetics but can be found in any standard textbook.

Considerable space has been devoted to the problem of chi-squared. This has been done for two main reasons. Firstly, if elementary genetics is to be taught in a way that does not presuppose the answers (as has been recommended throughout this account) then it is important to bring home the idea of testing the validity of an hypothesis, i.e. testing for significant divergence from expected. Secondly, it is important that statistics is taught in a meaningful way and that its aims are understood and appreciated. There are two ways in

which the meaning can be brought home. The subject must be taught in the context of an experiment (e.g. with beads, or actual data) and it must be fully explained in the sense that, where possible, the intuitive basis for a test must be pointed out and equally any less obvious aspect of the test should be explicitly pointed out so that the student knows what to take for granted, and what to learn to appreciate.

III. COURSE DESIGN IN TEACHING BASIC GENETICS

1. INTRODUCTION

So far very little mention has been made of the content or planning of particular courses. Much of this will be determined by personal preference and availability of materials. Certain general principles should however be remembered. The teacher should be clear for what purpose each practical investigation is designed: is it to help the student understand the laws of genetics, is it to show that many organisms show a similar behaviour, or is it to relate elementary genetics to more advanced aspects of the subject? The investigation should be tailored to the purpose for which it has been chosen.

Investigations designed to explain the principles of gene behaviour should be kept as open-ended as possible. Even a simple investigation with a single pair of alleles should not be described as such, but as a cross between two organisms showing contrasting characters: whether the parents are from pure lines, whether one or many genes are involved and whether the genes are dominant or recessive is something that the student should be asked to discover.

Another essential is that the student should, after he has analysed his results, draw up a theoretical scheme to illustrate the mechanism involved. This scheme should indicate the genotype and phenotype of the parents and progeny, it should indicate the genotypes and ratios of the gametes, it should demonstrate random union of gametes, and finally a summary of ratios of the resultant genotypes and phenotypes. Clearly at a more advanced level many stages of the reasoning can be omitted, as long as the same general scheme of presenting the results is used.

Demonstrations may not appear at first sight to be amenable to such an open-ended project approach, but this is in fact not so. A demonstration should not be looked at with one fixed end point in view which is written down clearly for the student: he should be asked to observe, assess and comment. Fortunately this is readily possible in genetics, since the subject does have a logical theoretical basis.

The series of investigations outlined below are classified according to the

general educational level of the students concerned. The rationale behind these course suggestions is also explained, so as to give some guidance when it comes to including investigations not specifically mentioned in this account.

2. SCHOOL LEVEL

(a) Elementary

(i) Breeding mice differing in coat colour at one locus.
(ii) Demonstration of differences in various characters in human beings.
(iii) Examination of human pedigrees for fully expressive single gene characters.
(iv) Demonstration (e.g. groundsel ray/non-ray florets) of single gene segregation in plants.

This small series of investigations should serve to illustrate the main principles of heredity, and introduce them in the general context of biology. The investigation with mice could be a reasonably long-term one: in between the mice can be kept as pets. The first step is to show that say, black mice always produce black mice and brown mice always produce brown mice. Then a cross can be made between the stocks and carried on to the F_2 generation. This can be done throughout the school year not so much as a formal genetics experiment but almost 'just to see what happens'. The full explanation can then be given at the end of the year and then combined with the study of groundsel and human genetics in one or two simple practicals. Ideally the pupils should sow the groundsel F_2 themselves, but be provided with plants to show the parental and F_1 generations.

The human genetics can be part of a practical where chromosomes in general are examined and where there are photographs of human chromosomes, twins and other aspects of human genetics (e.g. sex determination).

(b) Advanced

(i) Breeding experiments with mice or *Arabidopsis*, differing in two pairs of unlinked alleles.
(ii) Demonstration of 9 : 3 : 3 : 1 segregation in peas, tomato seedlings or some other material.
(iii) Demonstration of complementary genes and an explanation in terms of development or sequence of biochemical reactions (e.g. maize cobs, cyanogenesis in clover).
(iv) An introduction to linkage, preferably an extreme case, to contrast this with independent genes in the F_2. Mention of backcrosses as a better way of assessing linkage.

(v) Allelic differences in crop plants, horticultural varieties, humans to show application of genetics.

This is a very similar scheme to the previous one but the two gene-case and its attendant complications of gene interactions (to be considered in biochemical terms rather than in abstract ideas of epistasis or complementation) and gene linkage have been introduced. Further extension is achieved by looking at the application of genetics to plant breeding: this can be done by showing the value of single gene changes, particularly with regard to dwarf varieties, flower colour, type of vegetable in the Brassicas, and disease resistance.

