PCR-based methods for identification of species of the Anopheles minimus group: allele-specific amplification and single-strand conformation polymorphism

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Abstract. We report two polymerase chain reaction (PCR)-based methods for distinguishing morphologically similar species based on amplification of a variable region of the 28S gene of ribosomal DNA. The four species we investigated are mosquitoes of the Anopheles minimus group: An. aconitus, An. varuna and An. minimus species A and C. The formally named species are vectors of human malaria parasites in south-east Asia but are difficult to distinguish with certainty on the basis of morphology. Allele-specific amplification was used to differentiate An. minimus A from An. minimus C. This technique has been widely used for the diagnosis of species. Single-strand conformation polymorphisms (SSCPs) were used to separate all four species. This technique, which has seldom been used for species identification, has many advantages: it does not require sequence information beyond that needed for amplification; it is ideally suited for the detection of heterozygotes; it utilizes more of the information in the PCR product than allele-specific amplification; it distinguishes all four species considered here and could easily be extended to other species; previously unknown intraspecific variation and additional species are likely to be detected. Thus, SSCP provides valuable population genetic information which allele-specific amplification does not.

Key words. Anopheles minimus group, allele-specific PCR, cryptic species, molecular systematics, polymerase chain reaction (PCR), ribosomal DNA, single-strand conformation polymorphism, Thailand.

Introduction

Many techniques for examining specific behaviour, ecology and genetics have been used to reveal, and subsequently identify, morphologically similar species of mosquitoes (Diptera: Culicidae). Chromosome differences are a commonly used means of identification of anopheline species, but the technique cannot be applied to all specimens: it is either sex or developmental stage specific. Considerable effort has been expended in finding alternative means of specific identification of mosquitoes, especially to distinguish between sibling species that may differ in their vectorial capacity. Allozyme electrophoresis (e.g. Mahon et al., 1976; Miles, 1979) requires fresh or frozen material. Cuticular hydrocarbon analysis using high-performance gas chromatography (e.g. Carlson & Service, 1979; Hamilton & Service, 1983; Phillips et al., 1990; Milligan et al., 1993) has not proved to be diagnostic and requires expensive equipment. Three DNA-based approaches have been utilized for mosquito species identification: hybridization assays based on species-specific sequences (e.g. Gale & Crampton, 1987), randomly amplified polymorphic DNA (RAPD, e.g. Wilkerson et al., 1993) and tests based on known, variable regions. The second and third options involve the polymerase chain reaction (PCR). We do not seek here to assess the relative merits of these approaches, but rather to assess two methods for analysing known variable regions: allele-specific amplification (ASA), which has frequently been used to distinguish species, and single-strand conformation polymorphism (SSCP) (Hiss et al., 1994), which has rarely been used for this purpose. Restriction fragment length polymorphism (RFLP) of PCR products was also considered for species identification, but suitable restriction sites were not found in the ribosomal RNA gene sequences studied.

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ASA utilizes primers that match, and therefore amplify, the sequence of one species, but mismatch other species. Primers are designed such that the products formed are of species-specific size and can be resolved on an agarose gel. Scott et al. (1993) devised a method of identifying five of six known species of the Anopheles gambiae complex using one universal and five specific primers to the intergenic spacer of ribosomal DNA (rDNA). Porter & Collins (1991) used a slightly different primer design: two external universal primers and two internal species specific primers. Their method distinguishes two species of the Anopheles maculipennis complex from the internal transcribed spacer 2 of rDNA.

SSCP analysis of PCR products was introduced by Orita et al. (1989). The technique can, in principle, detect single base pair mutations in a PCR product without any prior sequence knowledge beyond that needed for the PCR amplification. The product is denatured to form single-stranded DNA, and snap-cooled to form folded structures. Sequence differences affect the tertiary structures formed, which affect the mobility of the strands in a non-denaturing gel. Hence, two bands are expected from homozygotes and four bands from heterozygotes. Two identically migrating bands cannot be assumed to have identical sequences, because not all mutations will affect mobility. The technique has been used principally for human mutation detection, and therefore debate has centred on optimization of conditions in order to maximize detection rate. Hayashi (1991) found that, after optimization, SSCP could detect 99% of point mutations in DNA molecules 100–300 bp in length and 89% of mutations in molecules 300–450 bp in length. It is not so critical to detect every mutation when using SSCP to identify mosquito species: if taxa have been identified a priori, the pattern produced can simply be used as a marker.

