

Towards a new role for vector systematics in parasite control

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SUMMARY

Vector systematics research is being transformed by the recent development of theoretical, experimental and analytical methods, as well as conceptual insights into speciation and reconstruction of evolutionary history. We review this progress using examples from the mosquito genus *Anopheles*. The conclusion is that recent progress, particularly in the development of better tools for understanding evolutionary history, makes systematics much more informative for vector control purposes, and has increasing potential to inform and improve targeted vector control programmes.

Key words: Species complex, speciation, biogeography, vector systematics.

INTRODUCTION

Disease control directed at vectors such as ticks, flies and mosquitoes has proved to be a cost-efficient and successful approach (Michalakis and Renaud, 2009), but effective vector control may sometimes suffer setbacks from a lack of reliable identification of vectors (Monteiro *et al.* 2001; Marquardt, 2004). Correct identification of vectors directly relies on systematics research, such as research on species delimitation and reconstructions of evolutionary history (Manguin *et al.* 2008). From a control perspective, it may appear that the systematics of vectors is well understood, but for a large number of important vector species there is actually a lack of basic systematics knowledge. Among systematists, it is for instance known that several of the lower taxonomic levels of the genus *Anopheles*, such as Series, Groups, Subgroups and complexes, may not always represent the actual evolutionary history of the organisms (Foley *et al.* 1998; Krzywinski *et al.* 2001; Sallum *et al.* 2002; Harbach, 2004, 2007). Furthermore, for a large number of genetic species-level studies, one morphological taxon has turned out to be a species complex, where the species may differ in traits that are of importance for vector control, such as vectorial capacity, behaviour or ecology (Collins and Paskewitz, 1996; Manguin *et al.* 2008). Therefore, we suggest that vector control programmes could benefit greatly from an improved

knowledge of vector evolution and systematics, and from a better understanding of the uncertainties associated with systematics and methods of species delimitation. We outline some of the recent major advances in the systematics discipline and give examples of the recent research on cryptic species. We also discuss how recent development of systematics enables it to be more informative for targeted vector control strategies.

UNDERSTANDING SPECIES DIFFERENCES

Recent whole-genome scans of hundreds of thousands of genetic markers (Emelianov *et al.* 2004; Scotti-Saintagne *et al.* 2004; Nosil *et al.* 2007) have led to a better insight into how the genomes of two closely related species differ. The picture is emerging of a 'mosaic genome', with gradual accumulation of reproductive isolation across limited genomic regions (della Torre *et al.* 2002; Coyne and Orr, 2004; Wu and Ting, 2004; Via, 2009). If these cases represent common patterns of genome-wide differences, it seems possible that reproductive isolation can occur through incompatibilities in a small number of genes, while gene flow (Table 1) may still occur along other parts of the genome. Complex gene flow boundaries have already been found among the *An. gambiae* chromosomal forms (della Torre *et al.* 1997; Turner *et al.* 2005; Slotman *et al.* 2006; Lawniczak *et al.* 2010; Neafsey *et al.* 2010). For vector control, this may mean that there is a potential for transgenic elements or biocide resistance to spread across species boundaries (Djogbenou *et al.* 2008), so future monitoring programmes will perhaps need to extend

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Table 1. Definitions of terms used for the speciation process

Term	Definition
Speciation	The process whereby one species evolves into one or several new species. During a speciation process the taxon may accumulate morphological, behavioural and genetic differences that eventually lead to complete post-zygotic reproductive isolation.
Pre-zygotic reproductive isolation	Reproductive isolation which occurs before the formation of a zygote, for instance because two species do not physically meet, differences in reproductive timing or behaviour, or failure to recognise each other as mates.
Post-zygotic reproductive isolation	Reproductive isolation that occurs after the formation of a zygote, usually due to genetic or developmental incompatibilities. It is usually apparent when the species fails to produce reproductive offspring even during forced-mating experiments.
Gene flow	A measure of the rate by which alleles historically have been transferred between two populations or species. If reproductive isolation is complete, the gene flow is expected to be zero. A long-term cessation of gene flow appears to be essential for a speciation process to be complete.
Evolutionary history	Reconstruction of historical species formation, phylogenetic relationships and biogeographical history of a taxon.
Pattern-based species delimitation	Species delineation based on phenotypical or genetic differences.
Process-orientated species delimitation	Species delineation that takes the evolutionary and biogeographical history of species into account, and recognizes that speciation is not an instant event, but a sometimes reversible historical process.

monitoring of vectors across species boundaries in species complexes.