3. UNIVERSITY LEVEL

(a) Elementary

(i) Crossing mice, *Tribolium*, or *Arabidopsis* differing by two loci. An open-ended investigation where minimum information is given about the number of genes, their dominance or their linkage.
(ii) The behaviour of genes as illustrated by experiments with coloured beads. The segregation of genes during gamete formation, demonstrated by *Wx*/*wx* pollen. Demonstration of genetic ratios in a range of organisms (e.g. maize cobs, seedling characters, mice coat colours).
(iii) Demonstration of relevance and economic applications of genetics. Human genetics, pedigrees, counselling. Single gene differences of economic importance (e.g. flower colour, disease resistance, morphological changes).

Universities are usually faced with a very diverse intake, particularly with regard to the extent of background that the students have had in the biological sciences, and in genetics in particular. The courses therefore have to be designed to cater 'for all tastes'. The best compromise in this situation is to have a course which starts at a very elementary level but goes fairly rapidly into an area which is fairly advanced and beyond the level of teaching at school. This rapid transition can be dangerous, since genetics is a progressive logical subject and if the initial arguments are not fully understood there is subsequent deterioration in understanding of the subject as the course progresses.

For this reason, the first investigation is the most critical part of the course. This first investigation should be in the form of a project involving a cross between strains differing by two loci. Different students should investigate different pairs of genes and even different organisms. This may seem rather an 'advanced' way to introduce the subject of genetics but it has two main advantages (common to all types of open-ended investigation): firstly, the student is allowed to proceed at his own speed without being rushed through specific procedures, and secondly the interpretation of the results demands a

lot more thought and understanding simply because the answers are unknown.

The use of two genes in such a cross should not be too much of a complication if the student has done no genetics before, as long as he is told first of all to consider each character in turn. Each character considered separately should show in the F_2 a 3 : 1 segregation (assuming dominance is complete). This can be tested for using a chi-squared test. If there is deviation from a 3 : 1 ratio, then it suggests that one of the phenotypes is less viable than the other (see pp. 15, 75 for the full χ^2 analysis).

Two approaches to the investigation are possible. It may either start with a cross between a wild type and a mutant strain carrying two mutant genes, or it may start with a cross between two different mutant strains. The former approach is the conventional one, and has the advantage that if both the mutant genes are recessive, a backcross to the double recessive is possible and can be used for estimating linkage. Where two (recessive) mutant strains are crossed a backcross is impossible unless a double recessive pure line is available. Linkage has to be detected by a significant deviation from independent assortment (see p. 15). The problem of estimating linkage from the F_2 can be brought to the attention of the students and they can be shown the advantage of backcrosses.

An investigation which starts from a cross between two mutants has the advantage that one need only maintain a relatively few pure lines to obtain a large number of paired combinations of characters. Thus with six pure strains 15 different crosses can be performed. This type of cross is also unusual in its effects. If both the mutants are recessive then the F_1 will appear wild type, so demonstrating clearly how several genes may be hidden in the recessive condition.

The aims and relevance of the other investigations mentioned above have been outlined in the previous sections. The experiment with coloured beads can be used as a model for the experiment with the actual organisms, and it can be used to illustrate the effects of gene interactions of various sorts or the different coloured beads may be assigned quantitative values and so serve as an introduction to genetics of quantitative characters (see chapter 3). The experiment with *Wx*/*wx* pollen provides a striking illustration of 'different coloured beads' actually in nature.

The recommended demonstrations should serve to amplify the grounding the student gets from his main experiments and are therefore best considered towards the end of the course.

(b) Advanced

Teaching basic genetics at an advanced level in University is almost a contradiction in terms, since in more advanced courses the basic aspects are developed into considerations of other branches of genetics. These various

branches are considered in other parts of this book. Nevertheless teaching basic genetics at an advanced level has several aims.

(i) Revision exercises

Very frequently genetics courses do not run sequentially, and there is much to be said in favour of a few revision exercises when the subject is reintroduced at a more advanced level. Students can fumble through more advanced genetics courses without being aware of the basic principles of the subject, these having been left behind several terms previously. Several important aspects of elementary genetics have not yet been mentioned and these are suitable for more advanced instruction and revision.

(i) Three-point test crosses—this illustrates the process of genetic mapping, genetic interference and effect of double crossovers on recombination values. These are all important fundamental concepts. Suitable material is provided by mice, *Arabidopsis* and maize, although *Drosophila* is still probably the best material to use here (see chapter 1).

(ii) A study of a few clover populations polymorphic for V-shaped white leaf markings and cyanogenesis. This exercise can bring basic genetics into relation with population (chapter 7) and biochemical genetics (chapters 5 and 6).