The Anopheles minimus group of mosquitoes comprises eight formally named, morphologically similar species (Harrison, 1980). Three of the formally named species—An. aconitus Döntitz, An. minimus Theobald and An. varuna Iyengar—cannot be distinguished reliably by adult morphology. They are widespread across south-east Asia, sympatric across much of Thailand and vectors of human malarial parasites.

Behavioural and population genetic evidence indicates that An. minimus itself may be a complex of species. A mark–release–recapture experiment carried out in northern Thailand showed host-preference heterogeneities: individuals caught on humans or on bovids tended to return to the species from which they were first caught (Suthas et al., 1986). One explanation of this observation is that more species were present. Green et al. (1990) provided conclusive evidence for this hypothesis. They found the sympatric occurrence of homozygotes for two electromorphs, with an absence of heterozygotes at two localities in Thailand. The two species were informally designated species A and species C. A partially differential morphological trait was noted: An. minimus C adults were more likely than those of An. minimus A to possess a humeral pale spot on the costal vein of the wing. Both differed from ‘form B’ described from southern China on the basis of morphological variations (Yu & Li, 1984; Yu, 1987).

Materials and Methods

Mosquitoes

Adult females of the Anopheles minimus group were collected from humans and from bovids, in Kanchanaburi, Tak and Chiang Mai Provinces, Thailand, encompassing the known distribution of An. minimus C (Green et al., 1990; except that coordinates for Ban Phu Rat should read 14°17′N, 99°01′E). Progeny broods were raised from mosquitoes from Kanchanaburi Province and the larval and pupal exuviae of individual offspring were kept as a record of immature morphology. Immatures, and hence associated adults, were identified using the morphological keys of Reid (1968) and Harrison (1980). Immatures of An. minimus sensu lato were collected from Hanoi, Vietnam, and raised individually to adulthood.

Siblings of the allozyme-identified specimens of Green et al. (1990) were used to define An. minimus A and C. One individual of each species was sequenced for the D3 region. The diagnoses were corroborated by morphological evidence: species A tended to lack humeral pale spots whereas species C tended to possess humeral pale spots, as reported by Green et al. (1990).

Choice of DNA region

There are many advantages of using rDNA for species identification (Collins & Paskewitz, 1996). It has adjacent regions of conserved and variable sequences and is present in multiple copies, making it relatively easy to amplify by PCR; it is nuclear and therefore less likely to introgress than cytoplasmic DNA; and it evolves by concerted evolution, thus making one rDNA sequence largely representative of all the repeats within a population. Relatively small sample sizes can therefore be used for interspecific studies. Initially we studied the internal transcribed spacer (ITS) regions, but found ITS1 to contain a repeat structure, and ITS2 to be too variable to align between species (Sharpe, 1997). We therefore examined the less commonly studied third domain (D3) of the 285 gene, which is flanked by highly conserved regions. The PCR primers were designed by Litvaitis et al. (1994) to amplify platyhelminth DNA and have subsequently been used to amplify genomic DNA of mites (R. Thomas, personal communication) and nematodes (Despres et al., 1995).

Sequencing from genomic DNA

DNA was extracted from adult mosquitoes in two ways: a silica-based purification method (Boom et al., 1990; Höss & Piiäbbo, 1993) or a proteinase K digestion followed by phenol–chloroform extraction and ethanol precipitation (Sambrook et al., 1989). A negative control was included with every set of extractions. An 380-bp region was amplified by the PCR using primers D3a and D3b (Table 1). Amplification was performed in 25-μl volumes overlaid with one drop of mineral
oil in a Hybaid OmniGene thermocycler. Each PCR included mosquito DNA, 200 \( \mu \)M deoxynucleotide triphosphates (dNTPs), 600 nM of each primer, 1.5 mm-2.5 mm MgCl\(_2\) and 0.25 units of Thermoprime DNA polymerase (Advanced Biotechnologies, using manufacturer’s buffer IV). PCRs were initiated with a ‘hot start’ procedure: polymerase was added after an initial 5-min denaturation. Cycling conditions were 95°C 30 s, 55°C 30 s and 72°C 1 min for thirty-five cycles followed by a final 72°C extension for 4 min. Products were electrophoresed through ethidium bromide-stained 1% agarose gels in 1 × TBE (0.09 M Tris, 0.09 M Borate, 0.002 M EDTA, pH 8.3) and visualized under UV light. Products were cleaned using spin columns (Promega Wizard PCR Preps) and sequenced in both directions (AmpliTaq DNA polymerase, FS, Applied Biosystems on an automated sequencer, ABI 373). Amplification and sequencing primers were identical. Manufacturer’s instructions were followed, except that a phenol–chloroform cleaning step was included after the sequencing reaction in order to remove excess dye terminators. Sequences were edited manually and aligned using PILUP from the GCG suite of programs (Program Manual for the Wisconsin Package, 1994).

