Progress and knowledge gaps in Culicoides ecology and control

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Summary

The Bluetongue and Schmallenberg virus outbreaks in Europe between 1999 and 2013 stimulated progress in *Culicoides* field ecology and control. In responding to these outbreaks, however, the deficit of broadly trained entomologists became evident. *Culicoides* research has been uneven, and critical gaps remain in our knowledge of vector ecology. Certain aspects of adult midge biology have gotten more attention (biting rates and relationship to trap collections, seasonal activity and survey, taxonomy of critical species complexes). Some aspects of adult midge biology (survival, resting site selection, or direct dispersal measurements) have been understudied. More work is needed on *Culicoides* immatures (taxonomy and details of ecology). Control research has centred on insecticide treatments applied to animals. However, our true goal is not vector control, but reducing disease agent transmission. We require field vector control targets (*e.g.,* how far we might need to reduce biting rates) needed to interrupt transmission. The most critical need is for studies incorporating vector control experimentally into epidemiological studies in active arbovirus transmission zones. Such field interdisciplinary studies are absolutely required to understand transmission dynamics. Through the power of experimental field studies, we should be able to develop both theoretical and operational guidelines for disease management.

Progressi e lacune nella conoscenza dell'ecologia di Culicoides e del relativo controllo

Parole chiave
Bluetongue,
Controllo,
Culicoides,
Ecologia,
Vettore.

Riassunto

Le epidemie di Bluetongue e della malattia di Schmallenberg registrate in Europa tra il 1999 e il 2013 hanno stimolato gli studi sull'ecologia e sul controllo di *Culicoides*. Allo stesso tempo, la risposta alle emergenze epidemiche ha anche evidenziato il deficit di entomologi con conoscenze adeguate per gestire i focolai di malattia. La ricerca su *Culicoides* è stata discontinua, attualmente ci sono ancora importanti carenze nella conoscenza dell'ecologia del vettore. Alcuni aspetti della biologia degli insetti adulti hanno ottenuto maggiore attenzione, come il rapporto tra numero di punture ed esemplari catturati con trappole, l'attività stagionale, la tassonomia delle specie e dei complessi. Altri aspetti, invece, come la sopravvivenza, la scelta dei siti di riposo e le misure dirette di dispersione sono stati oggetto di minor interesse. Questo è anche il caso della ricerca sugli esemplari immaturi (tassonomia ed ecologia). Altre lacune sono emerse negli studi condotti sul controllo, prevalentemente incentrati su quello di tipo vettoriale, realizzato mediante il trattamento degli animali con insetticidi. Ciò ha determinato un disinteresse per lo studio della riduzione della trasmissione dell'agente patogeno che è il vero obiettivo della ricerca. Si ravvisa la forte necessità di fissare obiettivi per il controllo su campo dei vettori che permettano di interrompere la trasmissione del virus (ad esempio: è necessario stabilire una soglia minima di punture). La necessità più critica, comunque, riguarda lo sviluppo di studi che includano su base sperimentale il controllo vettoriale nelle zone in cui la trasmissione di arbovirus è attiva. Pertanto risulta di fondamentale importanza la realizzazione di studi interdisciplinari per la comprensione delle dinamiche di trasmissione e studi condotti su campo per sviluppare le linee guida teoriche e operative per la gestione della malattia.

Introduction

Since the first international meeting on Bluetongue (BT) in 1984 (Barber and Jochim 1984), great progress has been made in our understanding of *Culicoides* midge field ecology. We have also made some progress on control methodologies. Reviewing comprehensively such extensive progress is beyond the scope of this paper, as that has been done elsewhere (*e.g.,* Carpenter *et al.* 2008b, Purse *et al.* 2015). Rather, the present paper aims at categorizing relative progress in different areas of *Culicoides* field research and suggesting areas for future study. This exercise will be made with the assumption that our main goal is to better understand arbovirus transmission by *Culicoides* and thereby to interrupt or reduce that transmission.

