Enzyme genetics in taxonomy: Diagnostic enzyme loci in the spider genus *Meta**

Brian J. Pennington
Department of Zoology,
University of Edinburgh,
West Mains Road,
Edinburgh EH9 3JT**

Summary

This paper introduces a species identification method which is based upon genetic properties of randomly-breeding populations, which includes spiders. Genetic variations in the molecular structures of enzymes can be detected by starch gel electrophoresis. Gene frequencies and genotype frequencies at enzyme loci are characteristics of populations which tend to differ between species. The allozyme genotypes of individual animals can be used as species diagnostic characters for sorting mixed species samples into their component species. The method is illustrated here by results from four congeneric orb-web spider species, *Meta segmentata* (Cl.), *M. mengei* (Bl.), *M. merianae* (Scop.) and *M. menardi* (Latr.).

Introduction

The principal diagnostic characters of most closely related spider species, the morphology of female epigynes and male palpal tarsi, become visible only at the final moult. When these characters are as yet undeveloped in juveniles, or differ so slightly between related species that the ranges of morphological variation within the species overlap, it can be very difficult to identify spiders unambiguously. In surveys concerned with compiling species lists for localities this problem may be minimal, but it can be acute when the objective of a sampling programme is to characterise the life histories of a small number of related species, because here much of the relevant information is contained in pre-adult stages of the populations.

This paper is intended to introduce one solution to this problem. It is a species identification method which focuses explicitly on underlying genetic differences between species, using the technique of zone electrophoresis of enzymes. Enzymes are proteins, and all proteins consist of one or more long coiled and folded amino acid chains (polypeptides). Polypeptides are the primary products of gene translation, and many properties of enzymes are ultimately determined by the structures of the genes themselves. To appreciate fully how enzyme genetics can be applied to taxonomic problems, we must first focus on the mechanism of inheritance.

The genetic basis of variation

All inherited information is passed from one generation to the next in the form of nucleotide sequences in the heritable material, deoxyribose nucleic acid (DNA). The DNA macromolecule contained within each chromosome of the cell is a very long double stranded helix built from combinations of four different nucleotides, the basic building blocks of nucleic acids. The two strands run in opposite directions side by side along the helix (i.e. in antiparallel). Only one of these strands, called the sense strand, contains information which the cell interprets as instructions for building polypeptides. But the other strand, the antisense strand, is ultimately just as important, because the cell uses this strand as a template for building a new sense strand at the time of DNA replication. Similarly, the sense strand is the template for a new antisense strand. Thus the role of DNA is dual: it is the carrier of heritable information and it is its own template for replication and perpetuation over generations of cells and organisms.

Each nucleotide represents one letter of a series of three-letter words (codons) which form the vocabulary of the genetic code. Using several forms of RNA (ribose nucleic acid) and subcellular organelles called ribosomes, the cell reads the nucleotide sequence from one end of a genetic message to the other, one

*Editor's note:
This is thought to be the first time that electrophoresis has been used as a species identification method for spiders. Because of the interest and importance of this aid to taxonomic research and the technical nature of the subject, it was thought that some readers would appreciate a fuller account of the genetical background and methods than would normally be given in a paper on this subject.

**Address for correspondence:
14 Riverside Road,
Wormit,
Newport-on-Tay,
Fife DD6 8LS

codon at a time. For each block of three consecutive nucleotides the cell attaches a corresponding specific amino acid to the growing polypeptide chain. Since there are sixty-four possible three-letter combinations of four different nucleotides and only twenty amino acids, some codons stand for the same amino acid. There are also codons which are interpreted as "start reading" and "stop reading the message here". The gene coding for a particular polypeptide may be regarded as all of the information between and including the start and stop codons.

A long polypeptide is a physically unstable molecule, so when synthesis is either partial or complete it gains stability by acquiring more structure. The polypeptide's primary structure, the amino acid sequence, has been determined by the structure of the gene. Built into this primary structure are tendencies to coil (secondary structure) and fold (tertiary structure), and sometimes to bind together with other molecules (quaternary structure) or with cell membranes (quintenary structure). These higher orders of structure give the protein its biological function.

Proteins made by the cell under the direction of DNA act in concert with other molecules, some of which they themselves synthesise, to regulate the processes of development and differentiation of cells and organisms. Thus much variation at all levels of organisation, including physiology, morphology, behaviour and ecology, can ultimately be attributed to variation at the genetic level. We shall now consider how these variations are inherited.

The body cells of most animals contain two sets of chromosomes, one set inherited from each parent. For every chromosome of one set there is a corresponding or homologous chromosome in the other set (except for the sex-determining chromosomes which do not match in one sex of most species). Homologous chromosomes appear identical under the light microscope and carry essentially the same kinds of genetic information, whereas non-homologous chromosomes appear dissimilar and carry genetic information concerning entirely different processes. When the body cells divide in the process of mitosis, this "diploid" chromosome number is conserved and the daughter cells are genetically identical. But in the germ cell line the chromosome number is halved during meiosis, a complex form of cell division which usually involves exchange of material between homologous chromosomes and two successive cell divisions. (The reader is referred to a genetics textbook such as Whitehouse (1969) for a full account of meiosis and its role in genetic recombination). The important feature of meiosis which concerns us here is that the maternally derived chromosome and the paternally derived chromosome of a homologous pair part company and go into different daughter cells, the gametes. Thus the homologous chromosomes of the diploid set and the genes on them segregate at meiosis, and the gametes are genetically non-identical.

The gametes of a diploid organism therefore carry a "haploid" set of non-homologous chromosomes composed of a random assortment of maternally and paternally derived chromosomes, although on average 50% will have been inherited from each parent of the animal whose gametes they are. When a gamete of one sex unites with a gamete of the other sex at fertilisation, the diploid chromosome number is restored in the fertilised ovum, or zygote.

In genetics, the term "locus" refers to the location of a particular gene in the chromosome complement. Locus is frequently used abstractly, meaning a site of unknown location in the chromosome complement at which genetic variation occurs. A diploid organism can carry either two identical copies of a gene at a particular locus, one on each of the two homologous chromosomes on which it occurs, or two different versions (alleles) of the gene at that locus. To distinguish between these conditions we speak of the "genotype" of an individual animal with respect to a locus. A homozygous genotype is one in which the animal carries two copies of the same gene, e.g. A_1, and a heterozygous genotype is one in which two different alleles, e.g. A_1 and A_2, occur simultaneously at the A locus in an individual (one allele on each of the two homologous chromosomes). The three possible genotypic combinations of the two alleles A_1 and A_2 are usually written A_1A_1, A_1A_2, and A_2A_2.

Genotype and genetic locus are terms which refer to invisible underlying realities of an organism's genetic make-up. In contrast, an organism's "phenotype" refers to some observable characteristic which can actually be counted or measured. We cannot observe genotypes directly, only phenotypes, but we infer the existence of genes and genetic variation from the way in which character-differences are

inherited. Character-differences which segregate according to simple genetic laws are called Mendelian variations.

If there is a one-to-one relationship between genotypes and phenotypes with respect to a Mendelian character, and we know the genotypes of two parents, we can predict which genotypes will occur in their offspring, and in what proportions, simply by constructing a matrix to represent the cross. Let us suppose that in a hypothetical spider species three colour forms, blue, green and yellow, occur. This colour variation is an example of a polymorphism, and the genetic locus which controls body colour is called a polymorphic locus. Let us suppose that we have already conducted a number of breeding experiments and have found that single-pair matings between blue spiders always yield blue progeny, and that yellows also breed true. Accordingly, we postulate that blues are homozygous ($A_1 A_1$) for one allele at the colour locus and that yellows are homozygous ($A_2 A_2$) for another allele. Single-pair matings between greens, on the other hand, always yield several progeny of each colour, and we hypothesise that greens are heterozygous ($A_1 A_2$) for the two colour alleles.