**Allele-specific amplification**

Restriction sites suitable for distinguishing all four species were not present in the D3 products. Our ASA test aimed to distinguish An. minimus A from An. minimus C. It was not possible with the sequence data available to design a test that would also separate An. aconitus and An. varuna. The ASA utilizes an adjacent 2-bp difference between the taxa. An 380-bp product is always expected: it acts as an internal control for the PCR (Fig. 1). In addition, a 275-bp product is expected for An. minimus A, or a 75-bp product for An.

### Table 1. Primer sequences.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5’ – 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3a (forward)</td>
<td>GACCCGTCCTGAAACACCGGA</td>
</tr>
<tr>
<td>D3b (reverse)</td>
<td>TCGGAAGGAACCCGACTCTA</td>
</tr>
<tr>
<td>minimus A (forward)</td>
<td>GAAACCCACAGCGA</td>
</tr>
<tr>
<td>minimus C (reverse)</td>
<td>ACACCTGATTGTCTG</td>
</tr>
</tbody>
</table>

**DNA amplification**

Amplification was performed in 25-µl volumes overlaid with one drop of mineral oil using a Hybaid OmniGene thermal cycler. Considerable effort was expended to determine optimal conditions for the amplification. This included varying the concentrations of Mg\(^{2+}\) and primer, annealing temperature, number of cycles and type of polymerase. Finally, each reaction included mosquito DNA, 200 µM dNTPs, 720 nM of each of the four primers (Table 1), 2.5 mm MgCl\(_2\) and 1 unit of DyNAzyme DNA polymerase (Flowgen, using manufacturer’s Mg-free buffer). Cycling conditions were 95°C 30 s, 47°C 30 s and 72°C 1 min for thirty to thirty-five cycles followed by a 4-min final extension at 72°C. PCRs were initiated with a ‘hot start’ procedure. Products were visualized on an ethidium bromide-stained 1% agarose gel.

**Single-strand conformation polymorphism (SSCP)**

DNA was amplified using primers D3a and D3b (Table 1) in the same manner as for sequencing. Four microlitres of the PCR product were visualized on an ethidium bromide-stained 1% agarose gel to check for successful amplification. The remaining product was precipitated with isopropanol, dried at room temperature for 3 h to remove all traces of solvent, and resuspended in 10 µl of formamide loading buffer (formamide, 1 mM EDTA pH = 8.0, 0.1% bromophenol blue, 0.1% xylene cyanol).

**Electrophoresis to detect SSCP**

Non-denaturing polyacrylamide gels were cast using clean, non-siliconized glass plates (Bio-Rad Protein II system, 16 cm × 20 cm × 0.6 mm). Gels were made from 25% ‘acrylamide derived’ gel solution (Flowgen) and 0.6 × TBE; polymerization was initiated by adding 60 µl of TEMED and 300 µl of ammonium persulphate (10% w/v). Fifteen- or twenty-five-well combs were used. Gels were electrophoresed in 0.6 × TBE. Four positive controls were run on each gel: one each of An. minimus A, An. minimus C, An. aconitus and An. varuna. The PCR products were denatured (95°C for 5 min) and then plunged into ice water. Half of the sample was loaded onto the SSCP gel and the remaining half frozen in case the gel

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**Fig. 1.** Schematic diagram of the design of ASA. Primer names are given above the diagram. Approximate sizes are given below the diagram.

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needed to be rerun. Gels were run for 16–20 h at 4°C (in a cold room with cold tap water circulating the plates) with a constant voltage of 15 V/cm.

Silver staining of SSCP gels

Gels were stained in clean, shallow plastic trays. They were fixed for 10 min (50% methanol, 10% acetic acid), washed twice for 2 min (10% ethanol, 0.5% acetic acid) and stained for 10 min (0.1% AgNO₃ from a 10% stock). The silver was precipitated for as short a time as possible (1.5% NaOH, 0.001% Na borohydride from a 100-mg/ml stock, 0.002% formaldehyde, mixed together just before use), precipitated again until bands developed, washed in water and neutralized for 20 min (0.75% Na₂CO₃, from a 7.5% stock). All mixtures were prepared immediately prior to use with double-distilled water. Gels were transferred to filter paper, covered with plastic film, dried and scored.