Entomology training programs are necessary

The recent outbreaks of Bluetongue viruses (BTV) in Europe between 1999 and 2009 (Mellor *et al.* 2009) serve as a reminder of how necessary entomologists are. As the outbreaks began, many countries scrambled to marshal their scientific resources. They immediately encountered difficulties even in basic insect identification, but perhaps just as profoundly in how most efficiently to determine which *Culicoides* were likely transmitting viruses. To a non-specialist, such as a veterinarian charged with addressing this problem, it must have seemed inconceivable. How could we know so little about biting midges, in one of the most scientifically-advanced regions of the world? After all, surely this basic faunal information existed already. All the insects should be identifiable with a dissecting microscope and a few minutes work by anyone with an identification key!

Cuisance and Rioux (2004) discussed the serious lack of medical and veterinary entomologists in France, and their analysis can be considered representative of many countries around the world. However, this is merely a part of a more general global decline in entomological training programs, especially at the graduate level. In some cases, specialised training programs in medical and veterinary entomology have been developed (Baldet *et al.* 2011). Nonetheless, specialised training cannot substitute for comprehensive training in entomology PhD degree programs, including areas such as taxonomy, morphology, physiology, and ecology. An entomologist is much more likely to understand (and ask questions about) how insects actually function in their environment: how do they feed and develop eggs, resist environmental pathogens, respire in saturated soil or polluted water, disperse, or tolerate cold in winter? An entomologist is more likely to appreciate the connections between studies on disparate groups of arthropods. For example, the non-specialist would probably do a computer-based literature search for '*Culicoides*', thereby gaining access to recent papers that list '*Culicoides*' among the keywords (perhaps missing older and sometimes more robust articles). But they would be less likely to appreciate the fact that vast amounts of relevant literature was developed focusing on other insect groups, such as mosquitoes or even aphids (their dispersal has been studied and they are in the same size range as *Culicoides*), and to look there for guidance or insight.

The take-home message is clear and simple. The broader scientific community should be aware of the critical need for specialists in such a bewilderingly diverse group as insects. When your organization discusses eliminating yet another entomological unit, oppose it.

Progress and knowledge gaps

Taxonomy and systematics of *Culicoides*

In certain countries, such as the United Kingdom or France, significant survey and taxonomic work had been done by the late 1970's. This yielded a pretty good morphologically-based listing of *Culicoides* species present, though new species still are being discovered even in Western Europe (Ramilo *et al.* 2013). In other regions, including well-developed but large and diverse countries such as the US or Canada, the knowledge of faunal composition is still very patchy and incomplete. As mentioned above, trained entomologists are needed to understand the significance of species complexes, and to know how and where to look for them. Many new species await description, such as a new species in the same subgenus as the well-known BT virus vector *Culicoides sonorensis* recently described in the American West (Grogan and Phillips 2008). Even in a generally well-studied region such as California (Wirth 1952), specialized habitats frequently yield new species. *Culicoides* surveys from 1988-1990 in the desert mountains of Southern California, for example, revealed 19 *Culicoides* spp., itself rather surprising for an aquatic insect group in the desert. Amazingly, 5 of these (26%) were detected as undescribed by the eminent ceratopogonid taxonomist W. W. Wirth (Mullens and Dada 1992); the 3 most common ones have been given names (Wirth and Mullens 1992, Breidenbaugh and Mullens 1999). Only a true specialist would have spotted them; even most entomologists would likely just have placed them with the closest known species.

Borkent and Grogan (2009) estimated that, for the entire biting midge family *Ceratopogonidae*, we know perhaps 30% of existing species. The genus *Culicoides* is probably much better known than that, due to its blood-feeding habits. However, information about this genus is still scanty in many parts of the world such as Africa, South America, and Asia. Dedicated small groups of entomologists work in their regions to describe new species and provide precious scientific windows into the substantial global diversity of *Culicoides*, especially in the tropics (Spinelli *et al.* 2013).