To test this hypothesis we draw up the matrix in Fig. 1 and hypothetically cross a green ($A_1 A_2$) male with a green ($A_1 A_2$) female. We stipulate that the two alleles segregate at meiosis and that both sexes produce equal proportions (i.e. 50%) of A_1 and A_2-bearing gametes. The male's gametes unite with the

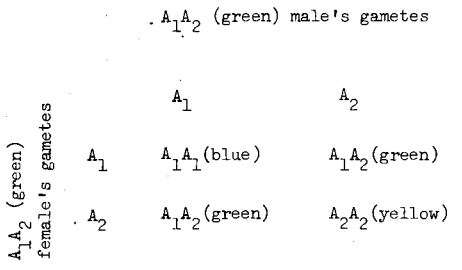

Fig. 1: Single-pair cross between a green ($A_1 A_2$) male and a green ($A_1 A_2$) female spider. Both parents produce A_1 and A_2-bearing gametes in equal proportions (50%), represented here by two columns and two rows. Four column-row combinations are possible, but two are identical. The expected genotype proportions in progeny of the cross are therefore $1(A_1 A_1) : 2(A_1 A_2) : 1(A_2 A_2)$.

female's gametes at random with respect to the genes they carry, and the matrix shows that four possible combinations of sperm and eggs occur. Two of them ($A_1 A_2$) are identical, so the expected blue ($A_1 A_1$) : green ($A_1 A_2$) : yellow ($A_2 A_2$) genotype ratio in the next generation is 1 : 2 : 1. If our observations agree with this prediction, we are satisfied that our interpretation of the genetic basis of the colour polymorphism is correct.

Mendelian characters sometimes exhibit dominance, i.e. the presence of a "dominant" allele, say A_1, in the heterozygote masks the expression of a "recessive" allele, A_2. The phenotypes of $A_1 A_1$ and $A_1 A_2$ genotypes might then be indistinguishable, and the observed phenotype ratio in the cross in Fig. 1 (this time between two heterozygous ($A_1 A_2$) blue spiders) would be 3 blues : 1 yellow. But it would still be possible to deduce the genotypes of all the blues by crossing each of them with a homozygous recessive ($A_2 A_2$) yellow spider of the opposite sex. Because the alleles segregate at meiosis, $A_1 A_2$ blues crossed with yellows yield both colours in the ratio 1 : 1, whereas $A_1 A_1$ blues crossed with yellows yield only $A_1 A_2$ blues.

Enzyme polymorphisms

Morphological polymorphisms are comparatively rare phenomena, and those which do occur, such as the wing pattern polymorphisms of Lepidoptera, often turn out to be genetically more complex than would at first appear. Morphological characters usually exhibit continuous (quantitative) variation, as opposed to the abrupt discontinuities characteristic of polymorphisms. Quantitative variations such as body weight and height are determined not by single-gene differences but by the cumulative small effects of genes at many polymorphic loci. Quantitative variation always contains elements of both genotypic and environmental variance, and the statistical techniques required to describe, separate and manipulate these components form the language of quantitative genetics (Falconer, 1960). Thus genetic polymorphism is fundamental to all forms of heritable variation. Observable polymorphisms are essential to the empirical study of the genetic basis of evolutionary change and, fortunately, technological advances over the last twenty years have revealed that

the paucity of morphological polymorphism is balanced by a great diversity of polymorphism at the molecular level (Lewontin, 1974).

We have already seen how the primary structure of a polypeptide is intimately related to the structure of the gene which codes for it. Enzymes are biological catalysts which are composed of polypeptides. A useful property of enzymes from the geneticist's point of view is that in solution they are electrostatically charged. This is because some of their amino acid side groups (the parts which make one amino acid different from another) ionize, either at low or high pH values depending upon whether they are basic (positive charge) or acidic (negative charge) respectively. Thus an enzyme molecule carries a net charge which is related to the pH of the medium and the sum of the charges of ionizing groups on its surface. Such a molecule will, like all charged particles, move in an electric field. Most importantly, molecules which differ in respect of as few as one amino acid can carry different charges, and these will move at different rates and hence separate in an electric field. Thus electrostatic charge and mobility in an electric field is an indicator of primary structure, and hence ultimately of gene structure.

Two commonly used methods of detecting differences in charge-related mobility of enzymes are starch gel electrophoresis (Smithies, 1955; Johnston & Den-

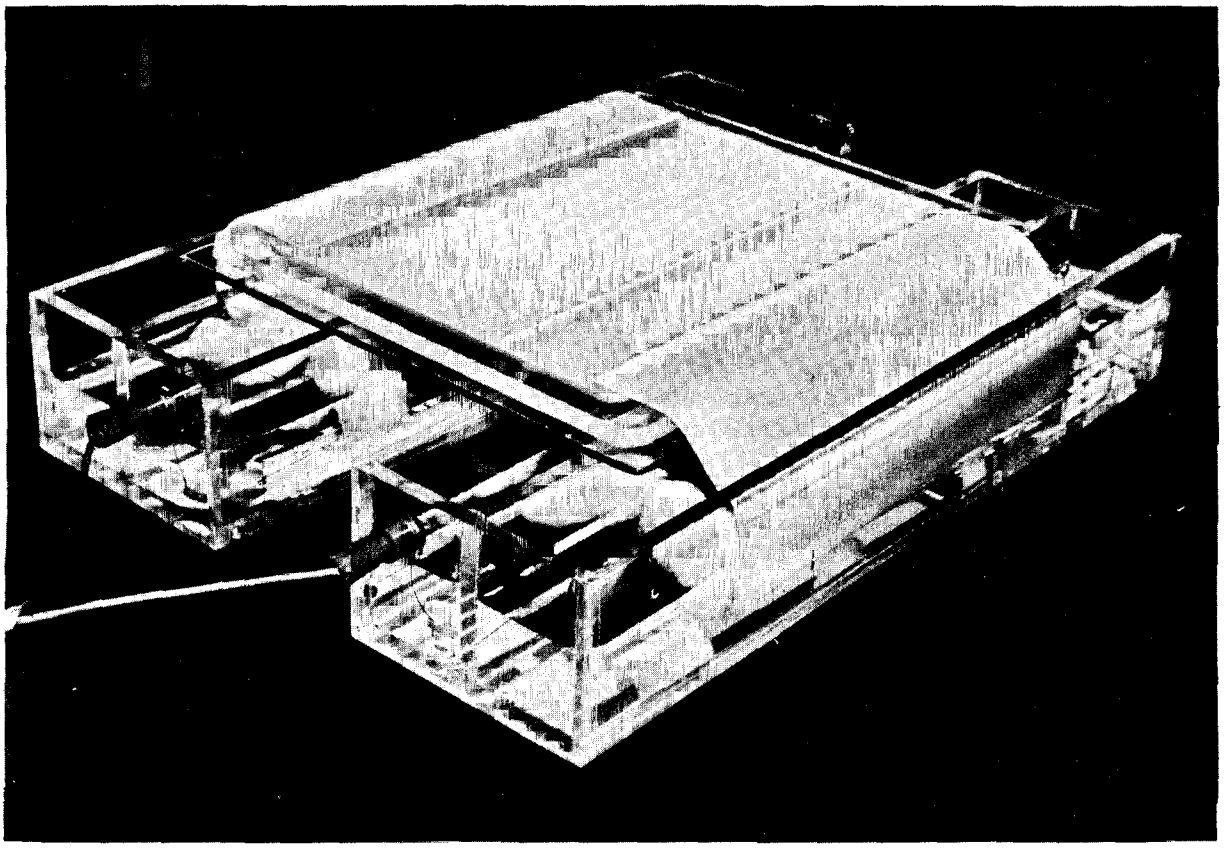

Fig. 2: Horizontal starch gel electrophoresis apparatus. The 20 x 15 x 0.6 cm starch gel has been prepared by pouring it hot into the perspex gel former on a glass plate, and has been sandwiched by a second glass plate, now removed, while cooling. The line of 25 sample-bearing paper inserts is at right angles to the electric field. The gel is electrically joined to the electrode buffers via filter paper wicks. The buffer in each buffer box is divided into two portions, which are connected by cotton wool plugs in the dividing walls of the boxes, in order to keep the sites of electrolysis distant from the gel. The gel will be covered with polythene to prevent dehydration, and the apparatus will be used in a cold-room to prevent excessive heating of the gel during electrophoresis. After electrophoresis the gel will be cut into several horizontal slices.

niston, 1964; Smith, 1968) and polyacrylamide gel electrophoresis (Prakash, Lewontin & Hubby, 1969; Johnson, 1977, and references therein). In horizontal starch gel electrophoresis, the method used to obtain the present data on *Meta*, fresh soluble extracts of individual animals (whole-animal homogenates in the case of small spiders) are absorbed onto small squares of chromatography paper which are then inserted into a starch gel. The paper inserts are a convenient way of confining the enzymes from individual animals in narrow zones in the gel. As many as twenty-five inserts are placed in a line which is perpendicular to the electric field (Fig. 2), so that when the current is switched on the charged molecules present in each sample move away from the origin and towards whichever electrode attracts them. Most enzymes migrate towards the anode, so the origin is usually located fairly close to the cathode. The gel is prepared from a mixture of hydrolysed starch and a buffer solution, and the electrodes are electrically joined to the gel via buffer-soaked filter paper wicks (Smith, 1968).