(iii) Genetics of mice using genes of variable dominance, expressivity or penetrance. This exercise shows the importance of genetic background and modifier genes. Similar phenomena can be shown in human pedigrees. It also raises questions about polygenes/major genes, developmental genetics, evolution of gene expression and dominance, and the difficulties of genetic analysis of such characters.

(iv) A study of the genetics and biochemistry of flower petal pigments. This can also serve to introduce the phenomenon of variable gene expression, and illustrate the value of genetic knowledge to plant breeding.

(v) A study of temperature sensitive mutants in maize or *Tribolium*. Another important exercise in developmental and physiological genetics, shows the importance of genotype × environment interactions (p. 120).

Several points of importance should be noted about such exercises. Firstly, they should not repeat an investigation that has already been undertaken, but should introduce either a new problem or a new organism. Secondly they should be approached as open-ended investigations, preferably with different members of the class investigating different aspects of the same general phenomena. Thirdly the investigation should be angled towards the area in which the more advanced course will specialize. Clover is of value for a general advanced course, or for placing emphasis on evolution. The mouse investigation would be suitable for the beginnings of a course in human or developmental genetics, and the flower pigment one for a course on physiological genetics.

(ii) Basic genetics research projects

The second value of basic genetics is that it can provide a useful source of ideas for minor research investigations. In many final year courses, and to an ever-increasing extent in lower years, the student is required to perform one or two minor research investigations and to write these up as a B.Sc. 'thesis' of one sort or another. Frequently basic genetics has by this time been left well behind, but there are many types of investigations related to basic genetics that could be suitable.

Investigation of genetics of characters in short-lived plants

Several plants species closely approach *Arabidopsis* with regard to the length of their life cycle, but few have mutants readily available although natural populations may show variation for some characters. These plants could be investigated with regard to the induction of mutations. A list of such short-lived plants (or tachyplants) is given by Postlethwait & Enochs (1967).

Inheritance of varietal differences in horticultural plants

The inheritance of flower colour, colour pattern and morphology in many horticultural varieties is imperfectly understood and an investigation of one or more aspects of such plants would provide an exacting project. Normally it would be long term but the student could be presented with preserved material (pressed, dried or deep frozen) covering several generations. Suitable ideas and techniques can be obtained by consulting McQuown (1963) which gives techniques for growing and crossing the following: *Dianthus*, *Campanula*, *Epiphyllum*, *Dahlia*, *Iris*, *Rhododendron*, *Rosa*, *Gladiolus*, *Narcissus*, *Tulipa*, *Hydrangea*, *Ilex*, *Lycopersicum*, *Primula*, *Pelargonium*, *Lathyrus*, *Lilium*, *Delphinium*, *Chrysanthemum*, *Fuchsia*. Crane M.B. & Lawrence W.J.C. (1954) *Genetics of Garden Plants*, 4th edn., published by Macmillan, London, is also useful.

IV. CONCLUDING REMARKS

This chapter has attempted to show that it is possible to teach basic genetics using examples taken solely from the higher plants and animals, and without recourse to the classical organism, *Drosophila*. This has been done mainly in an attempt to bring the many diverse examples from higher organisms into a coherent plan and to indicate what type of investigation each organism can be used for, and where such a type of investigation can be put in a course. It is almost certainly not desirable to teach basic genetics without recourse to other organisms such as, of course, fungi and *Drosophila* which have distinct

practical advantages; they are further illustrations of the range of organisms which are covered by the elementary laws of genetics, and it is important that students should handle organisms that are central to research and teaching. This chapter, although it has been presented in a comprehensive manner, should therefore not be considered in isolation.

The whole topic of basic genetics is a story of variations on a theme, that theme being the fundamental laws of genetics as proposed by Mendel. The understanding of this theme (and its more obvious variations such as linkage) requires that it be explained, demonstrated and extended into related fields, and this can best be done through the medium of practical work. The most difficult part of teaching genetics is that of explanation: it is here that practical work can be of paramount importance. A full explanation should result in a complete understanding, but this understanding can really only be achieved by active participation by the student. The importance of this participation has been recognized frequently in the form of theoretical problems presented to the student during the course. The practical work has generally been looked upon rather differently, even though its overall aims should be the same. This chapter has tried to emphasize the importance of maintaining the 'problem approach': every investigation should be regarded as a minor open-ended project, and practical schedules should contain the minimum of information about the expected results (and even in some cases about the types of cross the student should perform). The student should be allowed to work out for himself what must be going on in inheritance and if possible (as in a general school investigation on mice as pets) it is well worth while students having to make crosses without any prior knowledge of genetics, and then trying to get them to deduce what is happening from their results. Normally a theoretical and practical course are concurrent, so students have prior knowledge of the laws of inheritance, but as has been mentioned, their understanding is detached, and takes on a different complexion when it comes to solving a problem for themselves.