With European BTV vectors in the *Culicoides* subgenus *Avaritia*, we already knew from older work that we had an identification problem; that subgenus has been problematic worldwide (Garros *et al.* 2014). Males of the several Western European species could be separated morphologically, but the females were another matter. It took the BT outbreak to really stimulate (through increased funding) molecular studies to allow females of the important Obsoletus group to be separated successfully. Sustained progress in this area in Europe now has resulted in an interactive identification key (Mathieu *et al.* 2012). Similar efforts would be very useful in other parts of the world; for example we still lack a US key to *Culicoides* species. Integrating molecular and morphological techniques has helped immensely in our understanding of species boundaries (Bellis *et al.* 2014), and these 2 approaches should be extended in tandem geographically, beyond the rather limited areas investigated so far.

Taxonomy of *Culicoides* immature stages lags far behind our understanding of the adults. Borkent (2014) noted that, among all *Culicoides*, only 3% of species are known as eggs, 13% as larvae, and 17% as pupae. While immatures of a few more *Culicoides* species continue to be described (Ronderos *et al.* 2010), even basic morphological description is lacking for most species. Of 144 *Culicoides* spp. known from the US in 1991, for example, only about 1/3 (49) were known as larvae even after the monumental work of Murphree and Mullen (Murphree and Mullen 1991). The North American situation has not changed much since then. Astoundingly, even the immature morphology of the main North American BTV vector, *C. sonorensis*, which has been in colony for many years, is only now finally being studied in any detail. Morphologically-based descriptions would likely include studies of larval mouthpart structures and other features also useful in understanding their ecology (Murphree and Mullen 1999, Ronderos *et al.* 2010). So far molecular identification techniques have not been widely applied to studies of *Culicoides* immatures, although they have the potential to help immensely with ecological studies (Schwenkenbacher *et al.* 2009, Yanase *et al.* 2013). Ultimately, immature taxonomic work has much to offer to our understanding of *Culicoides* and Ceratopogonid systematics (Glukhova 1979, Borkent 2014). For example, some aspects of current sub-generic classifications are somewhat controversial and based mostly on adult characters.

Assuming we can define systematic relationships correctly, they have much to offer to our understanding of the ecology of the species groupings. This in turn relates to control prospects. For example, the subgenus *Monoculicoides* includes the well-known North American BTV vector, *C. sonorensis*. Other species in the subgenus (*e.g., Culicoides nubeculosus*, *Culicoides riethi*, *Culicoides parroti*) tend to be found in similar types of larval habitats. In this case, studies on biology or control done with *C. sonorensis* (population dynamics, habitat manipulation for control of immatures) might transfer reasonably well to other members of that subgenus. But *C. sonorensis* studies might be irrelevant for other groups such as the subgenus *Avaritia*.

Ecology of *Culicoides* immatures

If we could pick one factor that dominates abilities of *Culicoides* spp. to exist in different geographic regions or on local scales in numbers sufficient for pathogen transmission, it should be the nature and abundance of suitable immature habitats. Our profound ignorance of this aspect of *Culicoides* biology continues to cripple our abilities to understand their distribution and factors regulating their abundance.

Culicoides spp. utilize a variety of moist microhabitats for oviposition and larval development, and recent farm surveys have been helpful to identify where immatures may be located (Gonzales *et al.* 2013, Harrup *et al.* 2013, Zimmer *et al.* 2014). Many of these habitats are found in association with livestock, although importance of more natural habitats is less studied with regard to virus vectors. It is well known that *C. sonorensis* develops in the edges of wastewater ponds on farms. However, the species may also take advantage of other ephemeral water sources for development (*e.g.,* puddles, irrigation leaks, trough spillover) (O'Rourke *et al.* 1983), including pristine edges of unpolluted desert streams or seeps (Mullens, unpublished). The importance of these habitats for maintaining *C. sonorensis* populations may have been underestimated. That species is famously associated with polluted ponds, but a dairy in Southern California that did not have a wastewater pond had unusually high numbers of *C. sonorensis* adults, and irrigation runoff on pasture supported large numbers of larvae (McDermott, unpublished). In Northern Spain, composting manure and rotting leaf litter produced high numbers of the potential BTV vectors, *C. obsoletus* and *C. scoticus*, respectively, and the greatest diversity of *Culicoides* spp. emerged from samples collected from ponds and areas of water runoff (Gonzalez *et al.* 2013). Similar microhabitats were identified during a survey of a Belgian cattle farm, including silage residue, old manure, flooded pasture, and ponds (Zimmer *et al.* 2013a). The chemical composition of these habitats may indicate their suitability for *Culicoides* (Uslu and Dik 2010). In silage residue, high lignin content was positively related to larval *C. obsoletus* and *C. scoticus* development, while high levels of calcium and magnesium were negatively related (Zimmer *et al.* 2013b). A better understanding of which habitats are most important for the development of vector species, and of what, specifically, makes these habitats attractive or suitable could provide new avenues for better vector control.