During electrophoresis, molecules which are electrostatically neutral at the pH value of the gel remain in the narrow zones in which they were first placed. The charged molecules also remain in narrow zones (in plan view they appear as narrow bands), but these move in bands across the gel towards the electrodes, one band for each group of similarly charged molecules.

After electrophoresis, the locations of bands are determined by selective staining of horizontal slices of the gel in a process which resembles photographic development. Since there could have been many different kinds of protein coded by many different genes in the original extracts, each stain employed narrows down the possible array of bands to as few as the products of one genetic locus by picking out one specific kind of catalytic activity. For instance, only bands containing the enzyme lactate dehydrogenase (LDH) stain up on gel slices incubated in a buffered solution of lactic acid and NAD (nicotinamide adenine dinucleotide), i.e. the substrates of LDH, and a reagent mixture which changes from colourless to blue and precipitates on the gel in the presence of local concentrations of the reducing agent NADH (reduced NAD), i.e. one product of the LDH reaction (the other being pyruvic acid). The pattern of bands which develops on the gel is called a zymogram. Figs. 6-10 are examples of zymograms in which each column of bands represents an individual spider's phenotype with respect to a particular enzyme.

Bands which represent the same enzyme in different individuals move equal distances from the origin. Enzymes which occupy non-homologous band positions are called isozymes. Isozymes are known to be structurally different enzymes which catalyse the same biochemical reaction because they have separated during electrophoresis, and hence must differ in net charge. Some isozymes on a zymogram may be the products of different alleles at a polymorphic enzyme locus, in which case they are called allozymes. Other isozymes might be quaternary structure variants of a polypeptide produced by a single gene, and yet others might represent the products of genes at non-homologous loci. It is usually possible to deduce from the pattern of variation in a population into which of these categories the bands detected fall, and consequently to deduce individuals' genotypes. It is often helpful to test such hypotheses with breeding experiments in the same way as we hypothetically crossed colour phenotypes of spiders in Fig. 1 to establish whether or not our understanding of the genetic basis of the polymorphism was correct. Fortunately, enzyme polymorphisms, unlike morphological polymorphisms, do not exhibit dominance.

Gene frequencies as characters of populations

Charles Darwin recognised that heritable variation in populations is the raw material upon which natural selection acts to mould evolutionary change. But by focusing on quantitative variation he was wrongly led to suppose that numerous "gemules" which give parents their characteristics fuse and blend at fertilisation, whereas we know from the pioneering experiments of Gregor Mendel, a contemporary of Darwin, that the invisible "factors" (i.e. genes) responsible for inherited character-differences do not blend, but segregate intact and continue to exert their influences on the phenotypes of subsequent generations. Modern population genetics contains essential elements of both Darwinian evolution and Mendelian genetics. Natural selection is now seen as a process (or set of processes) which brings about evolutionary change by altering gene frequencies at polymorphic loci.

Genotypes are properties of individuals which form at fertilisation and continue to exist for only as long as individuals exist. But the genes themselves can achieve virtual immortality by disengaging from genotypes at meiosis and voyaging from one generation of genotypes to the next via the gametes. A population is not just a collection of static genotypes, it is also a breeding unit, a dynamic field in which genes temporarily cooperate in genotypes, dissociate, and reassociate in new cooperatives as generations of individuals elapse. We capture this sense of dynamic continuity by describing the genetic constitution of a population not in terms of genotypes, but in terms of the relative frequencies at which specific alleles, e.g. A_1 and A_2, occur at particular loci carried by the population. A gene frequency is a measure of how often an allele is represented at a locus in a population. For example, if A_1 and A_2 are the only alleles carried at the A locus in a population, and A_1 is represented 18 times in the 100 genes carried by 50 diploid individuals, the gene frequency of A_1 is 0.18 and the gene frequency of A_2 is 0.82.

Gene frequencies are related simply to genotypes by the Hardy-Weinberg Law, which states that if in a large randomly-mating (panmictic) population the gene frequencies of the A_1 and A_2 alleles are p and q respectively, so that $p + q = 1$, the relative proportions of A_1A_1, A_1A_2 and A_2A_2 genotypes in the population are given by the binomial expansion $p^2 + 2pq + q^2 = 1$. This relationship can be derived in two ways, both of which feature the transmission of genes from one generation to the next (Falconer, 1960). One derivation is illustrated in Fig. 3.

A population remains in Hardy-Weinberg equilibrium only so long as certain conditions are satisfied. These are that the genotypes are equally "fit" in the face of natural selection (i.e. that the alleles are selectively neutral), that no migration of genotypes to or from the population occurs, that the mutation rates of A_1 alleles to and from A_2 are negligible, and that no assortative mating of genotypes occurs. If the genotype frequencies observed in a population do not agree with the genotype frequencies expected on the basis of the observed gene frequencies, we conclude that one or more of these conditions has been breached. Thus the Hardy-Weinberg Law is a useful formula for investigating these conditions themselves.

Many population surveys have shown that a proportion of enzyme loci in most populations are polymorphic (Lewontin, 1974). Gene frequencies at these loci are very useful characters for comparing populations. It is often found that gene frequencies at homologous loci differ between geographically isolated populations of the same species, though such gene frequency differentiation is usually relatively limited and confined to a few loci. The genetic differentiation observed between very closely related species, for example morphological sibling species (i.e. species which look very similar indeed), is usually more marked; more loci are involved, and to a greater extent. At this stage of phyletic divergence in a group, unique alleles might be detected at a few loci. These are alleles which are not detectable in other species. As more distantly related congeners are compared, the degree of gene frequency divergence and the number of unique alleles in each species tend to increase.

This observed parallel between gene frequency divergence and phyletic divergence has given rise to the concept of genetic distances between species (Nei, 1972), an idea that is analogous to the numerical taxonomist's concept of taxonomic distances between species (Sneath & Sokal, 1973). A measure of genetic distance essentially quantifies the degree to which the identities and frequencies of alleles at homologous loci are correlated between a pair of species. Avise (1976) reviews genetic differentiation

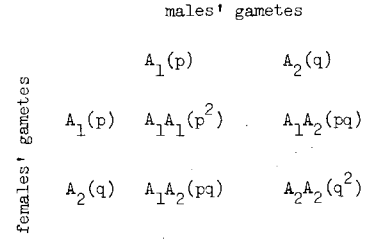

Fig. 3: This illustrates one of two ways of deriving the Hardy-Weinberg Law. The relative proportions of A_1 and A_2-bearing gametes produced by each sex in a population depend upon the gene frequencies, p and q, of the A_1 and A_2 alleles. Provided the sexes mate at random with respect to genotype, the relative genotype frequencies in the next generation will be the products of the frequencies of the genes which combine in the genotypes: $p^2(A_1A_1)$, $2pq(A_1A_2)$, and $q^2(A_2A_2)$.

during speciation and measures of genetic distance between populations and species.

