

Progress and knowledge gaps in *Culicoides* genetics, genomics and population modelling: 2003 to 2014

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Summary

In the 10 years, since the last international meeting on Bluetongue virus (BTV) and related Orbiviruses in Sicily, there have been huge advances in explorations of the genetics and genomics of *Culicoides*, culminating in the imminent release of the first full genome *de novo* assembly for the genus. In parallel, mathematical models used to predict *Culicoides* adult distribution, seasonality, and dispersal have also increased in sophistication, reflecting advances in available computational power and expertise. While these advances have focused upon the outbreaks of BTV in Europe, there is an opportunity to extend these techniques to other regions as part of global studies of the genus. This review takes a selective approach to examining the past decade of research in these areas and provides a personal viewpoint of future directions of research that may prove productive.

Traguardi raggiunti e da raggiungere nella genetica, genomica e modelli di popolazione dei *Culicoides* dal 2003 al 2014

Parole chiave

Bluetongue,
Ceratopogonidae,
Orbivirus,
Peste equina africana,
Virus della Bluetongue,
Virus della Peste equina
africana.

Riassunto

Nei dieci anni seguenti il Convegno Internazionale di Taormina (CT) sulla Bluetongue e *Orbivirus* correlati, sono stati fatti notevoli passi avanti nella ricerca sulla genetica e la genomica dei *Culicoides*, culminati nell'imminente rilascio del primo sequenziamento completo del genoma di questo *Genus* mediante assemblaggio *de novo*. Durante lo stesso decennio, i modelli matematici per la predizione della distribuzione, stagionalità e dispersione di *Culicoides* adulti sono diventati più sofisticati, riflettendo i progressi in ambito computazionale. Nonostante questi progressi siano avvenuti a seguito delle epidemie del virus della Bluetongue (BTV) in Europa, è possibile implementare queste tecniche anche in altri continenti per contribuire allo studio globale del genere *Culicoides*. Questo articolo esamina gli ultimi dieci anni di ricerca in questo campo e fornisce una visione personale riguardo le direzioni future che potrebbero rivelarsi produttive.

Introduction

From 2003 to 2014, there have been significant changes in the global epidemiology of Bluetongue virus (BTV). These were most notable in Western Europe, which experienced the most costly series of epizootics in the recorded history of the virus (Carpenter *et al.* 2009b, Maclachlan and Mayo 2013, Purse *et al.* 2015, Tabachnick 2013). As a result, since 2003, quite a high number of studies focusing on *Culicoides* have been conducted in Europe. Studies were initially

based in the Mediterranean basin but, starting with 2006, they increasingly focused on North-Western Europe following the incursion of BTV serotype 8 (BTV-8) into the Netherlands. This review examines how novel techniques in genetics, genomics, and mathematical modelling were integrated in Western Europe within a rapidly expanding community of entomologists studying *Culicoides*. In addition, research from outside Europe, which broadened the range of techniques when studying the *Culicoides*

genus, or those that demonstrated novel approaches within the disciplines addressed by the review are also examined.

Due to the volume of relevant literature published since 2003, it is inevitable that this paper would be selective. Critical reviews are available providing more detailed examinations of advances in certain subject areas. The genetic analysis of *Culicoides* has been reviewed with focus on taxonomy and phylogenetics (Harrup *et al.* 2015) and of vector competence (Carpenter *et al.* 2009b). Host preference has also been summarised in a recent opinion piece (Martinez-de la Puente *et al.* 2015), with a detailed description of the studies to date provided by Logan and colleagues (Logan *et al.* 2010), while reference to the impact of *Culicoides* on public health has been articulated by Carpenter and colleagues (Carpenter *et al.* 2013). Use of genome-based techniques has also been examined specifically for analysing *Culicoides* by Nayduch and colleagues (Nayduch *et al.* 2014a), who also provided a review of colonization as a useful prerequisite for study and a more theoretical discussion of this area has also been provided (Tabachnick 2013).