With the exception of several studies on the effect of temperature on development, which will not be discussed here, very few manipulative studies have been done with immature *Culicoides* to determine their physiological tolerances or responses to insecticides. One study examined the desiccation tolerance of the eggs of *C. sonorensis* (McDermott and Mullens 2014). Contrary to the popular belief that *Culicoides* eggs are completely susceptible to drying, some eggs were able to withstand losses of over 50% of their initial water weight and most could tolerate at least a 20% loss. Many *Culicoides* spp. probably exist in ephemeral habitats, so tolerating at least short-term desiccation might be more widespread than we expect. That we still have no idea of the function of the bumps or stalks, called ansullae, which are prominent on many *Culicoides* eggs, highlights our lack of knowledge of this immature stage. Current research on *Culicoides* pupae is similarly lacking, with the exception of a few new morphological descriptions (*e.g.,* Ronderos *et al.* 2013). Pupal ecology, beyond their being found in surface layers of substrate, has been mostly overlooked.

The literature regarding larvae is slightly more robust. The infectivity of several strains of entomopathogenic fungi (genera: *Metarhizium, Beauveria, Isaria,* and *Lecanicillium*) for *C. nubeculosus* larvae was examined by Ansari *et al.* (2010). One species in particular (*M. anisopliae*) was found to be highly pathogenic to larvae, although its use in control programs may be limited by the need for supplemental inoculations. Of *Culicoides* natural enemy groups, pathogens are probably most likely to seriously impact field populations (and have promise for management), although predators or parasites such as mermithid nematodes may be more important than we appreciate. A small study by Reeves (2010) showed that in the presence of a common freshwater predator - *Hydra littoralis* - *C. sonorensis* larvae spend more time actively swimming than they do in the absence of a predator, indicating a possible avoidance behaviour that might cost larvae considerable energy and reduce survival. Environmental contaminants might also be manipulated for control of *Culicoides*.

Our greatest need with immatures is to understand their basic ecology. What do the larvae eat, for example, and how do they manage that? How well do they overwinter? Knowing how *Culicoides* interact with their environment would greatly improve our understanding of distributions and population dynamics, and could be extremely helpful in informing control practices. Biodiversity surveys of these habitats could turn up new natural enemies or pathogens of *Culicoides* and help us understand the significance of natural mortality factors. Studying how eggs, larvae and pupae respond to environmental challenges, like freezing and desiccation, could help predict the potential ranges of vector species. Choosing to largely ignore three-fourths of the *Culicoides* life cycle (*e.g.,* focusing only on adults) is simply unwise.

Adult field biology

Because more work has been done in this area recently, we will highlight a few important areas specifically to increase awareness of them.

Temperature effects on diel host-seeking activity

For many *Culicoides* species, peak host-seeking activity occurs near sunset and/or sunrise with reduced activity throughout the night, and limited or no activity during daylight hours (Kettle 1969, Barnard and Jones 1980, Mellor *et al.* 2000). This crepuscular feeding habit offers opportunities for reducing attack rates on animals by moving them indoors in late afternoon (Meiswinkel *et al.* 2000) or by applying repellents or feeding barriers to animals prior to the start of the evening activity period. However, as evening temperatures begin to cool seasonally, adult activity may shift into daylight hours, when temperatures are still warm enough for flight. For example, the host-seeking activity of *C. obsoletus* in Northeastern Spain during September was greatest in the period immediately before sunset, but peak activity shifted to as much as 90 min before sunset on colder days (Gerry *et al.* 2009). Diurnal host-seeking activity of crepuscular species has been noted previously (Barnard and Jones 1980, Mullens 1995, Carpenter 2008a) and may be common during cooler weather when nighttime temperatures restrict adult activity. This shift to earlier host-seeking during cool weather may reduce effectiveness of cultural or chemical control techniques applied to reduce biting near sunset, unless timing of control measures is similarly adjusted.