Polymorphism applied to species identification

If the relative gene frequencies at a homologous locus differ between two species, it follows that the genotype frequency distributions will also differ between the species. We can use this fact to guess the specific identity of individual animals from their genotype with respect to that locus. The hypothetical data in Table 1 will clarify this point.

In our previous discussion of the colour polymorphic spider species we were concerned only with deducing the outcome of single-pair matings and did not consider the distribution of the colour forms in the population as a whole. Let us suppose that those spiders were collected from a small island on which this species was the only representative of its genus, but that this species (species X) and a close relative (species Y) coexist on the mainland and we have decided to embark on a research programme aimed at finding out how they coexist without competing for resources. To begin with we want to examine their life histories, but we find that we can identify only adults (by their sex organs) and that no reliable diagnostic character exists for identifying juveniles. We notice, however, that the same three colour forms occur in both species, although most blue and green adults are species X and most yellow adults are species Y. Exactly how reliable would body colour be as a diagnostic character of juveniles?

To answer this we first obtain estimates of gene frequencies by taking random samples of adult spiders from both species, counting the number of times each allele occurs in the samples, and expressing these numbers as proportions of the totals counted in each species. We then compute expected Hardy-Weinberg genotype frequencies. Provided the assumptions of the Hardy-Weinberg Law hold for this example, the expected frequencies will closely match the "observed" genotype frequencies from which we calculated the gene frequencies. However, in order to avoid invalidating our conclusions, we continue to use the expected frequencies. The results are summarised in Table 1a.

What we want to know is, once we have assigned a blue or green spider to species X or a yellow spider to species Y, what is the probability that we are wrong? The answer is P = 0.0442. This figure is reached by the following argument. The right hand side of Table 1a shows that a small proportion (0.01) of species X occur in the "wrong" colour class (i.e. yellow); similarly, small proportions (0.0016 and 0.0768) of species Y occur in "wrong" colour classes. The sum of these three frequencies (0.0016 + 0.0768 + 0.01 = 0.0884) is the proportion of overlap (Ayala & Powell, 1972), and this represents the composite probability of being wrong in either of two ways: wrongly identifying blues or greens as species X, and wrongly identifying yellows as species Y. But since we identify only one spider at a time, we can be wrong in only one way at a time; so the appropriate figure is ½ of 0.0884 = 0.0442. This means that fewer than 1 in 20 species identifications made on the basis of genotype at the diagnostic colour locus will be wrong. In statistical terms this locus is "diagnostic at the 0.05

	"Observed" gene frequencies		Expected genotype frequencies		
	A_1	A_2	A_1A_1 (blue)	A_1A_2 (green)	A_2A_2 (yellow)
(a) species X	0.9	0.1	0.81	0.18	0.01
species Y	0.04	0.96	0.0016	0.0768	0.9216
(b) column averages	0.47	0.53	0.4058	0.1284	0.4658
expected genotype frequencies in "species" Z			0.2209	0.4982	0.2809

Table 1: (a) The gene frequencies of the A_1 and A_2 alleles in the hypothetical species X and Y were calculated from the numbers of alleles "observed" to occur in imaginary population samples. The genotype frequencies corresponding to these gene frequencies are those expected of populations which are in Hardy-Weinberg equilibrium.
(b) When species X and Y mix together in a composite "species" Z, the "observed" gene and genotype frequencies are the arithmetic averages of the two true species. The genotype frequencies expected on the basis of the "observed" (average) gene frequencies differ markedly from the "observed" (average) genotype frequencies in "species" Z.

or 5% level". Thus in this example colour is quite a good indicator of species.

The existence of such a diagnostic colour locus in reality is improbable, but a survey of, say, ten polymorphic enzyme loci would almost inevitably reveal at least one, and perhaps several diagnostic loci, even in very closely related species. In fact, diagnostic loci can turn up unexpectedly and point to the existence of more than one species in a population sample. For example, Webster & Burns (1974) describe the electrophoretic detection of a group of *Anolis* lizard sibling species which were formerly thought to be geographic variations of one species. Had it not been for a number of polymorphic enzyme loci exhibiting marked deviation from Hardy-Weinberg equilibrium, these species would have remained undetected and undescribed. We have seen how to press the Hardy-Weinberg Law into the service of species identification under one set of circumstances, namely when gene frequencies measured in one age class of a species pair are used to predict identity in another age class, which is a straightforward procedure. Now let us develop the technique to allow us to proceed when less initial information is at our disposal, i.e. when no morphological criterion exists to indicate unambiguously that the sample of adult and juvenile spiders contains more than one panmictic species.

Had there been no diagnostic morphological character to distinguish between adults of species X and species Y, we might have assumed that the sample contained one species only, "species" Z. In this case the "observed" gene and genotype frequencies in "species" Z would have been the column averages of the data in Table 1a. These averages are shown in Table 1b with expected genotype frequencies calculated for these gene frequencies (0.47 and 0.53) in a truly panmictic species. The composite "species" Z is quite obviously not in Hardy-Weinberg equilibrium: compared with the expected frequency distribution there is an excess of homozygotes and a paucity of heterozygotes. Several possible explanations exist for this kind of deviation from Hardy-Weinberg equilibrium. In this case the correct explanation is a form of assortative mating, namely that the animals show marked preferences for breeding only with their own species.

This result alerts us to the heterogeneity of the sample and we attempt to resolve it into its con-

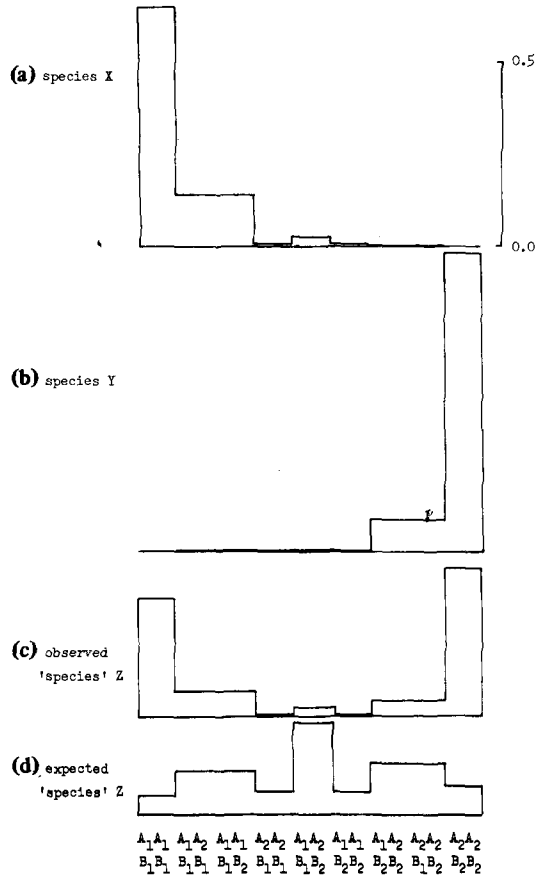

Fig. 4: If two A locus alleles, A_1 and A_2, are inherited independently of two B locus alleles, B_1 and B_2, then nine genotypes will occur with respect to these loci in a population containing these alleles. These are listed in arbitrary order of similarity along the horizontal axis. The relative frequencies of these genotypes in a population can be deduced by multiplying together the frequencies of the component genotypes (e.g. freq. $A_1A_1B_1B_2$ = freq. A_1A_1 x freq. B_1B_2). The A locus genotype frequencies expected in species X and Y, which are in Hardy-Weinberg equilibrium, are given in Table 1a. The B locus alleles occur in these hypothetical species at the same frequencies as their counterparts at the A locus, so B locus genotype frequencies are the same also. The histograms in (a) and (b) illustrate the two-locus genotype frequency distributions of species X and Y respectively. Fig. (c) is the average of these distributions, and represents the frequency distribution "observed" in the composite "species" Z. By comparison, the frequency distribution expected of a truly panmictic species Z which is in Hardy-Weinberg equilibrium is shown in (d).

stituent components, species X and species Y. This would be very simple if the sample consisted of homozygotes only (i.e. a mixture of A_1A_1 and A_2A_2), because the only reasonable interpretation of the "observed" variation would be that a different allele was fixed (i.e. frequency = 1.0) in each of the two species. Genotype would then be a completely unambiguous indicator of species. Unfortunately, under the given circumstances the best we can do is to argue that A_1A_1's are species X and A_2A_2's are species Y: heterozygotes must remain undefined, because without an unambiguous diagnostic character we cannot resolve the average gene frequencies (0.47 and 0.53) into their constituent components (i.e. the left hand side of Table 1a), and hence we have no way of knowing what the underlying structures of the average genotype frequencies really are.

