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The application of molecular markers in the study of diversity in acarology: a review

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Abstract. The application of molecular markers to the study of ticks and mites has recently yielded new insights into their population structures and taxonomic relationships. Ticks have been studied at individual, population and species level. Mites are a more diverse group and those that have been studied to the same degree as the ticks include the Tetranychidae (spider mites), Phytoseiidae (predatory mites) and the Eriophyidae. Population variation has also been studied in the important bee parasitic mite *Varroa jacobsoni* Oudemans. The methods used to study these organisms have much in common. At the individual level these range from general approaches, such as AFLP, RAPD or DALP, to highly specific microsatellite analysis. Although these markers also work at the population and species level, additional analysis of specific nuclear or mitochondrial genes has been conducted either by RFLP or sequencing. Molecular applications have had particular success in facilitating the identification of taxonomically difficult species, understanding population structures and elucidating phylogenetic relationships.

Key words: AFLP, allozymes, DALP, DNA sequencing, genetic structure, microsatellites, mitochondrial DNA, mites, molecular systematics, PCR, phylogeny, RAPD, RFLP, ribosomal DNA, ticks

Introduction

Describing, quantifying and classifying diversity is a long-standing task for biologists. Morphological description has been widely used for such purposes in acarology and has given rise to much successful research. The techniques involved generally use direct observation of phenotypic differences between organisms. Studies of tick and mite chromosomes have led to an interesting hypothesis that ticks and mites have distinct origins (Oliver, 1977), an observation that is a suitable challenge for a molecular phylogenetic analysis. Other studies of the total genome of ticks include analysis of the base composition of *Amblyomma americanum* (L.) and the estimate of the genome size

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as 1.04×10^9 nucleotide base pairs (Palmer *et al*., 1994). Of this 35.8% was unique sequence and most of the rest (60%) was either moderately or highly repetitive.

Towards the end of the 20th Century the advent of molecular techniques has generated the potential to investigate DNA at the individual base-pair. This is clearly a much more direct way of measuring and quantifying the genetic variation within and between species. This review is focused on the recent applications of molecular markers for describing genetic diversity at both inter- and intra-specific levels in the Acari. The molecular biological approach has been particularly undertaken in Acari of economic interest in the agricultural, medical and veterinary sciences. Among the phytophagous mites, the families Tetranychidae and Eriophyidae have been central to this type of DNA-based research. The molecular biology approach is, however, of increasing importance in two other economically important taxa, the Varroidae and the Phytoseiidae. In medical and veterinary sciences knowledge of the genetic diversity of the Ixodidae has been considerably enhanced by the application of molecular techniques. This review will mostly focus on these diverse groups. It will describe different biochemical and molecular techniques used as well as the principal genome regions studied. Following this a general survey of the results obtained in this field is presented.

Biochemical and Molecular Techniques Used in Genetics of the Acari

The development of markers is a prerequisite for studies of genetics. Since the appearance of one of the first compilations of molecular biology techniques applied to insects and mites (Hoy, 1994), the panel of available methodologies has substantially increased. We describe here techniques that are currently being used, or are of potential, in detecting genetic polymorphism (Table 1). These techniques can be further used in studies of molecular systematics and evolutionary genetics (see Hillis *et al*. (1996) for an overview of DNA techniques and data analysis applied to molecular systematics).

Allozymes

Protein electrophoresis has been an effective technique for the detection of genetic polymorphism for over three decades (see Pasteur *et al*. (1988) for detailed information of methods). Enzymatic polymorphism detected by electrophoresis has been widely used on ticks and mites and an exhaustive review is not provided here. The most commonly studied enzymatic system in mites is that of the esterases (for example Sula and Weyda, 1983; Gotoh and Takayama, 1992; Osakabe and Komazaki, 1996). Multilocus studies

Table 1. Common molecular techniques useful for evolutionary studies, and selected examples in the Acari