Results

Mosquitoes

Collections comprised An. minimus sensu lato, An. aconitus and An. varuna. D3 sequence data corroborated the morphological identifications (Sharpe, 1997). Morphological identifications gave conflicting results for both An. varuna families, highlighting the problems of such means of identification. One family had one sibling with characteristics normally associated with this species, and another with characteristics intermediate between that of An. varuna and An. minimus. Another family had one offspring with characteristics of An. varuna and two offspring with characteristics of An. pampanai Bittiker & Beales, another member of the An. minimus group. Both families had identical D3 sequences and have been treated as specimens of An. varuna.

Sequencing from genomic DNA

Sequence data obtained from the D3 locus are given in Table 2 and Fig. 2. Thirty-six individuals were sequenced: 5 An. aconitus, 18 An. minimus A, 10 An. minimus C, 2 An. varuna and one individual (referred to as No. 157) that resembled An. minimus A and C, but could not be assigned to either species. Geographic origins are given in Table 3.

The five An. aconitus individuals each possessed more than one sequence of D3 (Table 2); these could be due to heterozygosity or to differences between copies of the rDNA cistron. Base 32 is consistent with both hypotheses. Base 30, however, is inconsistent with the hypothesis of heterozygosity as the chances of sampling five heterozygotes from a population with the maximum proportion of heterozygotes possible (50%) is 3.1% (0.5²). The hypothesis of heterozygosity can be ruled out if rDNA is assumed to be X-linked in An. aconitus as it is in An. gambiae: two of the individuals with two sequences are male, and therefore would be hemizygous for the X chromosome.

Allele-specific amplification analyses

A photograph of an ASA gel is shown in Fig. 3. Where only one species-specific band amplified, it always proved to be that of the ‘correct’ species. Occasionally products representing both or neither species amplified; when repeated, these samples usually produced just one species-specific band. It was because of this problem that the use of SSCP was investigated. Such products are to be expected with ASA if the window of conditions under which species-specific amplification occurs is narrow.

Single-strand conformation polymorphism analyses

SSCPs can overcome the difficulties associated with ASA. Diagnostic, repeatable differences were observed between all four species (Fig. 4). Specimen No. 157 gave a band of intermediate mobility between that of An. minimus A and C. More than two bands were often visualized per sample. These are most likely to be due to alternative stable conformations of the PCR product, but could also be due to alternative PCR products, for example if copies of rDNA differ in sequence. The additional bands were species specific and reproducible: they aided rather than hindered identification. All An. aconitus specimens gave three strong bands; in addition, An. aconitus haplotype 2 (see Table 2) gave two extra, strongly staining bands, providing additional evidence to support the sequence data. Five individuals gave unexpected SSCP patterns. Four were sequenced and all proved unique (GenBank accession numbers AF114020–AF114023). None resembled other members of the An. minimus group sequenced. They can be aligned with other Anopheles sequences but not with Culex or Aedes sequences, providing evidence that they do represent anopheline mosquito sequence. We feel that the most likely explanation is that they represent misidentified anopheline species.

Discussion

Species distributions

Anopheles aconitus was recorded from Kanchanaburi, Tak and Chiang Mai Provinces. Although not previously recorded from Kanchanaburi or Tak, Harrison (1980) regards it as ‘ubiquitous’ in Thailand. Only two specimens of An. varuna were recorded in this study, and some doubt as to their identity remains. Both specimens originated from Kanchanaburi Province. There are only two recordings of An. varuna in Thailand, from Lampang and Chiang Mai Provinces (Harrison, 1980). This probably reflects limited sampling rather than rarity.
Anopheles species identification by PCR

**Table 2.** Variable sites in ~313bp of the D3 PCR product. Bases are numbered relative to An. minimus A (Fig. 2). Base 136a refers to an insertion between bases 136 and 137. Dots represent identity to An. minimus A; dashes represent indels. Y=C and T; M=A and C. Subscripts refer to different alleles obtained from the one species. Good sequence was obtained only in one direction from the two An. varuna individuals; bases 1-22 are unknown. The sequences have been deposited in the GenBank database (accession numbers AF114014–AF114019).