What is needed to improve upon this situation is for the frequency of the ambiguous genotype, A_1A_2 heterozygotes in Table 1b, to be diminished to a point where the data closely approach the ideal of two non-overlapping homozygous genotypes (i.e. A_1A_1 and A_2A_2). This can be achieved surprisingly easily, by screening for another polymorphic locus showing significant deviation from Hardy-Weinberg equilibrium in the mixed population. For simplicity, let us suppose that the alleles B_1 and B_2 segregate at the B locus in species X and Y at exactly the same frequencies as their counterparts at the A locus, so that all that has been said in respect of the A locus applies equally to the B locus. Furthermore, B alleles are inherited irrespective of the identities of the A alleles inherited, and *vice versa*. Because A and B alleles are inherited independently, the probability of an A_1A_2 heterozygote being also a B_1B_2 heterozygote is $(0.1284)^2 = 0.016$. (The frequencies of the nine possible two-locus genotypes we will observe in the composite "species" Z can be deduced by constructing a matrix to multiply A locus genotype frequencies by B locus genotype frequencies, using the "observed" average genotype frequencies of Table 1b). Fig. 4c illustrates the comparative rarity of this ($A_1A_2B_1B_2$) and two rarer ($A_1A_1B_2B_2$ and $A_2A_2B_1B_1$) ambiguous genotypes. The genotype frequency distribution now splits naturally into two virtually non-overlapping genotype groups which correspond to species X and species Y. Only a small proportion of genotypes remain undefined (the three just mentioned). Figs. 4a and b illustrate the comparative genetic structures of these species, and Fig. 4d shows the genotype frequency distribution expected of the composite "species" Z had it been in Hardy-Weinberg equilibrium. Further analysis is unnecessary because, except for about 2% of the sample, the species identification problem has now effectively been solved. All that remains to be done is to name the two species which have emerged from "species" Z. Fig. 5 summarises the operations we have examined so far.

The data that emerge from the left and right hand sides of Fig. 5 differ in one important respect, although both sets of operations solve the original problem, namely to detect and distinguish between two or more panmictic populations mixed together in one sample. On the left (1-6, 12) we define the species on some criterion (of adults) and measure gene frequencies at one locus. If gene frequencies at this locus are sufficiently differentiated between the species, we predict the genotype frequencies in the juveniles, using the Hardy-Weinberg Law, and sort the observed genotypes of juveniles into groups which, we argue, correspond to the species. Since these groups have been defined by their genotypes, and hence must possess very similar gene frequencies to the adults, we cannot measure the gene frequencies of the juveniles with a view to comparing the genetic constitutions of the species. To do so would be tautological.

It would also seem tautological, therefore, to measure gene frequencies and compare the genetic compositions of the species which emerge from the right hand side of Fig. 5 (1-2, 7-12), because here the species are entirely genetically defined. But this is not so. The difference is that now we define the species on the basis of two or more independently inherited characters (genotypes), none of which is necessarily particularly reliable on its own, but which together leave little doubt as to the affiliations of individual animals. So having defined each individual on the basis of a cluster of characters, we can proceed to estimate gene frequencies at each locus in the populations as wholes. This is how the data in Table 2 and Tables 4-7 were obtained (in the latter four Tables observed phenotype frequencies rather than gene frequencies are reported).

Diagnostic loci in *Meta*

The rest of this paper concerns the detection of diagnostic polymorphic enzyme loci in *Meta* and a brief discussion of the genetic basis of the variation detected at each locus.

Sampling the populations

The research from which the present data are extracted began with a survey of enzyme polymorphism in *Meta segmentata* (Clerck) and evolved into a study of the comparative life histories of *M. segmentata* and three sympatric congeners, *M. mengei* (Blackwall), *M. merianae* (Scopoli) and *M. menardi* (Latreille) (Pennington, 1977). The study area was on the shores of Loch Sween in Argyll. The main collecting sites were situated on a climax oak-wooded (*Quercus robur* L.) peninsula ridge in this locality (NM 761864: NM 754873 was also sampled to supplement (b) below). Spiders were collected from two major habitats:

(a) In October 1973 mainly adult *M. segmentata* and *M. mengei* were collected by hand from field layer vegetation. Field layer sweep samples were subsequently taken at three-week intervals on fine days from April-November 1974 and 1975, and March-August 1976. Depending upon the season, all species were represented in these samples, though neither *M. merianae* instar 7 nor *M. menardi* instars 4-7 were detected in the field layer (*Meta* species leave their eggsacs in the 2nd instar and mature in the 7th instar).

(b) Representatives of all *M. merianae* instars were collected from beneath rock and vegetation overhangs on the steep uneven slopes of the ridge by searching with torchlight after dark. Adult and a few intermediate-sized *M. menardi* were found at the entrances to, and within, deep cave-like chambers ("micro-caves") formed between large partially-buried rocks. Adult *M. menardi* could be detected only at night, but adult *M. merianae* could be shaken from overhanging vegetation during the day.

The spiders were kept alive in 6 x 1.5 cm clear plastic tubes for up to three days, killed by freezing, and stored in a deep-freeze in the laboratory. For analyses of the age structures of the populations the cephalothorax length and, where possible, the sex and species of each specimen were recorded. Spiders which could not be identified easily were prepared for enzyme electrophoresis (many spiders of known identity were also analysed for comparison). These were individually homogenised with 1-2 volumes of distilled water. Homogenates were absorbed onto 5 mm chromatography paper squares which were stored frozen until used. The horizontal starch gel electrophoresis apparatus (Smith, 1968; Bathgate, 1978) is described in Fig. 2.

The enzymes which were sufficiently active to be assayed routinely were: non-specific esterases, malate dehydrogenase (MDH), lactate dehydrogenase (LDH), α-glycerophosphate dehydrogenase (α-GPDH), glutamate-oxaloacetate transaminase (GOT), and phosphoglucoisomerase (PGI). These could all be assayed on Tris-Citrate pH 6.2 gels, but esterases were routinely assayed on Tris-EDTA-Borate pH 8.6 gels (Pennington, 1977).

Detecting Meta *species by diagnostic loci*

The first hand-collected sample of adult *Meta* (October 1973) was originally thought to consist entirely of *M. segmentata.* The basis for this assumption was that Locket & Millidge (1953) described *M. mengei* as a subspecies of *M. segmentata*: sexually mature subspecies cannot coexist in time and space without eventually merging into one genetically homogeneous population, so the two "subspecies" could not co-occur in autumn. However, partly because they co-occur in autumn, Chrysanthus (1953) subsequently argued that *M. mengei* is a true species (Locket, Millidge & Merrett, 1974).

Chrysanthus' revision was impressively upheld by the first batch of zymograms. I had expected electrophoresis to reveal a population (of *M. segmentata*) in Hardy-Weinberg equilibrium with respect to each polymorphic locus detected. In fact, every polymorphic locus (Est-1, Est-2, MDH, LDH and GOT) exhibited significant deviation from Hardy-Weinberg equilibrium, of the kind described in Table 1b. No MDH or LDH heterozygote was detected. When the data were considered as a whole (as in Fig. 4), it was clear that two genetically non-overlapping populations were represented in the sample. Closer examination of the remaining intact adult males of the sample confirmed that the two genetic groups corresponded to *M. segmentata* and *M. mengei*.