TOWARDS A SYNTHESIS OF SYSTEMATICS AND EVOLUTIONARY HISTORY

Genomic research has advanced our understanding of how species are formed, but it has also highlighted strong discrepancies between what we understand a species to be, and how species have been described and delimited—a rift that current systematics is trying to suture. A formal species description requires that the new species is given a valid name following the *International Code of Zoological Nomenclature* (ICZN), and a holotype specimen is designated (ICZN, 1999). Information must also be included that distinguishes a new species from related species, but the *Code* imposes no limit on the quality or nature of the information (morphological

characters, DNA sequences, chromosome banding patterns, or a combination of different types of information) (ICZN, 1999). The quality of evidence presented in vector species designations therefore varies widely and has evolved with technological advancement; early 20th century species delimitation was conducted through a brief comparison of key morphological differences to similar species (Wilson, 2004) (e.g. Theobald, 1901–1910; Dönitz, 1903). For species descriptions of later date, the delimitation may also include a wider geographical sampling, ecological information and chromosome, allozyme or molecular evidence (e.g. White, 1985; Baimai *et al.* 1987; Wilkerson *et al.* 2004; Linton *et al.* 2005). Regardless of the evidence, a species description remains a hypothesis, which may or may not be correct.

The problem of species delimitation using pattern description

Many of these attempts at species delimitation are, as in Linnaean times, implicitly essentialist; regarding species as fixed entities, preserved through time and space, and they may neglect biologically relevant within-species heterogeneity and gene flow across species boundaries. Pattern-based species descriptions are simple comparisons of similarities and differences in morphology or DNA markers, rather than on in-depth study of evolutionary history. Some possible artefacts can be avoided by integrating results from several sources of data to reach an overall conclusion, a so-called ‘total evidence approach’ or ‘integrative taxonomy’ (Puillandre *et al.* 2009; Roy *et al.* 2009). However, the problem remains that many such studies do not state *a priori* criteria for identifying species boundaries, so they interpret each clade as a separate species, irrespective of the biogeographical history of the clade (Sites and Crandall, 1997; Wiens, 2007). This has the effect that the hypothesis of ‘new lineage equals new species’ is rarely rejected, even when it is unclear if the lineages are real or a result of short-term allopatric fragmentation or incomplete geographical sampling (Zarowiecki *et al.* 2011). This may lead to taxonomic instability, which makes it more difficult to use the designated species in other studies (Godfray, 2002; Wiens, 2007), including those for vector control purposes.

Enhanced species delimitation using a process-oriented approach

A better fit between species definition and description could perhaps be achieved by conducting mating experiments, which investigates postzygotic reproductive isolation. Unfortunately, mating experiments are not feasible for many vector taxa, and do

not consider geographical, pre-zygotic or ecological barriers to reproduction (Table 1). A promising alternative to create more coherence between species and species delimitation is to use a process-oriented approach, which evaluates current and historical gene flow (Table 1). By thoroughly reconstructing the entire evolutionary history of a species; determining historical gene flow, population structure and biogeography, systematics research provides a much more comprehensive understanding of the species, and more information than just vector identification, some of which may be relevant for vector control. For this purpose, DNA sequence markers have an advantage over cross-mating experiments, because they can be used to detect long-term gene flow and biologically relevant sub-populations in the vector species, not only current post-zygotic mating barriers between individual populations. This might be not only of theoretical value, but also ubiquitously useful, as seen over time and space, one could argue that all species in fact are 'in speciation', i.e. they may contain several independently evolving lineages, which may become completely reproductively isolated sometime in the future – or indeed may not be maintained when the ecology, geography or climate changes (Lamont *et al.* 2003; Seehausen; 2006), as long as complete post-zygotic reproductive isolation has not occurred (Nosil *et al.* 2009).

