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REVIEW: APPLICATION OF MOLECULAR MARKERS IN THE STUDY OF GENETIC DIVERSITY IN ACAROLOGY

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Abstract-Mites are a more diverse group includes the Tetranychidae (spider mites), Astigmatidae, Phytoseiidae (predatory mites) and the Eriophyidae. Population variation has also been studied in the important bee parasitic mite Varroa jacobsoni. 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 had particular success in facilitating the identification of taxonomically difficult species, understanding population structures and elucidating phylogenetic relationships. The application of molecular markers to 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.
Keywords: AFLP, microsatellites, phylogeny, RAPD, RFLP, sequencing, ticks.

1. INTRODUCTION

In recent years, DNA-based marker systems have been increasingly employed in diverse areas of biology including phylogenetic studies, evolution, ecology, population genetics, population dynamics and genetics of complex traits in both plant and animal systems. This has been possible because of the rapid advances in molecular biological methods and bench-level protocols for wider application (Morin et al. 2004). The utility of molecular markers as additional tools in basic and applied entomology has led to 'molecular entomology', also referred to as 'precision entomology' (Ananthakrishnan 2005). Over a long time, significant contributions have been made in the field of insect systematics through morphometric traits, wherein a number of difficulties were encountered due to genotype-environment interactions (Cruickshank 2002). The limitations in using morphological, physiological and cytological markers for assessing genetic diversity and population dynamics have been largely circumvented by the developments in DNA-based markers. Molecular markers by nature are neutral to the stage of development, physiological status and environmental influences (Heckel 2003). Isozymes and other proteins as markers are often expressed codominantly and discriminate homozygous and heterozygous individuals. However, the limited number of proteins and isozymes as markers and requirement of different protocols for each enzyme/ protein limit their utility. Unlike morphological and protein-based markers, several DNA based markers are available to elicit the differences between individuals and populations, or they can be developed for each specific purpose. Although a large number of samples can be analyzed quickly, a number of other factors such as cost, speed and requirement of technical skills are the major concern. DNA-based markers can generate large amount of high quality data compared to several biochemical marker marker systems, but degree of polymorphism detected and the statistical dependability of the results vary among marker systems (Weising et al. 1995). Polymorphism is a general term used to describe the difference between individuals either at morphological or at molecular level. The techniques described include restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs),microsatellites DNA sequencing technology has evolved to a point where user friendly kits are available for the entire process and it is no longer necessary to have specialised molecular expertise in order to produce molecular data for phylogenetic analysis. I hope this review will encourage the wider use of molecular techniques in systematic acarology.

2. MATERIAL AND METHODS

2.1 Collection and Storage of Specimens for Molecular Work

Evidence from insects suggests that the most effective method for preserving specimens for molecular work is ultracold freezing (-80ºC) of live specimens (Dillon et al., 1996), however, this is not always practicable. An acceptable alternative is storage in 100% ethanol. Mites from other media should be transferred to 100% ethanol and stored at -20ºC as soon as possible. Lower concentrations of ethanol give variable results and should be avoided if at all possible, however, mites stored in ethanol as low as 70% for many years can give good yields of DNA. Drying is

not generally good for DNA and therefore parasitic mites collected from museum specimens may not be suitable for molecular work.

2.2 DNA extraction

Klompen (2000) found it necessary to use 2-5 individuals in each extraction whereas Anderson & Trueman (2000) used only leg tissue dissected from individual mites. If possible it is preferable to extract DNA from single individuals to prevent mixing of distinct genotypes, particularly if there is any question about the identity of the mites. This should be possible using all but the very smallest of mites. Extracted DNA should be stored at -80ºC.When extracting DNA it is usual to slide mount a few individuals as voucher specimens so that they are available for examination subsequent to DNA sequencing. Ideally the voucher specimen should be the individual that is sequenced but this individual is usually completely destroyed during the extraction process.

2.2 Properties of the Ideal Molecular Marker

Single-copy (or multiple homogenous copies)
The ideal molecular marker should be present as a single copy in the haploid genome. If more than one copy is present it is possible that sequences obtained from different individuals in the phylogeny will be from different (paralogous) copies of the gene.

2.3 Substitution Rate is Low Enough to Avoid Excessive Numbers of Multiple Substitutions

If too fast a gene is used then there will be sites at which two or more substitutions have occurred. Since subsequent substitutions mask previous ones it will become increasingly difficult for most methods of phylogenetic analysis to reconstruct the tree. This is called saturation.

2.4 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 (Hubbard et al. (1995) for ticks pre756 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, (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.

2.5 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 (Yli-Mattila et al., 2000).

CONCLUSION

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).

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