The technique (Fig. 5: 1-6, 12) was then used routinely to identify juvenile and adult female

M. mengei and *M. segmentata* in the life history study (it is difficult to distinguish between adult females as well as juveniles of these sibling species). In May 1974, however, two additional genetically distinct groups of juveniles appeared in field layer sweep samples. One of these was identified as being *M. merianae*, but the other remained a puzzle until its identity was finally established by comparison with the zymograms of an adult female *M. menardi*. These spiders had been difficult to identify because the first two free-living instars of *M. menardi* disperse widely in the field layer and are coloured quite differently from later instars, which are confined to microcaves. This aspect of *M. menardi*'s life history is outlined

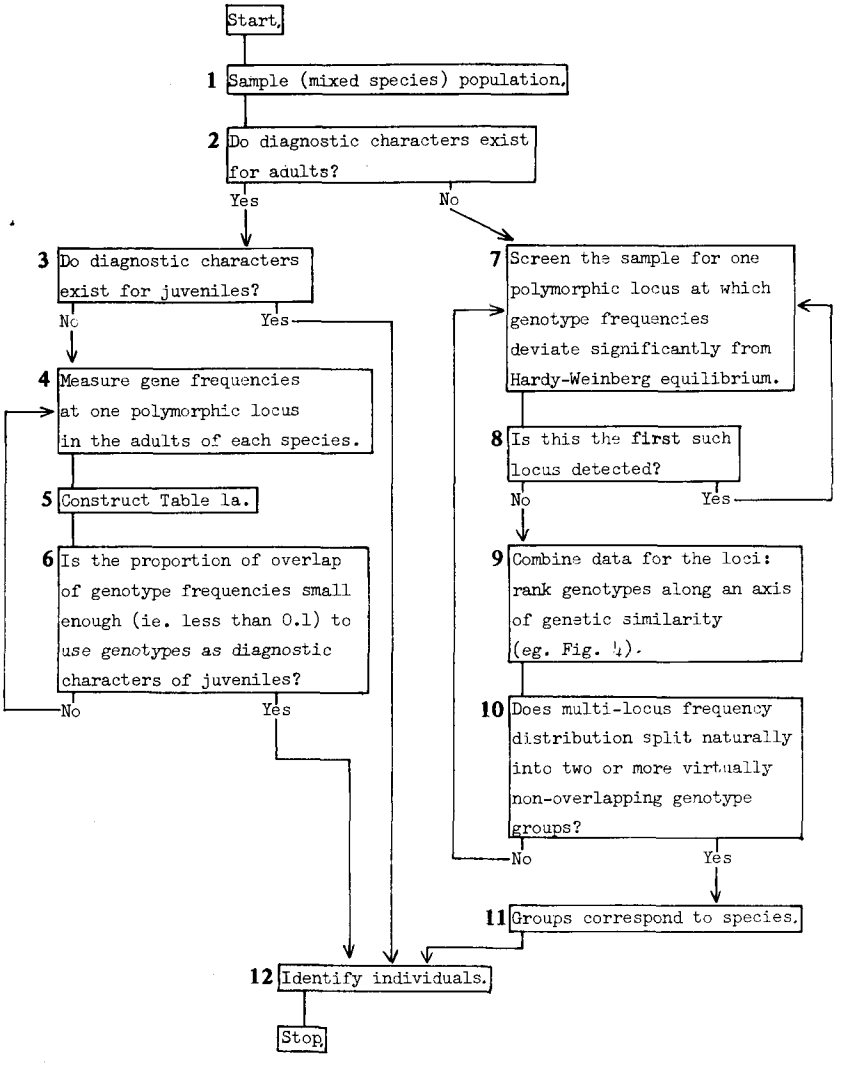

Fig. 5: Two ways of identifying spiders genetically are discussed in the text. The distinctions between these two sets of operations are summarised here. The primary difference is that on the left (1-6, 12) the species are defined by conventional characters, whereas the species are defined genetically as well as identified during the operations on the right (1-2, 7-12). The operations on the right (1-2, 7-12) do not necessarily begin with prior knowledge of the number or names of species represented in the sample. This is one way of sharply defining known species, or searching for new species; it is also how new species are detected accidentally during investigations of polymorphism at the molecular level.

	Est-1a	Est-1b	Est-1c	Est-1d	Est-2a	Est-2b	N
M. mengei	–	–	0.279	0.721	0.936	0.064	39
M. segmentata	0.014	0.684	0.295	0.007	0.01	0.99	150

Table 2: Gene frequencies observed at two polymorphic esterase loci in *M. mengei* and *M. segmentata* collected in October 1973. The genotype frequencies observed at these loci were in close agreement with Hardy-Weinberg expectations in both species.

elsewhere (Pennington, 1979).

Having found that each of the species is typified by a unique combination of isozyme phenotypes at four (MDH, LDH, GOT and PGI) of the six polymorphic loci examined, the following genetic profiles were used as the bases of subsequent species identifications: MDH3, LDH3, GOT5, PGI3 (*M. mengei*); MDH1, LDH4, GOT3, PGI3 (*M. segmentata*); MDH1, LDH3, GOT1, PGI3 (*M. merianae*); MDH2, LDH4, GOT1, PGI1 (*M. menardi*). The nomenclature used to describe these phenotypes (not genotypes), and the reason why esterases are not included, are explained in the following section.

The genetic bases of the polymorphisms of **Meta**

It is not always easy to interpret the underlying genetics of isozyme phenotypes (individuals' zymograms), especially when it is not practicable to test each interpretation with breeding experiments of the kind illustrated in Fig. 1. As it happens though, it does not really matter what the genetic bases of the individual differences reported here are, because the species identification method was arrived at pragmatically; so a simple description of the phenotypes observed should suffice. For the sake of thoroughness, however, I shall discuss my interpretations of the data for each locus in turn. Figures 6-10 are diagrammatic representations of zymograms showing the relative electrophoretic mobility of isozymes. Each vertical column of parallel bands represents the phenotype of one spider with respect to one enzyme.

Figure 6 illustrates esterase polymorphisms detected at two homologous loci in *M. segmentata* and *M. mengei*, and Table 2 reports the gene frequencies observed at these loci in the October 1973 sample. The interpretation of the variations observed at these loci is straightforward: the four faintly-staining bands correspond to allozymes (alleles) at one locus (Est-1), and the two darkly staining bands correspond to alleles at another locus (Est-2). All four Est-1 alleles segregate in *M. segmentata*, and two of these, Est-1c and Est-1d, also occur in *M. mengei*. The Est-1c allele is at similar frequencies in the two species. Est-2 gene frequencies are considerably more differentiated between the species and, as Table 3 argues, the Est-2 locus is almost diagnostic at the 1% level for *M. seg-*

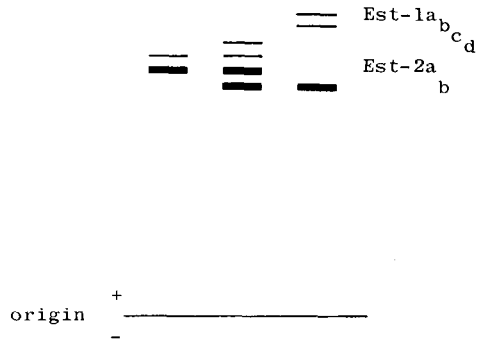

Fig. 6: Esterase allozymes observed at two homologous polymorphic loci in *M. mengei* and *M. segmentata* in October 1973. Each vertical column of parallel horizontal bands represents the isozyme phenotype (zymogram) of one spider. All of the isozymes illustrated here have moved from the origin towards the anode during electrophoresis. Thin bands are Est-1 allozymes and thick bands are Est-2 allozymes. From left to right the genotypes of these spiders are: Est-1dd, Est-2aa (*M. mengei*); Est-1cd, Est-2ab (*M. mengei*); Est-1ab, Est-2bb (*M. segmentata*).