Teaching practical genetics therefore not only has techniques that need to be learnt but also certain attitudes and aims, all of which go towards the production of a satisfactory course. In this chapter some of these attitudes have been emphasized since teaching genetics at a basic level is normally not just a technological problem but also an educational one.

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VI. REFERENCES AND FURTHER READING

CLARKE C.A. *et al.* (1966) Prevention of Rh haemolytic disease: results of the clinical trial. A combined study from centres in England and Baltimore. *Brit. med. J.* **ii**, 907–914.

CREED E.R. (1966) Geographic variation in the two spot ladybird in England and Wales. *Heredity* **21**, 57–72.

DARLINGTON C.D. & BRADSHAW A.D. (1963) *Teaching Genetics in School and University*. Oliver & Boyd, Edinburgh.

FLOR H.H. (1956) The complementary genic systems in flax and flax rust. *Adv. Genet.* **8**, 29–54.

FORD E.B. (1953) The genetics of polymorphism in the Lepidoptera. *Adv. Gent.* **5**, 43–88.

FORD E.B. (1967) *Moths*. New Naturalist Series. Collins, London.

FORD E.B. (1971) *Ecological Genetics*. Chapman and Hall, London.

FOSTER M. (1965) Mammalian pigment genetics. *Adv. Genet.* **13**, 188–339.

GLUECKSOHN-WAELSCH S. (1951) Physiological genetics of the mouse. *Adv. Genet.* **4**, 2–52.

GRANT V. (1956) The genetic structure of races and species in *Gilia*. *Adv. Genet.* **8**, 55–87.

HEAD J.J. & DENNIS N.R. (1968) *Genetics for 'O' level*. Teachers' Guide. Oliver and Boyd, Edinburgh.

KEER W.E. & LAIDLAW H.H. (1956) General genetics of bees. *Adv. Genet.* **8**, 109–153.

KOMAI T. (1956) Genetics of ladybeetles. *Adv. Genet.* **8**, 155–188.

KRUG C.A. & CARVALHO A. (1951) The genetics of *Coffea*. *Adv. Genet.* **4**, 127–158.

LEVINE P. (1954) The genetics of the newer human blood factors. *Adv. Genet.* **6**, 183–234.

MCQUOWN F.R. (1963) *Plant breeding for gardeners: a guide to practical hybridizing*. Collingridge, London.

MAHONEY R. (1966) *Laboratory Techniques in Zoology*. Butterworth, London.

NAGAO S. (1951) Genic analysis and linkage relationships of characters in rice. *Adv. Genet.* **4**, 181–212.

NUFFIELD BIOLOGY TEACHERS' GUIDE V. (1967) *The Perpetuation of Life*. Longmans/Penguin, London.

OLDROYD H. (1963) *Collecting, Preserving and studying insects*. Hutchinson, London.

POSTLETHWAIT S.N. & ENOCHS N.J. (1967) Tachyplants—suited to instruction and research. *Plant Science Bulletin* **13**, 1–2.

RAE A.L. (1956) The genetics of the sheep. *Adv. Genet.* **8**, 189–266.

REMINGTON C.L. (1954) The genetics of *Colias* (Lepidoptera). *Adv. Genet.* **6**, 403–450.

RICHEY F.D. (1950) Corn breeding. *Adv. Genet.* **3**, 159–192.

RICK C.M. & BUTLER L. (1956) Cytogenetics of the tomato. *Adv. Genet.* **8**, 267–382.

ROBINSON, R. (1971) *Lepidoptera genetics*. Pergamon Press, Oxford.

SAWIN P.B. (1955) Recent genetics of the domestic rabbit. *Adv. Genet.* **7**, 183–226.

SEARS E.R. (1948) Cytology and genetics of the wheats and their relatives. *Adv. Genet.* **2**, 239–270.

SHRODE R R. & LUSH J.L. (1947) The genetics of cattle. *Adv. Genet.* **1**, 209–261.

TANAKA Y. (1953) Genetics of the silkworm, *Bombyx mori*. *Adv. Genet.* **5**, 239–317.

WHITE M.J.D. (1951) Cytogenetics of Orthopteroid insects. *Adv. Genet.* **4**, 267–330.

WHITING A.R. (1961) Genetics of *Habrobracon*. *Adv. Genet.* **10**, 295–348.

ZIEGLER I. (1961) Genetic aspects of ommochrome and pterin pigments. *Adv. Genet.* **10**, 349–403.

INFORMATION SERVICES

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