Temperature thresholds for adult activity are poorly studied for most *Culicoides*. Temperature thresholds resulting in reduced activity have been determined for some important BTV vectors, including *C. sonorensis* at 10°C (Nelson and Bellamy 1971) and *C. brevitarsis* at 18°C (Murray 1987). Exceptionally low daytime temperatures may restrict adult host-seeking activity altogether with midges remaining in daytime resting locations awaiting warmer temperatures. For some species there is evidence for wintertime activity on warm days. This suggests that overwintering adults will return to host-seeking behaviours under permissive temperature conditions, but thermal thresholds for this activity are not known. If adult *Culicoides* present at the end of a seasonal pathogen transmission period are surviving in wintertime resting sites and simply waiting for a suitable flight temperature, the first warm day could pose considerable risk of pathogen transmission for animals in the vicinity of these resting sites! The idea is supported by a recent study (Mayo *et al.* 2014), who demonstrated presence of BTV in day-active, host-seeking (CO_2 suction traps) *C. sonorensis* collected in February in California's Central Valley.

Adult daytime resting sites

One of the great mysteries for most *Culicoides* species is identifying these midges' resting sites (Mullens *et al.* 2004). On dairies in California, for example, biting rates of *C. sonorensis* on cattle can be in the hundreds to thousands of bites per evening. While we have not made an intense effort, the limited scouting that we have done has not revealed their common daytime resting sites, despite very high midge populations. Resting sites for a few *Culicoides* have been identified (Bidlingmayer 1961, Carpenter *et al.* 2008a), but these may be site-specific and perhaps not generalizable across habitats even for the same species.

For many control programs focused on other vectors, management efforts target the resting adult population to provide immediate reduction in biting rates and pathogen transmission risk. This is a particularly common control strategy against many mosquito species of concern to public health. If daytime resting sites for *Culicoides* spp. were well characterized, control efforts, including applications of pesticides, could be similarly targeted to these sites. Trapping of adults at resting sites might also provide a means to monitor adult *Culicoides* abundance without the need for deploying semiochemical-baited traps. We additionally must keep in mind that the time spent resting vastly exceeds time midges spend flying and looking for hosts or oviposition sites. Knowledge of microhabitat conditions in the resting sites (particularly temperature versus typical weather data), is therefore absolutely critical for accurate modelling of population dynamics, temperature-dependent virogenesis, survival estimates, etc.

Dispersal and short-range movement

There has been considerable interest lately in evaluating the potential for long-range dispersal of *Culicoides*, with wind-aided movement of midges recorded (or more often inferred by circumstantial evidence) over hundreds of kilometers (Garcia-Lastra *et al.* 2012, Burgin *et al.* 2013, Eagles *et al.* 2014, Kelso *et al.* 2014). These studies are intellectually stimulating and may help to explain the introduction of exotic midge species and exotic viruses into new areas. However, the need to evaluate short-range movement of *Culicoides* is perhaps of greater direct importance to mitigating disease outbreaks and to reducing pathogen transmission in locations where midges and viruses already occur. While it is generally presumed that most *Culicoides* exhibit a limited flight range and remain near development sites (Mellor *et al.* 2000), the few studies to actually measure short-range movement of *Culicoides* suggest that midges can readily disperse over 1-2 km per night, travelling an average of >2 km over several nights (Lillie *et al.* 1981, Brenner *et al.* 1984, Kirkeby *et al.* 2013). Whether *Culicoides* tend to fly upwind following attractive host odours, as suggested by Kirkeby *et al.* (2013), or omnidirectionally, as suggested by others (Brenner *et al.* 1984, Sedda *et al.* 2012), is unknown and may depend upon the midge species, presence of hosts, and environmental characteristics. However, it seems unlikely that midges would fly upwind orienting toward odours from hosts 1,750 meters away, as suggested by Kirkeby *et al.* (2013), a result that may have more to do with trap placement near hosts at this distant location than with direction of flight by marked midges.