	Est-2aa	Est-2ab	Est-2bb
M. mengei	0.876	0.12	0.004
M. segmentata	0.0001	0.0198	0.98

Table 3: Expected Est-2 genotype frequencies in *M. mengei* and *M. segmentata*. If Est-2aa and Est-2ab genotypes are taken as diagnostic for *M. mengei* and Est-2bb diagnostic for *M. segmentata*, the proportion of overlap between the species is (0.0001 + 0.0198 + 0.004) = 0.0239. The probability of wrongly identifying a spider on the basis of genotype is therefore P = 0.012. Thus this locus is nearly diagnostic at the 0.01 or 1% level.

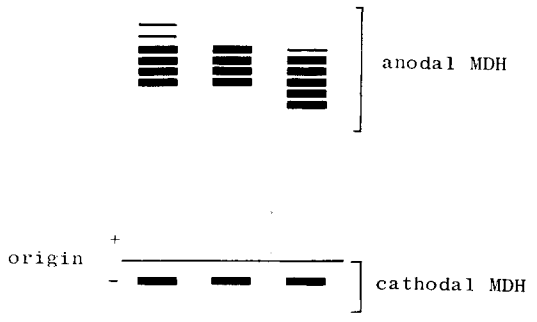

Fig. 7: Malate dehydrogenase (MDH) phenotypes observed in *Meta*. Two sets of MDH isozymes, one at the anodal side of the origin and one at the cathodal side of the origin, were observed in the genus. These probably correspond to two distinct genetic loci. The anodal band patterns only were recorded. From left to right the phenotypes are named: MDH1 (*M. segmentata* and *M. merianae*); MDH2 (*M. menardi*); MDH3 (*M. mengei*). The order of phenotypes here corresponds to the order in Table 4.

mentata and *M. mengei*. Thus, as errors of species identification will occur at a rate of approximately 1 in 100, Est-2 genotypes are good indicators of species.

The esterase loci were not used routinely in species identification because, unfortunately, the homologues of these loci could not be identified in *M. merianae* and *M. menardi*. Identifying homologous loci in different species is largely a matter of guesswork which is greatly eased when at least some band positions are common to the species. This is true of the remaining four loci studied, although in these cases all of the enzymes have quaternary structures (i.e. they are built from two or more polypeptides, each of which may vary genetically) and the relationships between phenotypes and genotypes are subject to doubt. Consequently, I refer to isozyme phenotypes for these enzymes, rather than genotypes as in the case of esterases. Sample sizes differ among the loci because some zymograms resolved poorly and the data were not recorded. Band positions are numbered in order of electrophoretic mobility.

Two sets of MDH isozymes were detected in the genus: anodal MDH activity and cathodal MDH activity (Fig. 7). These probably represent two MDH loci (cf. the MDH isozymes of *Limulus*: Selander *et al.*, 1970). The cathodal locus was polymorphic in all

MDH phenotype	MDH1	MDH2	MDH3	N
Genotype	aa	bb	cc	
M. merianae	1.0	–	–	298
M. segmentata	1.0	–	–	233
M. menardi	–	1.0	–	206
M. mengei	–	–	1.0	569

Table 4: MDH phenotype frequencies and assumed genotypes.

of the species, but homologous band positions were difficult to identify, even within species, because the several allozymes separated only slightly. The genetics of the variation at this locus were not deduced and the position of the commonest cathodal band only is illustrated in Fig. 7. No intraspecific variation at the anodal MDH locus was detected (Table 4), but in the genus as a whole three distinct phenotypes were recognised. *M. segmentata* and *M. merianae* are both characterised by a six-banded MDH phenotype (MDH1) which migrates two band positions further than a similar six-banded *M. mengei* phenotype (MDH3). The *M. menardi* phenotype (MDH2) is composed only of the four bands common to MDH1 and MDH3. The simplest interpretation of the interspecific variation is that each isozyme phenotype is made up of a series of quaternary structure variations of a single polypeptide, i.e. monomer, dimer, trimer, etc., and that a different allele for the polypeptide primary structure (which determines charge and possible sizes of combinations) is fixed in each species. Thus *M. segmentata* and *M. merianae* are genetically the same at this locus (Table 4).

The GOT enzyme is a dimer (i.e. consists of two polypeptides) which can form 2- or 3-banded heterozygous phenotypes, depending upon which two

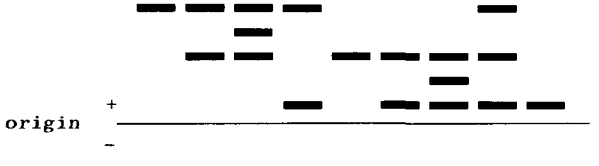

Fig. 8: Glutamate-oxaloacetate transaminase (GOT) phenotypes observed in *Meta*. The band positions are numbered in order of electrophoretic mobility. From left to right the phenotypes are: GOT1; GOT13; GOT123; GOT15; GOT3; GOT35; GOT345; GOT135; GOT5. These phenotypes appear in the same order in Table 5.

GOT phenotype	1	13	123	15	3	35	345	135	5	N
Genotype	aa	ad	ab	ac	bb	be	bc	cf	cc	
M. merianae	0.98	0.004	0.007	0.007	0.004	–	–	–	–	298
M. menardi	0.995	–	–	0.005	–	–	–	–	–	190
M. segmentata	0.004	–	0.004	–	0.98	0.004	0.004	–	0.004	233
M. mengei	–	–	–	0.019	0.044	–	0.067	0.004	0.907	569

Table 5: GOT phenotype frequencies and assumed genotypes.

polypeptides occur together (Fig. 8). For example, when the a and b GOT alleles occur together in a heterozygote three kinds of active enzyme molecule are produced: homodimers aa and bb, and a heterodimer, ab; but in heterozygotes for the a and d alleles, no heterodimer is formed. Heterodimers appear as the middle "hybrid" band in 3-banded heterozygotes: band positions 2, 3 and 4 are positions of heterodimers, whereas band positions 1, 3 and 5 are the locations of homodimers (Table 5). Several alternative genotypic interpretations exist for the observed GOT polymorphisms. Table 5 reports one of these.

The LDH enzyme is also a dimer which forms 2- and 3-banded phenotypes in heterozygotes (Fig. 9, Table 6), but here there is the additional complication of minor LDH band activity on clearer zymograms. For convenience of presentation these are illustrated in Fig. 9 for LDH3 and LDH4 phenotypes only. Minor band activity did not appear to be tissue specific (leg and heart muscle and haemolymph were tested separately), and I assume here that these represent LDH subunits (polypeptides) which have some catalytic activity of their own and which are split into two groups *in vitro* by presence or absence of some attached charge modulator. Because their minor bands have different electrophoretic mobilities, *M. segmentata* LDH4 is an allozyme of *M. menardi* LDH4 (i.e. they are composed of different polypeptides). Alternatively, the major and minor LDH band systems may correspond to two of the three *Limulus* LDH loci identified by Selander *et al.* (1970).

The majority of PGI assays yielded deeply stained streaks rather than sharp bands, and after a while this assay was discontinued. PGI is also a dimer (Fig. 10, Table 7). Figure 10 also illustrates the relative band positions of α-GPDH, which appeared to be monomorphic in the genus.

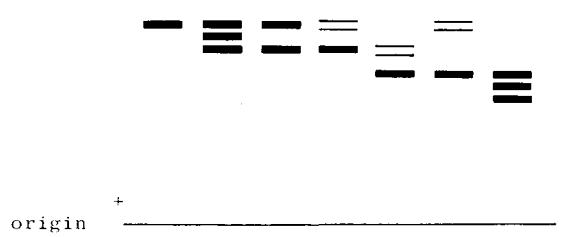

Fig. 9: Lactate dehydrogenase (LDH) phenotypes observed in *Meta*. The thick bands represent the major LDH locus observed in every individual, and the thin bands represent the minor LDH activity observed on many well-resolved zymograms. Minor band positions are illustrated for three phenotypes only, to indicate their relative positions. The genetic interpretation of the minor bands is uncertain. From left to right the phenotypes are: LDH1; LDH123; LDH13; LDH3 (with minor bands); LDH4 (of *M. segmentata* with minor bands); LDH4 (of *M. menardi* with minor bands); LDH456. These phenotypes appear in the same order in Table 6.