<table>
<thead>
<tr>
<th>Base number</th>
<th>Haplotype</th>
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<tbody>
<tr>
<td>An. minimus A</td>
<td>18</td>
</tr>
<tr>
<td>An. minimus 157</td>
<td>1</td>
</tr>
<tr>
<td>An. minimus C</td>
<td>10</td>
</tr>
<tr>
<td>An. varuna</td>
<td>2</td>
</tr>
<tr>
<td>An. aconitus 1</td>
<td>4</td>
</tr>
<tr>
<td>An. aconitus 2</td>
<td>1</td>
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</table>

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Geographic origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>An. aconitus haplotype 1</td>
<td>2</td>
<td>Kanchanaburi, Thailand</td>
</tr>
<tr>
<td>An. aconitus haplotype 1</td>
<td>1</td>
<td>Tak, Thailand</td>
</tr>
<tr>
<td>An. aconitus haplotype 1</td>
<td>1</td>
<td>Chieng Mai, Thailand</td>
</tr>
<tr>
<td>An. aconitus haplotype 2</td>
<td>1</td>
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</tr>
<tr>
<td>An. minimus A</td>
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<td>Hanoi, Vietnam</td>
</tr>
<tr>
<td>An. minimus A</td>
<td>10</td>
<td>Kanchanaburi, Thailand</td>
</tr>
<tr>
<td>An. minimus A</td>
<td>4</td>
<td>Tak, Thailand</td>
</tr>
<tr>
<td>An. minimus A</td>
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<td>Chieng Mai, Thailand</td>
</tr>
<tr>
<td>An. minimus C</td>
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<td>Kanchanaburi, Thailand</td>
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<td>2</td>
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</tr>
<tr>
<td>An. minimus No. 157</td>
<td>1</td>
<td>Kanchanaburi, Thailand</td>
</tr>
<tr>
<td>An. varuna</td>
<td>2</td>
<td>Kanchanaburi, Thailand</td>
</tr>
</tbody>
</table>

**Fig. 2.** D3 sequence from An. minimus A.

**Fig. 3.** Agarose gel showing ASA products. Lanes 1 and 8 are 100-bp DNA size marker; lanes 2–4 are An. minimus C; lanes 5–7 are An. minimus A.

An. minimus C appears to have extended its distribution to Chieng Mai Province, in the north of Thailand. Green et al. (1990) did not detect this species in 229 individuals of An. minimus sensu lato examined. Suthas et al. (1986) conducted their mark–release–recapture experiment showing host-preference heterogeneities at this village, and it was therefore curious that Green et al. (1990) did not detect species C there. Mosquito species are known to undergo periodic booms and busts in population size and therefore it is not surprising that distributions sampled at different times do not fully agree.

**Sequence data**

Our observations reveal low levels of geographical sequence variation, though we stress that this is from limited parts of the species’ ranges: An. minimus A, An. aconitus and An. varuna are known to have much wider distributions.

Anopheles minimus No. 157 could represent intraspecific variation of either species A or species C, or could represent...
that it has been tested against. The possibility of finding exceptional individuals that do not fit the previously observed pattern always exists. This is especially true for species with a wide distribution. It is stressed that the sequence data used here come from specimens from a small part of the species' ranges. However, this is typical of other studies and highlights the advantages of SSCP as unexpected variation is detectable.

**Design of allele-specific amplification**

The use of ASA is widespread. There are, however, several possible designs for such tests, which are each expected to have different merits, and these have not been widely discussed. The ideal attributes and the properties of various designs (Fig. 5) of such tests are considered below. Some of the desirable attributes are mutually exclusive.

The test must, above all, be species specific. The mismatch must be diagnostic, and the test must be maximally refractory to amplification with a mismatched primer, preferably under a wide range of PCR conditions. Maximum discrimination of the PCR may be achieved in a number of ways. First, it is commonly known that primer length, annealing temperature and various PCR conditions affect the probability of amplification and must therefore be optimized. Second, some base-pair mismatches are less likely to be extended by the PCR than others (Kwok et al., 1990; Huang et al., 1992). Two adjacent base-pair mismatches are less likely to be extended than one base-pair mismatch (Kwok et al., 1990). Third, the use of specific primers on both positive and negative strands (design 4) aids specificity. If one mismatched primer does amplify, it will result in linear rather than exponential amplification. Fourth, competition in the PCR process is expected to aid specificity. For example, Paskewitz & Collins (1990) designed a three primer test (Fig. 5c) to distinguish *An. gambiae* and *An. arabiensis*. They reported the results of preliminary tests using only two of these primers: the universal and one of the specific primers (Fig. 5a). Primers matching both species were tested for both primer positions. Three of the four primers produced strong, reliable and correctly sized products with the 'correct' species. However, all of these also amplified from the wrong species, albeit more faintly or occasionally.