Understanding long-distance dispersal of virus-infected midges, *e.g.* aided by wind, would help us identify periods of greater risk, which in turn might trigger enhanced surveillance. Identifying short-range movement patterns for putative vectors is critical in order to developing local control strategies and useful quarantine restrictions to employ in the event of an outbreak (Sedda *et al.* 2012). Distances travelled, direction of travel relative to prevailing winds, and distance range for host odour detection are important factors that must be evaluated to provide the data needed to produce accurate models predicting transmission risk. Additionally, the age structure of the dispersing

Culicoides population is important. Dispersing nullipars may be of little consequence to spread of a pathogen, while a greater than expected proportion of dispersing individuals that are parous would have important ramifications for modelling efforts.

Relationship of surveillance methods to biting rates

Key vectorial capacity parameters include survival, competence, and biting rate, which operate in the context of the environment (Mullens *et al.* 2004). *Culicoides* survival studies to date in nature have mostly been based on inferences from parity profiles (Gerry and Mullens 2000, Lysyk 2007). Of the vectorial capacity parameters, survival of *Culicoides* has been relatively ignored by researchers recently; more use of direct survival estimates, such as mark-release-recapture, would be beneficial. Assuming adequate survival, the degree of virus transmission by *Culicoides* is likely to be predominantly a function of vector competence and the biting rate of each species. An increase in the biting rate of *C. sonorensis* in California was predictive of seasonal BTV transmission to cattle (Gerry *et al.* 2001). Documentation of host selection and use by *Culicoides* should be among the very first field activities done by researchers who wish to understand arbovirus transmission in a region.

Accurate measurement of biting rate can be difficult for hematophagous insects that feed on animals. Traps exposing and then enclosing animals for defined periods may be used (Schmidtmann *et al.* 1980b, Mullens and Gerry 1998, Carpenter *et al.* 2008c, Mayo *et al.* 2012), biting insects can be sampled by mechanical aspiration directly from an exposed animal (Schmidtmann *et al.* 1980a, Gerry *et al.* 2009, Scheffer *et al.* 2012), or insects landing on animals may even be caught directly using adhesive (Viennet *et al.* 2013). Because using bait animals can be difficult, relative midge abundance is often measured using traps baited artificially with host semiochemicals (usually CO_2) or UV light. However, the relationship of midge capture by these traps to the true animal-biting rate is usually purely speculative. When specifically compared, biting rates on animals have often been far greater than collections in artificially-baited traps (Mullens and Gerry 1998, Carpenter *et al.* 2008c, Gerry *et al.* 2009). This illustrates the complexities and likely synergistic nature of the various cues involved in host location by vectors (visual, chemical, heat, etc.), and we are only just beginning to study this with *Culicoides* spp. Importantly, the relationship between actual biting rates and artificially-baited surveillance traps differs by trap type and by midge species (Gerry *et al.* 2009), so that this relationship must be determined for each midge species and trap type in relation to each host of interest. Recently, additional researchers have begun to look more carefully at these relationships (Scheffer *et al.* 2012, Elbers and Meiswinkel 2014), but further work is needed particularly to identify variation among trap captures and host biting rates among available vector species.

UV light traps, or traps using other attractive wavelengths such as green (Bishop *et al.* 2006), are perhaps the simplest and most cost-effective surveillance tools (Elbers and Meiswinkel 2014). However, care should be used to interpret capture rates for pathogen transmission modelling until comparisons of capture rates to host biting rates have been conducted. These comparisons must be performed seasonally as well, given that light traps will only function during darkness and *Culicoides* activity may shift to daylight hours during cooler weather as described above. Alternatively, development of host semiochemical-baited traps with improved attraction to a range of midge species, perhaps using CO_2 in combination with attractive host odours (Harrup *et al.* 2012), may provide a more universal surveillance system with an improved relationship to actual host biting rates.