Research question	Techniques	Advantages	Drawbacks	Examples
Phylogenetic relationships (family, genus)	rDNA sequencing (genes)	Suited to resolve “deep phylogenies” Conserved sequences for PCR primer annealing. Multi-copy genes therefore many targets for PCR	Less informative for resolving recent divergences	Black *et al.*, 1997 Klompen *et al.*, 2000 Dobson and Barker, 1999
	mtDNA sequencing	Conserved regions facilitate primer design for PCR. Multiple copies in each cell, therefore many targets for PCR	Saturation of the mutations due to high rate of evolution	Navajas *et al.*, 1999 Black and Piesman, 1994 Crosbie *et al.*, 1998
Phylogenetic relationships (species, populations)	rDNA sequencing (Internal Transcribes Spacers)	Small regions which lie between conserved genes useful for PCR primers Highly variable	Sequence alignments may be difficult. Incomplete homogenisation can mean that there is intra-individual variation producing ambiguous sequences	Zahler *et al.*, 1995 Fenton *et al.*, 2000
	mtDNA sequencing	Strict maternal inheritance No recombination Population structure can be defined at the geographical level Introduction traced	Only the maternal lineages are traced Ideally use both mitochondrial and rDNA	Anderson and Trueman, 2000 Navajas *et al.*, 1998

Table 1. continued

Research question	Techniques	Advantages	Drawbacks	Examples
Population studies (populations, individuals)	PCR-RFLPs	Relatively inexpensive and simple Also useful in species diagnostics	Low variation	Gotoh *et al.*, 1998 Fenton *et al.*, 1995
	Microsatellites	Highly polymorphic Loci easily scored Used for mapping genes	Expensive and technically difficult to isolate Mostly species-specific loci	Delaye *et al.*, 2000
	DALPs	Co-dominant markers Only polymorphic loci are isolated Unlimited number of markers No prior genetic information required	Species-specific loci	Perrot-Minnot *et al.*, 2000a Perrot-Minnot *et al.*, 2000b
	AFLPs	No prior genetic information required Unlimited number of markers Used for mapping genes	Dominant marker, heterozygotes can't be detected	Weeks *et al.*, 2000
	RAPDs	Relatively inexpensive and simple No prior genetic information required Unlimited number of markers	Dominant marker, heterozygotes can't be detected. Great care required for repeatability	Hemandez *et al.*, 1998 de Guzman *et al.*, 1997, 1999 Edwards *et al.*, 1998
	Allozymes	Inexpensive and simple Protocols available to detect many enzymes	Low variation Large quantities of material required Low number of loci detected in small animals	Dunley and Croft, 1994 Kain *et al.*, 1997

have been used to study the dispersion of the phytoseiid *Typhlodromus pyri* Sheuten (Dunley and Croft, 1994), to trace the origin of a major pasture pest *Halotydeus destructor* (Tucker) from its putative source in South Africa to Australia (Quin, 1997), for species identification in the Oribatida (Avanzati *et al*., 1994) and to study the population structure of the spider mite *Tetranychus urticae* Koch in glasshouses (Tsagkarakou *et al*., 1999) and in open fields (Tsagkarakou *et al*., 1997, 1998). In ticks, several enzymatic systems can be resolved from single individuals, however, diverse studies have reported low polymorphism of the resolved loci (Delaye *et al*., 1997; Kain *et al*., 1997).

Although protein electrophoresis is still a useful method for studying genetic polymorphism (Lewontin, 1991), it has important limitations: (1) the biological material must be kept alive or be frozen until used, and (2) at a given locus the technique reveals only a fraction of the actual genetic variation. Moreover, the small size of many mite species seriously limits the number of enzyme systems that can be detected per individual. Modification for increased efficiency and for work specifically with small organisms using isoelectric focusing on cellulose acetate membranes has been described by Kazmer *et al*. (1991) and adapted for *T. urticae* by Tsagkarakou *et al*. (1996), making it possible to reveal up to five loci per individual in a single run. The technique has been applied to investigate the persistence of a predatory mite *Neoseiulus fallacis* (Garman) introduced in apple orchards during biological control programs in Canada (Navajas *et al*., 2000b).

Direct analysis of proteins

Highly sensitive techniques for separating proteins, such as two-dimensional electrophoresis (O'Farrell, 1975), have not yet been widely applied to study ticks and mites. However, there has been some use of SDS-PAGE in combination with general protein staining to investigate quantitative differences in proteins between organophosphate resistant and sensitive strains of *Boophilus microplus* (Canestrini) (Rosario-Cruz *et al*., 1997).