The test should include a positive control. In other words, the expected result should always be amplification, rather than no amplification, which cannot be distinguished from a blank due to error. Figure 5(b) shows an elegant means of achieving such an end. External, universal primers should always produce an amplified product which serves as a positive control for the PCR.

The chances of misamplification should be as equal as possible for all species. Figure 5(c) incorporates unequal competition amongst primers. Inner primers can prime against product produced by outer primers, but not vice versa. This could be tested experimentally.

The test should be able to be extended to other species if necessary, for example if more species in a complex are discovered, or if the range of one species expands so that it
Anopheles species identification by PCR

a) Two separate PCRs. Two primers in each, e.g. the preliminary two-primer tests carried out by Paskewitz & Collins (1990).

b) Two external universal primers; internal specific primers e.g. Ye et al. (1992), and Porter & Collins (1991).

c) One universal primer, several specific primers on the same strand, e.g. Paskewitz & Collins (1990) and Scott et al. (1993).

d) Several amplification reactions, all with separate specific primers, e.g. Walton et al. (1997).

Fig. 5. Schematic diagram of various designs of ASA. Solid lines indicate universal primers; dotted lines specific primers.

becomes sympatric with other species. This is easier with some designs than with others. For example, Fig. 5(b) has a limited number of bases between the external primers: there may not be sufficient variation available. Adding additional primers will produce unequal primer competition.

Comparison of allele-specific amplification and single-strand conformation polymorphism

In this study, ASA had a lower success rate than SSCP. This is not necessarily true for other ASA systems, because the success is predicted to depend on the design of the test adopted and on the individual nature of the primers. From the necessarily limited amount of sequence data available, it was not possible to design an ASA test that would distinguish all of the species of the An. minimus group in Thailand. Again, this does not necessarily apply to other ASA systems, although it is likely that there is a limit to the number of species that they can distinguish. This is a major disadvantage of the ASA test designed in this study. The SSCP, however, can distinguish all four species considered here, and it is likely that other members of the An. minimus group will also produce diagnostic patterns. The SSCP were substantially easier to develop than the ASA.

The design of ASA is constrained by the necessity of resolving products on an agarose gel: species-specific products cannot be too short and must be sufficiently different in size from other products. SSCP are not constrained in this manner.

SSCP require less sequence information than ASA and do not require the construction of primers for each species. All that is needed is a PCR product of a variable region. With the use of ‘universal’ primers, it is possible to eliminate most or all sequencing. This makes them considerably cheaper and less time consuming to develop than ASA. Primers are now available that will amplify most organisms. Simon et al. (1994) have published a compilation of universal mitochondrial primers. A range of both mitochondrial and nuclear insect
primers has been compiled by B. Crespi, J. Hobbs and members of the ‘bug-net’ discussion group. They are available from J. Hobbs (hobbs@unixg.ubc.ca).

SSCPs use more of the information in the PCR product: ASA relies on a difference of just a few base pairs, typically one base per species. As such it is particularly susceptible to intraspecific variation at these sites which would give erroneous species identification, but not to variation at other sites within the PCR product. The advantages of using the information in the whole PCR product are highlighted by the ability of the SSCP technique to detect individuals that do not belong to the An. minimus group. SSCP, however, demand little intraspecific variation in the PCR product if the technique is to be used for species identification. Thus, tests based on nuclear rather than mitochondrial DNA are to be preferred, and those based on tandemly repeated genes which are expected to be undergoing concerted evolution are ideal. SSCP are ideally suited to the detection of heterozygotes and thus, hybrids, or to the detection of heteroplasmy in a mitochondrial product.

SSCPs involve one more step than ASA: the running and staining of an acrylamide gel, which necessarily entails more time and expense. However, they can be used without any sequencing, and they require less effort to optimize PCR conditions. It is concluded that SSCP have great potential for providing critical population genetic information.

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References


Anopheles species identification by PCR


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