The use of mathematical modelling to predict *Culicoides*-borne arbovirus transmission or distribution and seasonal incidence of the genus has not received dedicated reviews. Useful summaries are provided as part of technical papers (Gubbins *et al.* 2008, Gubbins *et al.* 2014, Hartemink *et al.* 2009), which can be compared with earlier qualitative risk assessments for transmission in Europe (Sellers and Mellor 1993, Wittmann and Baylis 2000). In contrast, models used to predict the movement of adult *Culicoides* have been reviewed in relation to the detection of BTV incursion and spread (Burgin *et al.* 2013). In addition to these specific areas, more general reviews of *Culicoides* biology have examined many of these subjects in less detail as part of broad overviews of research (MacLachlan and Mayo 2013, Purse *et al.* 2005, Purse *et al.* 2015, Tabachnick 2010, Weaver and Reisen 2010, Wilson *et al.* 2008, Wilson 2008).

Progress and knowledge gaps in genetics

Molecular techniques developed for studying the genetics of *Culicoides*, their hosts, and the viruses that they transmit are increasingly accessible to entomologists, particularly the polymerase chain reaction (PCR)-based technologies. The most significant application has been the routine use of DNA sequencing of molecular markers for phylogenetic analysis of *Culicoides* and species recognition (Harrup *et al.* 2015). This use of molecular marker sequencing has also extended to the amplification and identification of host

DNA markers from blood-fed female *Culicoides* (Martinez-de la Puente *et al.* 2015). In addition to increasing the range of hosts identified for many species, this development also offers preliminary insights into host-preference. Finally, BTV RNA detection via real time RT-PCR (rtRT-PCR) paired with vector species identification has become commonplace, enabling the implementation of robot-based DNA and RNA extraction and high-throughput processing of individual *Culicoides* to determine infection status.

The major entomological response to BTV outbreaks in Europe has been the development of wide-scale surveys of *Culicoides* fauna, including identification of geographic and temporal incidence. These surveys are underpinned by a taxonomic framework of varying quality, which is usually almost entirely focused on livestock holdings. The primary motive for funding such research programmes is to obtain data that would enable authorities to demarcate risk of BTV transmission in space and time, in order to predict risk of virus incursion and spread (Carpenter *et al.* 2009a). These estimates of risk need to be sufficiently robust to permit drafting policies and legislation regulating the safe movement of ruminants (through identification of periods of low vector activity) and driving vaccination campaigns (through identification of peaks in vector abundance).

It is known that *Culicoides* vary in their vector capacity according to species and population and that this influences the distribution and spread of BTV. As an example, vector competence for BTV (the proportion of the population capable of developing transmissible infections) is known to vary at both a species and population level within *Culicoides* populations in the United States (Tabachnick 1996). Interspecific variation in vector competence is thought to drive BTV distribution in the Eastern states of the USA via the presence or absence of the confirmed vector, *Culicoides sonorensis* (Purse *et al.* 2015). Similarly, sustained BTV transmission on the East coast of Australia is entirely reliant upon the abundance of *Culicoides brevitarsis*, the presence of which determines risk of infection for ruminants (Bishop *et al.* 2004). However, the transmission of BTV in these countries occurs via a very restricted and well defined number of *Culicoides* species, in contrast to many other regions of the world that support diverse vector assemblages, including Europe.

In Europe, until 2006, research on *Culicoides* was aimed primarily at monitoring the geographic range of *Culicoides imicola*, by using light-suction trap surveys across the Mediterranean basin and Southern Europe (Mellor and Wittmann 2002, Wilson 2008). The scale, intensity, and longevity of systematic trapping conducted, in particular Italy and Spain, has been one of the major achievements

in *Culicoides* research since 2003 and the datasets on incidence produced are the most complete for the genus (Calvete *et al.* 2006, Conte *et al.* 2007). Following the 2006 incursion of BTV-8, however, the confirmation of earlier studies implicating other European *Culicoides* species in BTV transmission (Carpenter *et al.* 2006, Jennings and Mellor 1988, Savini *et al.* 2005) led many research efforts to focus on the areas of spread of BTV-8 where *C. imicola* was absent. While specific vector identification was limited to sub-genus level by technical issues, it is generally accepted that the major species involved in transmission of BTV within Northern Europe were within the subgenus *Avaritia* (Carpenter *et al.* 2006, Hoffmann *et al.* 2009).