Amplification of DNA using PCR

Allozyme data are now increasingly replaced by several types of DNA-based data. The advent of the polymerase chain reaction (PCR) (Mullis and Faloona, 1987; Saiki *et al*., 1988) made it possible to apply the DNA approach to small animals because only tiny amounts of initial material are necessary. Thus, most of the current techniques used to examine nucleotide variations are based on PCR to amplify sufficient quantities of DNA. Starting from DNA extracted by classic techniques (see Hubbard *et al*. (1995) for ticks pre-

served in alcohol), PCR uses the enzyme *Taq* DNA polymerase to synthesise a complementary DNA sequence from a single stranded DNA oligonucleotide (primer) hybridised to a specific part (target) of the DNA strand. A PCR machine (thermocycler) applies successive temperature cycles (typically 30–50 cycles) that include a denaturation cycle (typically 94°C) followed by an annealing stage (typically 40–60°C, but see also the 'touchdown' procedure (Don and Cox, 1991)) followed by a final extension stage under the effect of the polymerase (typically 72°C). The DNA fragment flanked by the two primers is thus duplicated exponentially to produce sufficient DNA quantities for further analytical procedures. Among the different techniques available, we describe those that are already used or are potentially of use in Acari.

Random amplified polymorphic DNA (RAPD)

The RAPD technique uses the PCR principle for random amplification of DNA sequences (Williams *et al*., 1990). Amplification is performed using a single primer with a very short sequence (8–10 base pairs) under temperature conditions (usually low) enhancing multiple binding at sites scattered throughout the genome. DNA molecules generated from the DNA of different individuals are then separated on an agarose gel matrix. Several DNA fragments are usually amplified and some of these may be present in a proportion of the individuals in a population. A large set of primers has the advantage of screening the entire genome. However, the interpretation of RAPD data is sometimes limited by poor repeatability of the results (Black, 1993), with the problem aggravated in the case of small species in which the quantity of DNA obtained per individual is reduced, thus preventing accurate assays of DNA concentration. Another limit of these markers is that the RAPD patterns display dominance, preventing identification of heterozygotes. Nevertheless, the RAPD technique is often used as the first source of information because results are generated quickly and easily. By cloning and sequencing RAPD products it is also possible to design new more-specific primers which will be more reliable (see Yli-Mattila *et al*., 2000). In addition, if the necessary precautions of verification of the heritability of the patterns obtained by RAPD are taken, the technique can be used successfully for the analysis of pedigrees and paternity exclusion (see Black (1993) for a review). Using the transmission patterns of RAPD markers Perrot-Minnot and Navajas (1995) showed that genetic material is transmitted from father to son in controlled crosses in a pseudo-arrhenotokous phytoseiid mite. Besides, the RAPD approach has been used to compare strains of *Tetranychus* sp. as a complement of fecundity and biometry studies (Hence *et al*., 1998). In ticks, Hernandez *et al*. (1998) used 120 RAPD primers to develop genomic probes for detection of acaricide

resistence in the cattle tick *B. microplus* and detected profiles differentiating susceptible and resistant strains.

Restriction fragment length polymorphism (RFLP)

Genome DNA regions isolated by PCR or by other means can be digested by restriction enzymes that generate RFLP patterns. This technique was used to differentiate between species of the genus *Panonychus* (Tetranychidae) (Osakabe and Sakagami, 1994) and to detect genomic variability in different populations of *B. microplus* (Passos *et al*., 1999). RFLP patterns of the products of PCR are also used for species diagnosis purposes as described below.

Microsatellites

Microsatellites are defined as short DNA fragments (≈100 bp) containing patterns with two to six base-pairs repeated in tandem. During DNA replication, repetition units are added to, or lost from, the microsatellite, causing the rapid evolution of these regions. There is generally a high variability in the number of repetitions at a given locus. This makes a large number of alleles per microsatellite locus available for population analysis (see Goldstein and Schlötterer (1999) for a recent review). These are co-dominant markers and the substantial polymorphism of these loci can be detected in a simple manner by measuring the size of the PCR-amplified fragments. Microsatellite loci are available for an increasing number of organisms and this new type of data has greatly stimulated the development of powerful statistical methods and computer programs for analyzing allele frequency data (Luikart and England, 1999). Microsatellites are indeed excellent for studies of population genetics (Jarne and Lagoda, 1996; Goldstein and Schlötterer, 1999).