IMPROVED PRACTICAL METHODS FOR SPECIES DELIMITATION

Species delineation has not only moved forward theoretically, but technology and statistics have developed as well. Newly introduced methods for species delimitation are often based on statistical testing of hypotheses, and an assessment of historical or current gene flow (Pons *et al.* 2006; Stockman *et al.* 2006; Stockman and Bond, 2007; Cummings *et al.* 2008). They have evolved along two different lines: the rapid large-scale methods that aim at charting mostly unknown territory (Monaghan *et al.* 2005; Pons *et al.* 2006) and the more specific methods that deal in depth with a small number of taxa (Templeton, 1998, 2001; Posada *et al.* 2000). These rapid methods are often based on information from a small number of genetic markers and are not expected to give completely correct species delimitation in every case. They provide instead statistical species delimitation, by trying to recover monophyletic clades in phylogenetic trees (Pons *et al.* 2006; Cummings *et al.* 2008), similarity above a certain threshold value (Hebert *et al.* 2004) or statistical clustering (Schloss and Handelsman, 2005, 2006). Although some statistical methods have been criticised for oversimplifying species delimitation (DeSalle *et al.* 2005; Ebach and Holdrege, 2005), such methods have proved useful when applied to real problems, especially in combination with

morphology and biogeography (DeSalle *et al.* 2005; Vogler and Monaghan, 2007). They can, for instance, rapidly reveal if there is a discrepancy between species delimitation resulting from different data (e.g. DNA, morphology, polytene chromosomes), and indicate clades of apparent taxonomic inflation or cryptic diversity. One caveat is that they are mostly pattern based rather than process orientated, so they can only be seen as a first step towards a more complete understanding of the evolutionary history of a species (Vogler and Monaghan, 2007).

Biogeographical methods for species delimitation

In studies of vector systematics and species delimitation, a much more detailed understanding is often needed than can be provided by rapid methods. These methods nevertheless need to be logical, robust and amenable to scientific validation, e.g. through statistical testing. Several such methods are available, and usually depend on one or more properties one would expect species to acquire during a speciation process, such as monophyletic lineages (Baum and Shaw, 1995), significantly higher genetic divergence compared with intraspecific polymorphism (Highton, 1990; Good and Wake, 1992), lack of gene flow (Porter, 1990), lack of shared polymorphism (Sneath and Sokal, 1962; Davis and Nixon, 1992) and/or heterozygote deficiency (Doyle, 1995). Some tests combine two or more of these categories: morphological differentiation and genetic monophyly (Wiens and Penkrot, 2002), monophyly and ecological differentiation (Stockman and Bond, 2007) or monophyly, gene flow and ecological differentiation (Templeton, 1998; Posada *et al.* 2000; Templeton, 2001). These fine-scale methods are primarily developed from classic population genetics approaches, but make use of recently introduced biogeographical methods for estimation of population expansion, time of divergence (Drummond and Rambaut, 2007), levels of differentiation (Pritchard *et al.* 2000, 2002; Falush *et al.* 2003) and population structure (Dupanloup *et al.* 2002). The software LAMARC simultaneously estimates gene flow and population growth (Kuhner *et al.* 2005), and the 'isolation-with-migration' model (IM) simultaneously estimates gene flow and divergence time between two populations or species, using multi-locus data (Wakeley and Hey, 1997; Hey and Nielsen, 2004). As these methods need multi-locus data to give accurate estimates (Hey and Nielsen, 2004), they may be costly and technically challenging for non-model organisms (Knowles, 2004), but there is a growing number of such studies available for vector species (Loaiza *et al.* 2010; Morgan *et al.* 2010; Zarowiecki *et al.* 2011). These studies have an advantage over previous systematics research because they can give information about the evolutionary process,

e.g. estimates of times of divergence, species origin, population structure, selection on particular genes, population growth and gene flow heterogeneity across genomes – all of which may be important for understanding how the vectors evolved or how insecticide resistance can spread between incipient vector species.