It is notable that despite the huge economic impact of Bluetongue (BT) in Europe since the late 1990's, systematic attempts to characterize the *Culicoides* fauna within European countries using molecular marker methods have been limited both in scale and intensity. This issue was examined in a previous review, which highlighted the lack of a modern monograph for the Northern Hemisphere (Meiswinkel *et al.* 2004a). Studies in Italy (Gomulski *et al.* 2005, Gomulski *et al.* 2006), France (Perrin *et al.* 2006), and across Europe (Nolan *et al.* 2007) were aimed at characterizing putative vector species of BTV, primarily within the *Culicoides* and *Avaritia* subgenera. It is notable that all 3 studies sequenced different regions of DNA and no consensus has been reached regarding which and how many markers should be used. Although, for species recognition, the mitochondrial gene cytochrome c oxidase 1 is by far the most commonly used across vector groups (Garros *et al.* 2014).

While studies in Europe were broadly congruent with the morphological framework used to identify putative vectors, additional species were also proposed by the Italian studies, which have not since been integrated into standard surveillance reporting. This problem was again evident in the recent proposals of unrecorded *Culicoides* species within the subgenus *Avaritia* (Kirkeby and Dominiak 2014, Meiswinkel *et al.* 2015, Wenk *et al.* 2012) and the subgenus *Culicoides* (Lassen *et al.* 2012, Pages *et al.* 2009). While not guaranteed to be of epidemiological relevance in the transmission of BTV, the presence of such species may influence previous studies, which have used multiplex PCR assays to differentiate individuals, in addition to those using morphological identification.

Characterization of *Culicoides* species assemblages, even when restricted solely to livestock associated habitats, remains problematic worldwide. Identification of the fauna of South America, India, North America, and Russia is currently entirely based on expert morphological identification. While these areas have supported some of the best-regarded

taxonomists in the history of *Culicoides* research, it is clear that fundamental revisions using integrated molecular and morphological approaches will be required during the next 10 years. This process has advanced in Australia and South-East Asia, with recent studies utilising a high degree of quality control to explore species assemblages (Gopurenko *et al.* 2015). A major development is the increasing internet-based availability of both types of data with a degree of quality control, like for instance the Barcode of Life project (Ratnasingham and Hebert 2007). A key issue in the provision of databases with limited curation has been that the data uploaded are often incomplete (e.g. lacking original sequence chromatograms or accurate sampling location data). Adopting a more restrictive approach to quality standards and information required to upload data should yield more comparable results among regions of the world.

In addition to taxonomic identification, sequencing of molecular markers has also been used to examine intra-specific relatedness in *C. imicola*, as a means to assess the timescale of residence in specific regions (Dallas *et al.* 2003, Nolan *et al.* 2008). These studies were initiated by a Europe-wide examination of *C. imicola*, which confirmed its status as a single phylopecies and identified 11 haplotypes in populations across the Mediterranean basin and the Republic of South Africa, by using partial mitochondrial cytochrome oxidase subunit I (COI) gene sequences. Divisions in matrilineal structure were concordant with BTV incursions routes, with isolation between Eastern and Western populations (Dallas *et al.* 2003). While recent incursions of *C. imicola* into Europe were initially hypothesised a later study, using a panel of more rapidly evolving microsatellite markers, suggested that this species had been present in mainland Italy for a considerable period of time (Mardulyn *et al.* 2013). This contrasted with conclusions from Spain, where a recent colonization event was suggested through comparison of COI barcode sequences (Calvo *et al.* 2009). A coherent study using multiple molecular markers across the Mediterranean basin, Middle East, Asia, and Africa would be valuable in understanding the emergence of *C. imicola*, which has the widest range of any major vector species of BTV (Harrup *et al.* 2015).