The isolation of microsatellite loci involves the construction of DNA genomic libraries that are then screened with microsatellite-type repeated sequence probes (Estoup *et al*., 1993). Once a locus has been identified it is sequenced and primers are designed for the unique microsatellite flanking regions. This step can be omitted if primers known to work in one species amplify microsatellite regions in related species. Whereas microsatellite sequences were considered to be present in all the eukaryote genomes (Hamada and Kakunaga, 1982), Navajas *et al*. (1998) showed that they are extremely rare in two screened mite species (*T. urticae* and *N. fallacis*). Efforts have continued in the search for microsatellites for *T. urticae*, for which a library enriched in microsatellite sequences (Edwards *et al*., 1996) has been made, facilitating the isolation of several polymorphic microsatellites in mites (Navajas, unpublished data). Also the European tick, *Ixodes ricinus* (L.), has

been screened for microsatellite loci. Six polymorphic loci have been detected, including PCR primers hybridising with other members of the *I. ricinus* complex (Delaye *et al*., 1998). In a recent publication Evans (2000) provides information on nine microsatellite loci in the honey bee parasitic mite *Varroa jacobsoni* oudemans.

Direct amplification of length polymorphism (DALP)

The DALP method involves the use of random PCR primers to generate multi-band genome DNA patterns and enables the sequencing of polymorphic bands. PCR-specific bands are then defined and enable the study of distinct loci (Desmarais *et al*., 1998). This strategy combines the advantage of *a priori* definition of polymorphism and the reproducibility of the results by means of the use of specific primers. This new method has been applied to isolate five polymorphic loci in the phytoseiid mite *Neoseiulus californicus* (McGregor) as presented by Perrot-Minnot *et al*. (2000b). The Mendelian transmission of alleles and their co-dominance were verified, thus making DALP markers useful for genetic studies. The DALP technique has recently been used to study the pseudo-arrhenotokous reproduction system in *N. californicus*. In this study, the transmission patterns of parental alleles through two generations are reported at five loci, providing evidence for the retention in the male somatic tissues of most if not all the paternal chromosomes (Perrot-Minnot *et al*., 2000a).

Amplified fragment length polymorphim (AFLP)

The AFLP technique selects, by PCR amplification, restriction fragments generated from a total digest of genomic DNA (Vos *et al*., 1995). Using this method, sets of restriction fragments can be visualised by PCR without prior knowledge of the genome of the target species. As for other fingerprinting based methods, such as DALP or RAPD, it gives access to an almost unlimited number of genetic markers. However, in the AFLP technique heterozygote genotypes cannot be distinguished from homozygote genotypes and, as indicated previously for RAPD, this limits the use of these markers for population genetic studies. The AFLP method has been used in mites for the first time by Weeks *et al*. (2000).

Sequencing of DNA fragments

The DNA fragment amplified by any PCR can be quickly analysed for polymorphism either in the cleavage profile of restriction enzymes (RFLP), or sequenced by techniques that have now become routine and economical.

Obtaining the nucleotide sequence provides access to the ultimate detail of variation in the DNA. DNA sequences are collated and stored in data libraries (e.g. EMBL and GenBank) and are universally usable, powerful data. These also provide search facilities which allows unknown sequences to be identified by similarity search engines such as BLAST (Karlin and Altschul, 1993). Sequencing of the product can either be done directly (Navajas *et al*., 1998a) or after cloning into plasmid vectors (Fenton *et al*., 1997). The latter technique is more expensive, but is particularly important when investigating the extent of intra-specific variation in ribosomal genes (see molecular drive below). DNA sequences are used to assess the divergence between taxa or individuals and also enable the reconstruction of the phylogenetic relations between the taxonomic entities studied. The inference of phylogenies from molecular data requires the selection of the appropriate method from the many techniques that have been described (Swofford *et al*., 1996). The authors provide a comprehensive description of the analytical methods and summarise the different programs and software packages available for conducting phylogenetic and population analysis. In addition, several programs are devoted to multiple nucleotide sequences alignment, among these one of the most popular is Clustal W (Thompson *et al*., 1994). Alignment of sequences may be difficult because of length variation resulting from insertions/deletions in the nucleotide sequences. Although in the case of ribosomal sequences, the analysis of secondary structure to identify homologous positions may improve alignments (Kjer, 1995), structures may appear conserved irrespective of phylogenetic associations (Hancock and Vogler, 2000).

Different genomic regions can be analysed depending on the problem examined. In particular, these regions may have different rates of evolution and/or different modes of inheritance (maternal vs Mendelian). Rapidly evolving genes are useful for comparisons of closely related taxa and slowly evolving genes are useful for comparisons of distantly related taxa. Two of the most popular markers used in molecular evolution are mitochondrial DNA (mtDNA) and nuclear ribosomal DNA (rDNA). Information for Acari on both genomic regions is beginning to accumulate in the gene data-bases. The impact that this and related data has, or should have, in acarology is discussed next.