Control of adult and immature *Culicoides*

Culicoides may have direct effects on animal production (and human activities in some cases), especially when they reach very high numbers. While biting may impact animal production, we currently lack data to show such an effect. At the same time, while data on economic impacts of biting itself would be valuable, we are primarily interested in controlling the diseases associated with the pathogens that *Culicoides* transmit.

It is vital that we understand the critical distinction between incremental improvements in vector control on the one hand, and disease control via vector management on the other. We have made some progress with vector control techniques. However, we almost completely lack any experimental field studies, or even good field observational work, to link vector control to reductions in disease incidence. We have almost no idea how low we must get biting rates, for example, to have a reasonable expectation of reducing intensity of disease transmission. It is possible to have substantial success in reducing vector biting (say, by 50-80%), and still to have little or no impact on disease. Topical applications of insecticides such as permethrin were shown experimentally to reduce *C. sonorensis* blood feeding by about that much. Nonetheless, it is worthwhile stressing that field permethrin applications had no effect on season-long BTV seroconversion in a large California sentinel cattle herd (Mullens *et al.* 2001). However, it is clear that vector numbers or activity (expressed in trap catches and extrapolated biting rates) do drive transmission in the *Culicoides*-BTV system. In one especially intensive field study, vector populations and cattle seroconversion were monitored continuously on a California dairy farm for 3 years, to try to determine a threshold level of biting that was associated with BTV transmission (Gerry *et al.* 2001). CO_2-baited suction trap collections below about 16 *C. sonorensis* females/trap/night were equal to about 60 bites per cow per night, and were associated with little to no BTV transmission. Of course, other influential factors such as temperatures were changing simultaneously. Likewise, some dairies in California's Central Valley, a highly endemic zone for BTV, had such low *C. sonorensis* populations that Summer-Fall BTV transmission was undetectable (Mayo *et al.* 2012). The California BTV epidemiology studies above were observational in nature and illustrate that similar studies could and should be done in other regions. How much better would it be if we coupled experimental vector control with studies like this?

Why is it that we have done so poorly in this critical pursuit? There are many reasons, but we will highlight 3. First, areas where some of the best expertise exists (*e.g.,* the Pirbright Institute in the UK) are not necessarily in active transmission zones. Such studies typically require intense (and expensive) field study, and that is especially hard to accomplish if the researchers are far away from the active transmission areas. That said, there are several areas where detailed field epidemiology studies, and required lab support, might be marshalled in areas of dependable virus transmission by *Culicoides* (*e.g.,* California, Israel, India or South Africa). Second, such studies absolutely require interdisciplinary expertise and collaboration. Detailed field vector control experiments, at least beyond animal spraying with insecticides, tend to be designed and conducted by entomologists. Thus far, entomologists perhaps have not tried as hard as they could to connect with veterinarians, epidemiologists and virologists to accomplish more comprehensive studies. The collaboration, of course, goes 2 ways; more intentional effort to incorporate entomologists into such studies by other scientist groups would yield the kind of information we most need. Third, one must acknowledge that intensive, interdisciplinary studies are difficult and expensive. This is especially true in areas where more than 1 *Culicoides* species is probably involved in transmission, and where differences in species' biology (*e.g.,* diffuse breeding sites) present particular problems in applying certain types of control.

We must make the critical link between *Culicoides* control and orbivirus control; it is a matter of vision, resources, and will.

Carpenter and colleagues (Carpenter *et al.* 2008c) nicely reviewed control techniques for *Culicoides*, so we will try merely to supplement that review here. Control techniques fall into 4 general groupings:

1. chemical control (insecticides to kill vectors or strategic use of repellents to interrupt adult feeding);
2. biological control (use of natural enemies such as mermithid nematodes to reduce vector populations);
3. cultural control (stabling animals, habitat management such as eliminating wet areas, water fluctuation, manipulating pollution levels or edge slope in wastewater ponds to disfavor vectors like *C. sonorensis*); or
4. 'molecular' control (altering the vector's genetic makeup or physiology to interfere with vectorial capacity).