Despite these challenges, significant progress has been made in Northern Europe in linking species identification of *Culicoides* to vector competence, by both field and laboratory research. Initial studies in this region suffered from a lack of understanding of the process of infection and dissemination of BTV in *Culicoides* (Carpenter *et al.* 2009b). However, later experimentation was notable for the integration of robot-based technology and rtRT-PCR into screening of field collected *Culicoides* for BTV RNA, enabling

unprecedented speed of processing, as demonstrated by a study carried out in Germany, which screened 24,513 pools from 2007-2008 (Hoffmann *et al.* 2009). In addition, the use of rtRT-PCR to detect virus is far more robust than isolation of arboviruses, as it does not require a cold chain or specialised preservation media, and it is more easily paired with ethanol-based preservation, which is used by the vast majority of collectors. More recently, an elegant study in the Netherlands directly linked disseminated Schmallenberg virus infections to molecular marker haplotype sequences for the first time (Elbers *et al.* 2013), paving the way for more straightforward population-level examinations of arbovirus transmission. While an earlier, laboratory-based experiment had also linked virus isolation to identification using a multiplex PCR assay (Carpenter *et al.* 2008a), the additional level of detail provided by phylogenetic comparison of molecular markers will be of significant interest in future studies.

Transmission of BTV in many regions remains extremely poorly characterized, with little information available for many areas, including the majority of South America, India, China, and Africa (beyond extensive studies in South Africa). This is largely a consequence of either the limited impact of endemic BTV circulation in these regions and logistical difficulties in implementing techniques based on infectious BTV isolation. Very few laboratory-based studies of vector competence have been conducted outside Europe from 2003-2014, whereas systematic testing in South Africa has continued and has demonstrated the potential for species outside the primary vectors in the region to become infected with BTV (Del Rio Lopez *et al.* 2012, Venter *et al.* 2011) and African horse sickness virus (Venter *et al.* 2009; Venter *et al.* 2010). With the anticipated availability of PCR-based technologies in laboratories worldwide during the next decade, studies of vector competence in *Culicoides*, in both field and laboratory, should enable a clearer understanding of the role of species in transmission of BTV at a global scale.

The advent of molecular marker sequencing also enables routine analysis of blood-meal origin in *Culicoides* as a means of inferring host-preference as it has recently been reviewed (Martinez-de la Puente *et al.* 2015). The majority of putative vector *Culicoides* species in Europe have been found to utilise a wide range of mammalian hosts, including human beings (Carpenter *et al.* 2013). The separation between *Culicoides* species feeding on avian and mammal hosts remains relatively distinct. In this respect, it is worth noting that hypotheses have been put forward that this separation is reflected by morphological differences in antennae. While these studies have been informative in defining broad host species acceptability, they provide limited

information where they are not paired with detailed descriptions of host availability and ecology in the environment. To date, the best example of such studies have been conducted in Sweden, where *Culicoides* species identification was underpinned by COI barcoding (Pettersson *et al.* 2013). Similarly, an earlier study in Scotland attempted to identify the hosts of *Culicoides impunctatus*, using forage ratios (Blackwell *et al.* 1994, Blackwell *et al.* 1995). Another limitation is that blood-fed females almost always represent a relatively small proportion of light-suction trap catches and resting areas for these remain largely undescribed.

True manipulative studies of *Culicoides*' host-preference remain extremely rare worldwide, this is primarily due to the intensive effort required in conducting them. An exception in Europe was a recent ground-breaking study that used sticky trapping on hosts to define numbers of *Culicoides* attracted to poultry, a calf, sheep, a goat, and a horse (Viennet *et al.* 2013). In this case, the horse bait was found to attract 95% of the *Culicoides* collected within the trial, due in part to the large size of this host in comparison to other species used. A key area that requires further investigation in BTV epidemiology is *Culicoides*' host-preference between adult cattle and sheep. There is a large amount of anecdotal evidence that cattle attract greater numbers of BTV vector *Culicoides* and hence may act to drive BTV transmission. The impact of co-grazing sheep flocks with cattle herds in either increasing or decreasing *Culicoides* biting rates has not been examined, although earlier studies implied a reduced level of BTV transmission to sheep (Du Toit 1962). In addition, *Culicoides*' host preference for certain individuals, which has been characterized for *Culicoides impunctatus* and human beings in Scotland (Logan *et al.* 2009, Logan *et al.* 2010), has not been demonstrated for livestock. This could be approached through the use of rapidly evolving molecular markers for hosts, such as microsatellites (Torr *et al.* 2001), or single nucleotide polymorphism genotyping, which would allow for tracing blood-meals to a specific host individual.