Studies of Genome Characterisation

Mitochondrial DNA

The molecular biology and the patterns of evolution of animal mtDNA are well understood (Wolstenholme, 1992; Avise, 1994; Simon *et al*., 1994). The

mitochondrial genome of two ticks, *Ixodes hexagonus* Leach and *Rhipicephalus sanguineus* Latreille, has been sequenced entirely (Black and Roehrdanz, 1998). The gene arrangements of these two species, together with that of *B. microplus* (Campbell and Barker, 1999), have been established. The mtDNA of the spider mite *T. urticae* has also been characterised by a restriction map (Fournier *et al*., 1994). A study of 20 mite species belonging to the Tetranychidae and Tenuipalpidae shows that the characteristics and mode of evolution of the mtDNA are similar to those known for insects (genetic code, use of codons, base composition), and this is probably the result of ancestral characters shared between the two arthropod classes (Navajas *et al*., 1996). As in insects, the mitochondrial sequences of mites are extremely A+T rich (average 75%). However, variation in base composition has been shown between species within the same family (Navajas *et al*., 1996). This has important implications for the construction of phylogenies and requires the development of specific methods which take this type of variation into account (Galtier and Gouy, 1995). Several genes of the mitochondrial genome are increasingly used to assess phylogenetic relationships among animal taxa (see Simon *et al*. (1994) for a review). Evolutionary studies on mites have mainly surveyed the mitochondrial Cytochrome Oxidase subunit I gene (COI), whereas in studies on ticks mitochondrial ribosomal 16S is most popular.

Nuclear ribosomal DNA

Nuclear ribosomal DNA still provides one of the most complete tools for a multitude of molecular tasks. The main ribosomal locus in eukaryotic organisms consists of three genes encoding the 18S, 5.8S and 28S subunits of the ribosome. Between these genes are the internal transcribed spacers 1 (ITS1, between the 18S and 5.8S gene) and 2 (ITS2, between the 5.8S and 28S gene) (Hillis and Dixon, 1991). The three genes are reiterated in tandem and between each group lies the intergenic spacer. There may be more than one hundred copies of the ribosomal genes on a chromosome and they may be found on more than one chromosome (Wen *et al*., 1974). Reiteration is essentially a pre-amplification step which makes detecting these genes considerably easier than for single copy genes.

In the conserved ribosomal genes, PCR primers can be defined that work in a wide range of species (Kaliszewski *et al*., 1992). Sequences of the amplified fragments are useful for comparison of phylogenetically distant taxa. To complement this, the ITS regions are very useful for distinguishing between closely related taxa as they evolve more rapidly than the coding regions (Hillis and Dixon, 1991). To characterize the ITS regions it is possible to design PCR primers defined in the more conserved genes (28S, 5.8S and 18S) flanking the ITS. While these gene sequences provide primer sites for use in amplifying

ITS regions from many arthropods, including several species of ticks (Zahler *et al*., 1997; Fukunaga *et al*., 2000) and different groups of mites such as Psoroptidae (Zahler *et al*., 1995b, 1998; Essig *et al*., 1999), Sarcoptidae (Zahler *et al*., 1999), Eriophyidae (Fenton *et al*., 1993), Tetranychidae (Navajas *et al*., 1992) and Phytoseiidae (Navajas *et al*., 1999b), it is still possible for small changes in one or two bases to prevent amplification, as has been found in phytoseiids (Yli-Mattila *et al*., 2000) and eriophyids (Fenton *et al*., 1997). Once an ITS region has been successfully amplified it can then be analyzed by additional techniques.

The repetitive nature of ribosomal genes results in copies of ITS regions having the potential to vary within and between individuals. This can give rise to intra-specific diversity of ITS sequences, as has been found in some *Ixodes* ticks (McLain *et al*., 1995; Rich *et al*., 1997). However, this tendency to diversify is countered by a process known as molecular drive which ensures that the different copies of rDNA are the same (Dover, 1982; Polanco *et al*., 1998). In the phytophagous mites examined so far, this process appears to be very effective as they exhibit very low levels of intra-specific polymorphism (Fenton *et al*., 1997; Navajas *et al*., 1998a). Thus, in the phytophagous mites these regions have no problems associated with intra-individual diversity and can probably be widely applied.