Discussion

The practical applications of the procedures summarised in Fig. 5 are limited in this genus to identify-

Fig. 10: α-glycerophosphate dehydrogenase (α-GPDH) and phosphoglucoisomerase (PGI) phenotypes observed in *Meta*. α-GPDH was monomorphic in all species. From left to right the PGI phenotypes are: PGI1; PGI13; PGI123; PGI3. These phenotypes appear in the same order in Table 7.

LDH phenotype	1	123	13	3	4	4	456	N
Genotype	aa	ab	be	bb	cc	dd	cf	
M. merianae	0.008	0.119	–	0.87	–	–	–	243
M. mengei	–	–	0.003	0.995	–	–	–	383
M. menardi	–	–	–	–	–	1.0	–	206
M. segmentata	–	–	–	–	0.987	–	0.013	225

Table 6: LDH phenotype frequencies and assumed genotypes.

ing the more troublesome instars of the sibling species *M. segmentata* and *M. mengei*. Adult males and the smallest instars of these species, and all *M. merianae* and *M. menardi* instars, are quite distinctive once one has learned to recognise their characteristic features (colour pattern in juveniles). I do not hold that diagnostic loci should supplant conventional diagnostic characters where they exist; indeed, the cost and time involved in this method make it comparatively unattractive. But there must be many groups of taxonomically ill-defined varieties, subspecies and species of spiders (and of many other organisms) which could bear critical re-examination by defining species foremost as genetical processes, i.e. as panmictic populations in which the Hardy-Weinberg Law holds in respect of polymorphic loci. There must also be other examples of life history studies which would be greatly facilitated by the approach outlined here.

Acknowledgement

I am very grateful to Dr N. P. Ashmole for his support and many helpful suggestions during the preparation of the various manuscripts leading up to this paper.

References

AVISE, J. C. 1976: Genetic differentiation during speciation. *In* F. J. Ayala (ed.), *Molecular evolution*: 106-122. Sinauer Associates, Inc., Sunderland, Mass.

AYALA, F. J. & POWELL, J. R. 1972: Allozymes as diagnostic characters of sibling species of *Drosophila*. *Proc. natn.Acad.Sci.U.S.A.* **69**: 1094-1096.

BATHGATE, M. C. 1978: *Variations in natural populations of the Bank Vole,* Clethrionomys glareolus *Shreber* 1878. Ph.D. Thesis, University of Edinburgh.

CHRYSANTHUS, Fr. 1953: Is *Meta mengei* (Blackwall) a variety of *Meta segmentata* (Clerck)? *Zoöl.Meded. Leiden* **32**: 155-163.

FALCONER, D. S. 1960: *Introduction to Quantitative Genetics.* 1-365. Oliver & Boyd, Edinburgh.

JOHNSON, G. B. 1977: Characterisation of electrophoretically cryptic variation in the alpine butterfly *Colias meaddi*. *Biochem.Genet.* **15**: 665-694.

JOHNSTON, F. M. & DENNISTON, C. 1964: Genetic variation of ADH in *Drosophila melanogaster*. *Nature, Lond.* **204**: 906-907.

LEWONTIN, R. C. 1974: *The genetic basis of evolutionary change.* Columbia University Press (New York & London).

LOCKET, G. H. & MILLIDGE, A. F. 1953: *British Spiders* **2**: 1-449. Ray Society, London.

LOCKET, G. H., MILLIDGE, A. F. & MERRETT, P. 1974: *British Spiders* **3**: Ray Society, London.

NEI, M. 1972: Genetic distance between populations. *Am.Nat.* **106**: 283-292.

PENNINGTON, B. J. 1977: *Ecological and biochemical adaptation in four coexisting species of the spider genus* Meta *(Tetragnathidae).* Ph.D. Thesis, University of Edinburgh.

PENNINGTON, B. J. 1979: The colour patterns of diurnal *Meta menardi* (Latreille). *Bull.Br.arachnol.Soc.* **4**(9): 392-393.

PGI phenotype	1	13	123	3	3	N
Genotype	aa	ac	ab	bb	cc	
M. mengei	–	–	–	1.0	–	80
M. segmentata	–	–	0.018	0.982	–	57
M. merianae	0.059	–	0.118	0.795	–	34
M. menardi	0.92	0.074	–	–	0.008	122

Table 7: PGI phenotype frequencies and assumed genotypes.

PRAKASH, S., LEWONTIN, R. C. & HUBBY, J. L. 1969: A molecular approach to the study of genetic heterozygosity in natural populations. IV Patterns of genic variation in central, marginal, and isolated populations of *Drosophila pseudoobscura*. *Genetics, Princeton* **71**: 733-746.

SELANDER, R. K., YANG, S. Y., LEWONTIN, R. C. & JOHNSON, W. E. 1970: Genetic variation in the horseshoe crab (*Limulus polyphemus*), a phylogenetic "relic". *Evolution, Lancaster, Pa.* **24**: 402-414.

SMITH, I. 1968: Chromatographic and electrophoretic techniques **2**: Zone electrophoresis. Heineman.

SMITHIES, O. 1955: Zone electrophoresis in starch gels. Group variations in the serum proteins of normal human adults. *Biochem.J.* **61**: 629-641.

SNEATH, P. H. & SOKAL, R. R. 1973: *Numerical Taxonomy*. W. H. Freeman & Co.

WEBSTER, T. P. & BURNS, J. M. 1973: Dewlap colour variation and electrophoretically detected sibling species in a Haitian lizard *Anolis brevirostris*. *Evolution, Lancaster, Pa.* **27**: 368-377.

WHITEHOUSE, H. L. K. 1969: *Towards an understanding of the mechanism of heredity*. 1-447. Edward Arnold, London.

Bull.Br.arachnol.Soc. (1979) **4** (9), 392-393

The colour patterns of diurnal *Meta menardi* (Latreille)

Brian J. Pennington
Department of Zoology,
West Mains Road
Edinburgh EH9 3JT*

I wish to draw attention to the unusual colour patterns and diurnal habits of young *Meta menardi* (Latreille) in the vicinity of Loch Sween in Argyll. The possible (though unlikely) uniqueness of this population was referred to in the preceding paper of this journal (Pennington, 1979). A fuller description of the study area will receive attention elsewhere.

This orb-web spider is generally regarded as a cave-dwelling species which also inhabits man-made 'caves' such as cellars and disused well-shafts. In the absence of more spacious light-free situations, however, the chosen web sites of adult *M. menardi* in this locality are scattered among many small underground chambers formed between large, partially buried rocks which are to a large extent roofed over by a thick carpet of *Luzula sylvatica* (Hudson) Gaudin on the steeper, more shaded oak-wooded shores of the loch. None of these 'microcaves' extends more than a few metres from the field layer, but even so, intermediate age classes of the population largely remain hidden from the observer on the surface. However, adult *M. menardi* can be observed at night just inside the narrow entrances of microcaves, particularly in May and June when the males actively court the females. They are shy of illumination by torch-light and they retire to the darkness of the microcave interiors during the hours of daylight.

When they leave their eggsacs in late April, 2nd instar *M. menardi* journey from the microcaves into the field layer itself (*Meta* species moult once in the eggsac and mature in the 7th instar). There they disperse widely, and spin webs and feed during the day for a period of 2-3 months, living alongside populations of *M. mengei* (Blackwall), *M. segmentata* (Clerk), and juvenile *M. merianae* (Scopoli). For a short time they outnumber these species. At the end of the 3rd instar they abruptly vanish from the field layer, returning to microcaves to complete development.

Larger instars and adults bear only faint brown dorsal markings, a series of forward-pointing chevrons which stand against the shiny black abdominal background, but during these first two free-living instars the juveniles carry distinctive, contrasty black and white dorsal patterns (Fig. 1). At the anterior hump of the abdomen two large black spots join in a trans-

*Address for correspondence:
14 Riverside Road,
Wormit, Newport-on-Tay,
Fife, Scotland