Progress and knowledge gaps in genomics

Since 2003, a major advance has been the recent implementation of techniques based on genomics, although these are currently only applied to colony lines of the North American vector *C. sonorensis*. Transcriptomic analyses have already been deployed to examine the areas of developmental biology in *C. sonorensis* (Nayduch *et al.* 2014b) and aspects of innate immunity (Nayduch *et al.* 2014c). In addition, the first full genome construction for any *Culicoides*

has been initiated and is due for public release by The Pirbright Institute and the European Bioinformatics Institute during 2016 (Nayduch *et al.* 2014a). These exciting developments are likely to provide a major focus for studies in the upcoming decade and a wealth of opportunities for collaboration among entomologists, geneticists, and bioinformaticians.

While studies at present are entirely focused on colony lines of *C. sonorensis*, the upcoming decade is likely to see such techniques increasingly applied to other species. Among species that can currently be colonised with existing methods, *Culicoides nubeculosus* is an obvious target as a closely related species to *C. sonorensis*, but largely refractory to arbovirus infection. Similarly, *Culicoides riethi* also has a history of successful colonization (Boorman 1974) and, unlike *C. sonorensis* and *C. nubeculosus*, exhibits autogeny. Comparative genomics studies among these relatively closely members of the *MonoCulicoides* subgenus would provide a first glimpse of underlying mechanisms for very different phenotypes.

Among other major BTV vector species, *C. imicola* would be an obvious target for study as it possesses a vast geographic range and has a huge impact as a vector of arboviruses (Meiswinkel *et al.* 2004b). There are existing methods to blood-feed *C. imicola*, pioneered in the Republic of South Africa (Venter *et al.* 1991), which have the potential to act as a platform for genome-level analysis of vector competence. In addition, progress has been made in colonising *C. imicola* (Veronesi *et al.* 2009). Although, so far efforts did not result in consistent production. While not a prerequisite for many directions in genomic research, the production of *C. imicola* colony lines would enable straightforward investigations of areas such as vector competence, immunological responses, and physiological analyses. It would also assist in the production of cell lines specific to this species to complement those already produced for *C. sonorensis* (Nayduch *et al.* 2014a). Among the other primary vectors of BTV worldwide, there are several major challenges to be overcome before the full potential of genomic techniques can be realized. For many species, including all of those acting as major BTV vectors in Europe with the exception of *C. imicola*, blood-feeding techniques are only poorly effective and all previous attempts at colonization have failed (Nayduch *et al.* 2014a).

Progress and knowledge gaps in modelling of *Culicoides* population abundance and dispersal

The modelling of the spatial and temporal incidence of adult *Culicoides* has largely developed in parallel

with overarching assessments of BTV transmission risk. The use of these techniques played a major role in attempts to understand the drivers underlying the changing epidemiology of BTV in Europe. Within this subject, the influence of climate change on the transmission of BTV prompted a wide debate, which was brought into focus by an influential publication that integrated mathematical modelling, entomology, and virology (Purse *et al.* 2005). Earlier reviews proposed range parameters that might restrict the range of BTV in Europe, including climate, the presence of competent vectors and livestock susceptibility to infection (Mellor and Wittmann 2002, Sellers and Mellor 1993, Wittmann and Baylis 2000). However, the contribution of Purse and colleagues (Purse *et al.* 2005) to this debate remains unique in systematically and specifically assessing what potential mechanisms might be driving the current, and unprecedented, emergence of BTV in Europe. Hence, while at least 1 aspect of the paper (the hypothesised recent geographic spread of *C. imicola* Northwards in Italy) has been convincingly disproved, alternative regional-scale hypotheses to explain the dramatic shift in BTV epidemiology, and which do not invoke climate change, have not to date been produced.