It is a problem with many DNA techniques that a newly isolated marker could have come from a contamination event or a symbiotic organism and this is particularly the case with PCR. If DNA sequences are being investigated then it may be possible to use conserved regions to investigate the source of the sequence. The rDNA ITS regions are flanked by the 18S, 5.8S and 28S genes. These gene regions are evolving slowly and they contain phylogenetic information which can be used to search a sequence database for matches. Such a search will reveal sequences belonging to the taxon closest to the isolated sequence and this then helps confirm the source. A search with an Acarine sequence might identify another Acarine sequence. However, such sequences are still not very frequent and it is more likely that the conserved parts of the ribosomal genes will match other arthropod sequences. The methodology also facilitates the elimination of contaminating sequences. For example, fungal sequences have been found in aphids (Fenton *et al*., 1994) and more recently in mites (Yli-Mattila *et al*., 2000).

Other nuclear genes

The general characterisation of genes in the Acari is still in its infancy. However, two genes have been isolated and analysed from the cattle tick *B. microplus*. These genes encode the ecto-5′-nuclease and the octopamine-like G-protein receptor (Liyou *et al*., 1999; Baxter and Barker, 1999). The ge-

nomic sequence of the first gene contained no significant introns. It is not clear yet if this will be generally applicable to the Acari. Introns have proved useful in providing intra-specific markers in other organisms. The octopamine receptor gene was sequenced in amitraz-resistant and sensitive strains and no differences were found. This suggested that the resistance was not based on mutational differences in this gene. A third gene, the elongation factor-1α, has now proved to have potential for phylogenetic analysis of the Mesostigmata (Klompen, 2000).

In a fundamental study of mite developmental biology conducted by Telford and Thomas (1998), an oribatid mite was used to understand the evolution of the homologous zerknült (zen) gene of insects and the Hox 3 genes of vertebrates.

Overview of DNA Data

Phylogeny

Our knowledge of the phylogenetic relationships between organisms has recently greatly expanded through analysis of molecular data. DNA based phylogenies have been used to examine major taxonomic levels (mainly Ixodidae and Tetranychidae) that have challenged the established views of taxa relationships.

In the Ixodidade, exhaustive phylogenetic analysis based on sequences of the mitochondrial 16S rDNA (Black and Piesman, 1994; Norris *et al*., 1999), variable regions of the 18S (V4) and 28S (D1) ribosomal genes (Crampton *et al*., 1996) and the entire 18S region (Black *et al*., 1997; Dobson and Barker, 1999) covers several aspects of the evolution of the group. In a global approach that integrates nuclear and mitochondrial rDNA and morphological data sets, Klompen *et al*. (2000) present an evolutionary scenario of the relationships among tick genera and subfamilies. At a lower taxonomic level, the mitochondrial 16S sequences were used to examine the phylogenetic relationships in the genus *Dermacentor* (Crosbie *et al*., 1998) and *Rhipicephalus* (Mangold *et al*., 1998). In a recent review mainly devoted to the molecular detection of pathogen DNA in ticks, Sapagano *et al*. (1999) present a compilation of the published PCR primers used to amplified ixodid DNA.

In a comprehensive study of *V. jacobsoni* Anderson and Trueman (2000) used phenotypic and reproductive variation together with COI sequence polymorphism to examine populations collected from bees distributed throughout Asia. They demonstrated that this ectoparasitic mite is a complex of at least two different species. The authors suggest the creation of a new species, *V. destructor* n. sp. which is mostly applicable to findings of past research on *V. jacobsoni*.

Using a fragment of the COI in 20 species of phytophagous mites from nine genera and two families (Tetranychidae and Tenuipalpidae) to estimate phylogenetic relationships between taxa, Navajas *et al.* (1996) showed that molecular and morphological classifications are compatible as a whole, but some minor taxonomic revisions are called for. The data suggest, for example, that the genus *Oligonychus* is polyphyletic and enables better appraisal of the different morphological characters conventionally used in systematics.

In the case of plant-feeding mites phylogenetic relationships have been compared to molecular relationships of their host plants. In a recent study the molecular phylogeny of the highly host-specific phytophagous *Cecidophyopsis* gall-mite species was compared to that of their host plants. This found that the mite species had only very recently diverged onto their specialist hosts whereas the plants themselves had been separated considerably longer. This indicates that the host–plant interactions of these mites are not based on extended periods of co-evolution. The mites were related according to the physiological alterations they impose on the plant, i.e., mites which did not induce galls were very closely related, despite the fact that they had hosts that had been separated for millions of years (Fenton *et al.*, 2000).