WHY VECTOR EVOLUTIONARY HISTORY MATTERS FOR PARASITE CONTROL

Many otherwise well-studied parasite vectors have, through recent genetic studies, been revealed as cryptic species complexes (Collins and Paskewitz, 1996; Manguin *et al.* 2008). The species in a complex are morphologically indistinguishable under field conditions, but they are reproductively isolated (Table 1) and may differ significantly in ecology or behaviour. One of the most intensively researched vector species, the African malaria mosquito *Anopheles gambiae*, belongs to a well-known cryptic species complex, where some species differ widely in feeding preferences and their importance as malaria vectors (Gillies and Coetzee, 1987), but still new genetic forms are being discovered (Riehle *et al.* 2011). There are also, perhaps less well known, examples of where better species identification has been achieved through biogeographical studies: *Anopheles subpictus* is widely distributed across southern and eastern Asia, and is considered a major vector of malaria (Abhayawardana *et al.* 1996). In India, there are at least two species within this nominal taxon that differ in many traits, including vectorial capacity (Suguna *et al.* 1994; Sahu, 1998), but a recent study revealed several additional species in Southeast Asia, some of which separated several million years ago (Zarowiecki, 2009). The number of species that are actually malaria vectors is still unknown, but as several of the species in the complex are sympatric, it is certain that for a large number of previous studies on vectorial capacity, biting behaviour and insecticide resistance, it is unclear whether one or several species were studied. Rapid molecular identification methods for the *An. subpictus* complex are urgently needed to identify the vectors, so that their bionomics can be investigated and vector control methods properly focused. This is important, as mainland Southeast Asia has a relatively high incidence of *P. falciparum* malaria, confirmed parasite resistance to malaria drugs (Van Bortel *et al.* 2008), and restricted resources for vector control.

The *An. dirus* complex includes seven species (Harbach, 2004; Sallum *et al.* 2005). Of these, *An. baimaii*, *An. dirus* and *An. scanloni* are all major vectors of malaria in Southeast Asian tropical forests (Sallum *et al.* 2005). These three species are largely allopatric, but where they coexist there is a deficiency of heterozygotes in both polytene chromosomes and

allozymes (Baimai *et al.* 1987; Green *et al.* 1992). Post-mating reproductive barriers have been identified for all species, but some fertile offspring can be found between *An. dirus* and *An. scanloni* (Baimai *et al.* 1987). There are discrepancies between the phylogenies of these species constructed from nuclear and mitochondrial markers, and it is believed that mitochondrial introgression between *An. baimaii* and *An. dirus* could explain these observations (Sallum *et al.* 2007; O'Loughlin *et al.* 2008; Morgan *et al.* 2010). As a consequence, there is a concern that genes, such as those that confer insecticide resistance, could disperse across species boundaries (Morgan *et al.* 2010).

The *An. albitarsis* complex is broadly distributed in the Central and South America. Six species are currently recognised (Motoki *et al.* 2009), only three of which have been demonstrated to be vectors of malaria: *An. deaneorum*, *An. marajoara* and *An. janconnae* (Povoa *et al.* 2006). Post-mating barriers have been investigated only between *An. deaneorum* and the non-vectors *An. oryzalimnetes* (Klein *et al.* 1991) and *An. albitarsis* (Lima *et al.* 2004). As several members of the complex are sympatric (Brochero *et al.* 2007), behavioural, ecological and malaria transmission studies have been confounded by identification issues, but due to improved systematic techniques five species can now be reliably separated using PCR-RFLP of ITS2 (Li and Wilkerson, 2005; Brochero *et al.* 2007).

These examples reveal a range of genetic and behavioural diversity within species complexes. The basic systematics provides species-diagnostic tools, but also insights into the evolution of traits that are important for mosquito vectorial capacity and vector control, and allows for further research into the biology of vector species (Powell *et al.* 1999; Manguin *et al.* 2008).

CONCLUSIONS AND FURTHER DIRECTIONS

Some parts of more general vector control programmes (such as utilizing non-selective insecticidal spraying) can lead to biocide resistance and have adverse side effects on human health and ecosystems. This review has illustrated how understanding the evolutionary history of vector species is important for correct identification of vectors – which in turn is essential for developing efficient vector control programmes targeted at specific vectors. We have highlighted the difference between species descriptions, species delimitation and the current understanding of how species are formed, and suggest that correct species identification and delimitation should be underpinned by information on the evolutionary process of the vector species. For instance, systematics studies can provide information about genetic heterogeneity within the vector (Ramsey *et al.* 1994; Habtewold *et al.* 2008), allowing

vector control studies to incorporate vector genetic diversity. Detailed systematics data on gene flow of the vector are also crucial for transgenic vector control projects (Michalakis and Renaud, 2009). There are still significant challenges ahead for systematics, such as—in a standardised way—being able to incorporate evolutionary history in species descriptions, and allowing for differences in gene flow across different parts of the genomes of hybridising species. Nevertheless, it is clear that recent understanding of speciation and species delimitation renders systematics research increasingly useful for vector control studies.

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