Within wider attempts to understand the incidence of BTV, studies focusing on *Culicoides* ecology as a pre-requisite for transmission have advanced substantially since 2003. This is partly due to increasing availability of computer processing power and mathematical theory. Research on the distribution of *Culicoides* has been conducted on farm (Kirkeby *et al.* 2013), between farms (Kluiters *et al.* 2013), country (Conte *et al.* 2007), regional (Purse *et al.* 2007), and global scale (Guichard *et al.* 2014). Following the identification of BTV transmission in areas of Europe that did not support *C. imicola*, including Italy and the Balkans, assessing the risk of further Northward movement of the virus was in part reliant upon understanding the degree of similarity in species complement across the Palaearctic region. It was known from earlier studies that members of the subgenus *Avaritia* might be capable of BTV transmission (Jennings and Mellor 1988, Mellor and Pitzolis 1979). Nonetheless, identification to species level in samples was not entirely achieved either in these or in later studies both in the field (Caracappa *et al.* 2003, De Liberato *et al.* 2005, Savini *et al.* 2005) and in laboratory (Carpenter *et al.* 2006). This has meant that the relative involvement of *Culicoides* species in the transmission of BTV-8 remains unclear.

The datasets in later modelling studies of BTV transmission in Northern Europe also contained multiple species grouped as a single unit that were known to possess different ecologies. This contrasted with earlier studies of *C. imicola* in the Mediterranean basin, which was known to represent a single species

(Dallas *et al.* 2003). Initially, the majority of studies in Southern Europe focusing on BTV transmission in *C. imicola*-free countries did not differentiate female *Culicoides* of the subgenus *Avaritia* (Calvete *et al.* 2008, Purse *et al.* 2006). This was also the case with many later studies in Northern Europe following the BTV-8 incursion in 2006 (Clausen *et al.* 2009, Kluiters *et al.* 2013, Searle *et al.* 2014). Later studies distinguish between *Culicoides dewulfi* and *Culicoides chiopterus* from what is now commonly termed the *C. obsoletus* complex (*C. obsoletus*, *Culicoides scoticus*, and *Culicoides montanus*) using their morphology (Meiswinkel *et al.* 2014). Although morphometric techniques can be used to separate *C. obsoletus* from *C. scoticus* (Nielsen and Kristensen 2011), these have not been routinely employed for identification of surveillance samples where it is often the case that thousands of *Culicoides* need to be identified. Similarly, the application of differentiating assays remains unrealistic for very large studies given both time and direct cost of materials (Garros *et al.* 2014, Harrup *et al.* 2015), although studies involving several thousand individuals have been accomplished (Balczun *et al.* 2009, Carpenter *et al.* 2008b, Pili *et al.* 2010). A high-throughput rRT-PCR assay has been devised to differentiate the relative numbers of *C. obsoletus* and *C. scoticus* in homogenized pools of individuals (Mathieu *et al.* 2012). The test has the potential to be scaled to high-throughput format. However, it has not been fully integrated into surveillance sample identification.

The members of the subgenus *Avaritia* in the Western Palaearctic region are known to vary significantly in their biology and ecology. The most obvious difference is that *C. chiopterus* and *C. dewulfi* develop as larvae directly in dung (Kettle and Lawson 1952), whereas *C. obsoletus* and *C. scoticus* are far less specific and occupy a wide range of habitats (Harrup *et al.* 2013, Kettle and Lawson 1952). In addition, during trials using animal bait, it has become clear that *C. chiopterus* abundance is significantly underestimated by light-suction trapping (Carpenter *et al.* 2008b), although the underlying cause remains unclear.