A phylogenetic analysis of a portion of the 28S rDNA from a group of dermanyssine mites that contains arrhenotokous, peudo-arrhenotokous and ancestrally diplodiploid members was used to shed light on the evolutionary origins of haplodiploidy in this group of organisms (Cruickshank and Thomas, 1999).

Phylogeography and the genetic structure of the species

Distinct genotypes of *V. jacobsoni* have been described based on RAPD markers (Kraus and Hunt, 1995; de Guzman *et al.*, 1999) and mitochondrial COI data (Anderson and Fuchs, 1998; de Guzman *et al.*, 1998) and their geographic distribution world-wide assessed (see de Guzman and Rinderer (1999) for a review). Work on COI sequences has shown the genetic structuring of the species *T. urticae* throughout its distribution area. The COI sequences have made it possible to make a distinction between two lineages, and the results suggest on the one hand ancient colonisation of the Mediterranean region and on the other recent colonisation of the temperate regions in the northern hemisphere (Navajas *et al.*, 1998a). The phylogenetic information obtained for mitochondrial sequences of COI has not revealed divergences that can be correlated with ecological factors such as host plant or colour of *T. urticae* (Navajas, 1998), which in the past have led to subdivisions into subspecies or races (Gotoh *et al.*, 1993). A survey of allozymes together with ribosomal ITS2 sequence variation in populations of *T. urticae* originating from the Mediterranean basin allowed an evaluation of the role of the

host plant and the geographical distance in the genetic differentiation process (Navajas *et al*., 2000c). Such processes potentially lead to speciation.

In addition to the COI gene, the Cytochrome Oxidase III (COIII) gene has been used to assess population structure as demonstrated by the work by Kain *et al.* (1999) on populations of the tick *I. pacificus* from the USA. In a study of the tick *Ixodes scapularis* Say throughout its range, Norris *et al*. (1996) adopted a novel strategy to detect genetic variation that involves the use of single strand conformation polymorphism (SSCP) analysis. Using the SSCP approach to detect variation in a region of the 16S mitochondrial ribosomal DNA, Norris *et al*. (1996) estimated the frequency of haplotypes in various regions of the United States and determined phylogenetic relationships among these haplotypes.

Species discrimination

The small size of most mite species implies that a limited number of morphological characters are available for systematics. In addition, it is common that intra-specific variability of these characters complicates the determination of taxa. Molecular markers can help to discriminate between species that are morphologically very close, as has been reported for the two sibling species *Tetranychus pueraricola* Ehara and Gotoh and *T. urticae* (Ehara and Gotoh, 1996). Differences between the sequences of ITS2 of the two species consolidated the sibling species status and contributed to their unambiguous identification (Gotoh *et al*., 1998). The ITS2 sequence variation together with a cross-breeding experiment have also been used to establish the synonymy between two spider mite species: *Tetranychus kanzawai* Kishida and *T. hydrangeae* Pritchard and Baker (Navajas *et al*., 2000a). Edwards *et al.* (1998) examined variable RAPD markers in three *Typhlodromalus* mites and found several bands that could be used individually to distinguish phytoseiid species.

Evidence of the reproductive isolation of *Dermacentor marginatus* (Sulcer) and *D. reticulatus* (Fabricius) was supported by ribosomal ITS2 sequences as a complement to cross-breeding and morphological studies (Zahler *et al*., 1995a; Zahler and Gothe, 1997). These results were corroborated by a comparative study of inter- and intra-specific polymorphism of both tick species that exhibit overlapping phenotypes (Zahler *et al*., 1995b). The same ribosomal region was used to establish the taxonomic status of species belonging to the *Rhipicephalus sangineus* group (Zahler *et al*., 1997) and the taxonomic status of two ticks, *Ixodes neotomae* Cooley and *I. spinipalpis* Hadwen and Nuttall (Norris *et al*., 1997). Recently a molecular key has been described for 17 *Ixodes* species in the United States (Poucher, 1999).

In the Oribatida, analysis of a fragment of the mitochondrial COI gene served to establish the taxonomic status (synonymy) of *Steganacarus magnus* (Nicolet) and *S. anomalus* (Berlese) (Salomone *et al*., 1996). A COI fragment was also used to distinguish between four species of *Tetranychus* mites involved in quarantine problems associated with apple imports in North America (Lee and Lee, 1997).