We now have much better data on protecting animals from *Culicoides* biting via insecticide use than we had at the 2003 Taormina Bluetongue Symposium. Protection studies are far more accurate and realistic when we use bait animals and measure natural midge attack and successful engorgement (see adult activity studies above). In some trials, pyrethroids (the dominant insecticidal chemical class currently available for on-animal treatments) can reduce successful field midge feeding significantly and sometimes for periods of up to a few weeks (Wieher *et al.* 2014). Other uses of insecticides, for example treated screen barriers deployed around animal pens, have been tested with less obvious effects (Calvete *et al.* 2010). Repellents such as fatty acids also have potential (Venter *et al.* 2011), but there is still a critical need for testing on live animals.

Biological control research is almost nonexistent for *Culicoides* spp., and little is specifically known of natural mortality factors in general. One small-scale trial showed reduction in emergence of *C. sonorensis* (treated field enclosures) caused by experimental release of the mermithid nematode *Heleidomermis magnapapula*, and natural mortality from this nematode is around 50% in California wastewater ponds (see Mullens *et al.* 2008). While the commercially successful bacterial mosquito larvicide *Bacillus thuringiensis israelensis* seems not to harm *Culicoides* or *Leptoconops* larvae (Lacey and Kline 1983), other *Culicoides* pathogens are known and may have promise for control. Strains of commercially-viable entomopathogenic fungi, such as *Metarhizium anisopliae*, may have promise for suppressing either immatures or adults of *Culicoides* in certain circumstances (Nicholas and McCorkell 2014).

As opposed to treating animals or habitat with insecticides, cultural control, including habitat manipulation, requires greater understanding of the biology of the target species. Stabling, placing animals into enclosed night time quarters where they are less likely to be bitten, remains an intriguing proposition (Viennet *et al.* 2012). *Culicoides* species differ substantially in biting cycles, host preferences, and their tendencies to avoid or enter buildings. Given recent interest in the European *Avaritia* which develop in cow manure pats, small scale tests have begun on effects of physical pat disturbance (Luhken *et al.* 2014b) or the ability of *Culicoides* to withstand drowning (Luhken *et al.* 2014a). Surprisingly little has been done to try to manipulate immature *Culicoides* habitats for control. This is unfortunate, for such manipulations, successful or not, also tell us a lot about the field biology of the insects. Further, even where water level fluctuation, pollution responses, or slope manipulations have been tested experimentally against *C. sonorensis* (Mullens and Rodriguez 1988), the next steps have not been taken. The techniques have not been applied scientifically elsewhere or confirmed to be effective in reducing midge activity or disease incidence. In 1 striking example, complete removal of a large California dairy pond, thought to be the main source of *C. sonorensis*, failed to result in substantial reduction in adult activity (Mayo *et al.* 2014). This illustrates how little we know about adult movement or use of smaller developmental sites that may be common at some animal facilities, and consequently the scale at which cultural control efforts might need to be implemented to be successful.

Finally, a major knowledge gap involves the interplay between natural habitats and domestic animal (farm) habitats. The simplistic transmission cycles on California confinement dairies feature essentially a single vector, *C. sonorensis* and a single ruminant host, cattle (Gerry *et al.* 2001, Mayo *et al.* 2012). They are matchless and invaluable model systems to study BTV or similar viruses epidemiologically. But we must recognize that this single vector situation is probably more the exception than the rule, globally speaking. In many areas, and perhaps most, both the viruses and multiple *Culicoides* species probably utilize wild hosts such as deer in addition to domestic hosts (Pettersson *et al.* 2013, Lassen *et al.* 2012). Successful control therefore may hinge on knowledge of both the wild and domestic arbovirus transmission cycles. The 2 types of cycles may very well have partially or even completely different key vectors.

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