Differences in phenology have also been recorded between *C. obsoletus* and *C. scoticus* in the limited number of studies where these species are separated and in studies that have examined male populations (which can be distinguished to species level) (Pili *et al.* 2010, Sanders *et al.* 2011, Searle *et al.* 2014). It is currently unknown what impact this lack of species-level differentiation has on the accuracy of models, although country- and regional-level models of distribution and seasonality are generally less robust than those constructed for *C. imicola*. As a vast number of collections of *Culicoides* have been made across countries in Northern Europe, targeted

studies to understand this issue would be useful in addressing the epidemiology of BTV. These will likely lead to more accurate prediction of the seasonality of transmission, which has been the major disease response policy impact of entomology in the region (Searle *et al.* 2014).

Models of *Culicoides* dispersal have also been produced and reviewed (Burgin *et al.* 2013). These originally employed air trajectories to predict long-range movement of *Culicoides* over water bodies (Sellers 1980). However, within the period of this review, they have become vastly more sophisticated through the use of simulation modelling. The most complex of these models is based upon the UK Met Office's Numerical Atmospheric-dispersion Modelling Environment (NAME), a Lagrangian particle model, which calculates dispersion by tracking simulated particles through a modelled atmosphere. Numerical Atmospheric-dispersion Modelling Environment has been adapted to predict *Culicoides* movement using knowledge of adult phenology (Sanders *et al.* 2011), diel periodicity (Sanders *et al.* 2012), activity under specific meteorological conditions (Sanders *et al.* 2011, Sanders *et al.* 2012), and probable flight range. This approach is now used consistently in retrospective analysis of the source of BTV outbreaks and in predicting vulnerable areas to this process (Eagles *et al.* 2012, Eagles *et al.* 2013). Due to the complexity of modelling movement over complex landscapes, NAME is most applicable to movement over water bodies (Burgin *et al.* 2013). Models that include virus surveillance data have also been used to try and quantify movement of *Culicoides* between farms. These have illustrated that long-distance dispersal over land is vastly less common than over water bodies (Hendrickx *et al.* 2008), and that upwind flight (amenotaxis) may be an important component in determining spread. To date, these models have not been integrated into those describing the transmission of BTV in the field and unrecorded animal movements remain a challenging confounding factor even in regions maintaining restrictions.

Conclusions

Entomologists are an integral part of the response to BTV incursion, but sustaining expertise in this area in the face of economic pressures in research remains a major challenge. Increasingly, specialist entomologists with skills in classical taxonomy and ecology are becoming rare and these areas are more likely to be approached by virologists, molecular biologists, or epidemiologists who have an interest in applying their skills to insects. Unlike traditional roles based on a single vector group, these scientists

are often discipline-based (e.g. specializing in molecular diagnostics) and far more able to adapt to fluctuations in funding availability, but they lack the depth of specialist knowledge of the traditional entomologist role. In one aspect this represents a natural process of evolution, without which the full implementation of novel and exciting technologies in *Culicoides* research would be impossible. A concern, however, is that this also results in a process of 'reinventing the wheel' where the community memory is limited to a few years, rather than the career spanning knowledge of specialists.

Within what is a relatively small community, connectivity among scientists is a major factor in determining the successful implementations of techniques based on genetics, genomics, and mathematical. Through the almost universal use of internet-based resources by scientists, communication among *Culicoides* experts has advanced hugely in the past decade and the increasing use of free-access resources¹ will become commonplace as alternatives to traditional publishing, alongside broader data repositories². A similar process will also occur with the upcoming release of the first genome build of *C. sonorensis*,

which will be a major landmark in the field and provide an impetus for a wide diversity of studies in the next decade. It is likely that public funding agencies will also increasingly demand public accessibility of datasets across research areas as part of their support and this may improve the current sharing of information. This may favourably impact the continuity of datasets which at present are fragmented across countries and regions, in part due to the way in which funding is obtained.

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¹ e.g. www.ibvnet.com; <http://avabase.cirad.fr/>; <http://www.iikculicoides.net/>.

¹ e.g. www.boldsystems.org/.

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