For eriophyid mites the problems associated with identification are particularly acute (Boczek and Griffiths, 1994). For the *Cecidophyopsis* mites an RFLP approach of the ITS regions was found to be initially successful (Fenton *et al*., 1995). However this has been superseded by the development of a multiplex PCR system which co-amplifies three bands from ITS1 which all vary in length between different species (Kumar *et al*., 1999). These are resolved on high resolution acrylamide gels (Kumar *et al*., 1999). This technique even works on single eriophyid mites which are the smallest of all the mites (Fenton *et al*., 1997).

Tracing introduction of species to new geographical regions

The study of the genetic structure of species is of practical application in the case of pests. An increasing number of pest species are introduced each year into new biogeographical areas. In some cases, it is important to determine the area of origin of these introductions, for example when predators are to be imported within the framework of biological control operations. The green cassava mite, *Mononychellus progresivus* Doreste, originated in the neotropical region and was accidentally introduced into East Africa in the 1970s. Study of the genetic diversity of the African and American populations of this mite based on sequences of mitochondrial COI and ribosomal ITS2 has shown that *M. progresivus* was probably introduced into Africa from Columbian populations or from a region bordering Columbia (Navajas *et al*., 1994).

Another important case of pest introduction is that of a parasite of bees, the mite *V. jacobsoni* in the Americas. Using RAPD markers, de Guzman *et al*. (1997) suggested a European origin for *V. jacobsoni* in the United States, whereas mites in Brazil and Puerto Rico are probably predominantly Japanese in origin.

Wolbachia *and reproductive incompatibilities*

As a complement to historical and ecological factors, incompatibilities in reproduction may play a role in the structuring of populations. In this context, it is important to note that a bacterial endosymbiont of the *Wolbachia* type was detected in several species of Tetranychidae and Phytoseiidae (Breeuwer and

Jacobs, 1996; Johanowicz and Hoy, 1996; Tsagkarakou *et al*., 1996a). These microorganisms are known to be involved in alterations in the reproductive system in arthropods (Stouthamer *et al*., 1999) including mites (Breeuwer, 1997; Johanowicz and Hoy, 1998), although it has no effect in some cases (Gotoh *et al*., 1995, 1999; Gomi *et al*., 1997; Navajas *et al*., 1999a). Natural populations of *T. urticae* have been found to be either infected or uninfected (Breeuwer and Jacobs, 1996). The dynamics of *Wolbachia* infection is not fully understood yet, however Johanowicz and Hoy (1999) showed that the infection did not spread rapidly through experimental laboratory populations of the phytoseiid *Metaseiulus occidentalis* (Nesbitt). The detection of *Wolbachia* infection in mites, as well as in a diverse array of arthropods, has been based in the PCR amplification of three genomic regions: a fragment of the 16S rDNA and part of protein coding genes, including the *ftsZ*, which is involved in cell division, and more recently the *wsp* which encodes a major cell surface coat protein (see Tsagkarakou *et al*. (1996) and Jeyaprakash and Hoy (2000) for PCR protocols). By removing the *Wolbachia* from its host through an antibiotic supplemented diet or heat treatment it is possible to study the effects that the bacteria had on their host (Van Opijnen and Breeuwer, 1999).

Concluding Remarks

In this review we have briefly described the current use of genetic markers in the study of ticks and mites. These markers have already been used to increase the accuracy of species identification as well as in helping identify previously unrecognised mite species. There are many more mite species to be identified. Phylogenetic relationships between taxa are now precisely established on the basis of DNA sequences, although there is still much to be learned. Detailed analysis of population structures has also been possible. Many of the methods described here are dependant on screening large numbers of individuals to fully understand the natural variation within a population. Clearly, this is time consuming, however the techniques are becoming routine and economical. Other model arthropods, mainly insects, have shown major advances in the understanding of their genetics and population structures (e.g. Walton *et al*., 1999). Much of this has been facilitated by the use of genetic manipulation to introduce marker genes which can also be selected e.g., the eye-colour system in *Drosophila* (Klemenz *et al*., 1987). These advances are slowly working their way into other arthropods and transformation has now been carried out with transgenic phytoseiid mites (Presnail and Hoy, 1992). This system may become available for the manipulation of other beneficial mites, increasing their effectiveness as natural predators.

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