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Scientific Note

Colonization of bison *(Bison bison)* wallows in a tallgrass prairie by *Culicoides* spp (Diptera: Ceratopogonidae)

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Culicoides biting midges (Diptera: Ceratopogonidae) are vectors of numerous animal pathogens worldwide and in the U.S.A. are most well-known for their role as vectors of two closely related viruses: epizootic hemorrhagic disease (EHD) virus (EHDV) and bluetongue (BT) virus (BTV) (Tabachnick 1996, Ruder et al. in press). EHD has long been considered one of the most significant diseases of white-tailed deer (*Odocoileus virginianus*) but can cause disease in cattle and other wild ruminants. BTV is a significant pathogen of domestic sheep and cattle, as well as numerous wild ruminant species. In the U.S.A., these viruses cause cyclical epidemics that can occasionally be widespread and severe (Ruder et al. in press). American bison (*Bison bison*) are among the ruminant species susceptible to infection with EHDV and BTV. While clinical disease is thought to occasionally occur, infection in most individuals is likely subclinical and the broader epidemiological role of bison is largely unknown (Vestweber et al. 1991, Ruder et al. in press).

Although BT and EHD are well-known diseases, the identity, ecology, and population dynamics of the vectors that cause disease outbreaks are generally not. Currently, *Culicoides sonorensis* (Wirth and Jones) and *Culicoides insignis* are the only confirmed vectors of BTV in the U.S.A. (Tanya et al. 1992, Tabachnick 1996), whereas only *C. sonorensis* is a confirmed vector of EHDV (Foster et al. 1977). Studies of *C. sonorensis* biology and ecology have focused on waste-contaminated sites resulting from agricultural or human activity (Jones 1959, Mullens and Rodriguez 1988, Mullens 1989); few studies have evaluated the ecology of *C. sonorensis* and other *Culicoides* species in natural environments.

Culicoides spp. develop in a wide variety of aquatic to semi-aquatic habitats (Borkent 2005) and ephemeral breeding sites are of unknown importance in *Culicoides* breeding. Variation in the amount and timing of rainfall may cause breeding sites to appear or disappear, and their persistence varies with season and rainfall totals (personal observation). For species that develop in mud on the border of bodies of water, adapting to variation in breeding habitats is a critical component of their life history. *Culicoides* in the Great Plains evolved in a variable environment with rivers and streams that could change radically in flow and depth during and between years. Creeks, springs, and seeps could appear and disappear depending on rainfall patterns and few permanent bodies of water were available for reproduction other than the larger rivers. *Culicoides* that evolved in this environment likely adapted to use ephemeral breeding sites.

The wallows of American bison are shallow depressions formed by bison repeatedly rolling and rubbing in one spot for many years (Reynolds et al. 2003) and are one form of ephemeral pool potentially available to *Culicoides*. Bison are considered a keystone species in the Great Plains and free-ranging and extensively managed populations can have great effects on their environment, including creating large areas of soil disturbance and nutrient input due to large quantities of fecal material (Knapp et al. 1999). Wallows formed by bison can also be found where bison have not occurred since nearly being eradicated from the U.S.A. These relict wallows do not share the recent history of disturbance or nutrient inputs of active bison wallows, but these sites are still easily recognized in intact grasslands and can also serve as ephemeral pools.

The objective of this study was to determine if *Culicoides* utilize bison wallows for reproduction and if there is a difference in utilization of active and relict wallows. Our study area, Konza Prairie Biological Station (KPBS), was located in the Flint Hills of northeastern Kansas (39°05' N, 96°3' W). The 3,487 ha area consisted of native tallgrass prairie on steep slopes characterized by shallow limestone soils. A minimally managed population of about 300 bison freely roam within a 961 ha enclosure on KPBS and bison wallows are common features on the landscape. Abundant rainfall from July 26 to August 12, 2013 filled wallows with water at KPBS. The wallows were dry before rains began on July 26 and filled with a 5.28 cm rain on July 29. Additional smaller rain events occurred intermittently through August 12. Total rainfall during this period (July 2-August 12) was 17.4 cm. Two relict wallows and five sites with at least one large active wallow were identified for sampling (Figure 1A). Relict bison wallows in grasslands vary in appearance and are often just shallow depressions containing vegetation. The two relict wallows identified for this study were well demarcated and devoid of vegetation in the wallow, had a nearly vertical bank when full, grasses overhanging the water, a longer period of water retention than active wallows, and little or no animal waste loading (Figure 1B). Active wallows were highly disturbed with shorelines with on average gentler slopes, generally devoid of vegetation and augmented with bison feces, urine, and hair (Figure 1C). Active wallows were all within the bison range area; the two relict wallows were in ungrazed areas 0.25 and 1.95 km from the nearest active wallow included in the study (Figure 1A). Relict wallows were single pools (35.6 and 57.2 m^2); active wallows were pools with a minimum of two associated smaller wallows (not sampled) ranging from 13.4 to 83.4 m^2. One of the five active wallow sites was a large complex of wallows (about 40) of varying sizes. Four pools were sampled from this complex on all dates where wallows contained water. Smaller wallows tended to dry out faster and were not sampled.

Mud at the waterline was collected weekly when wallows were
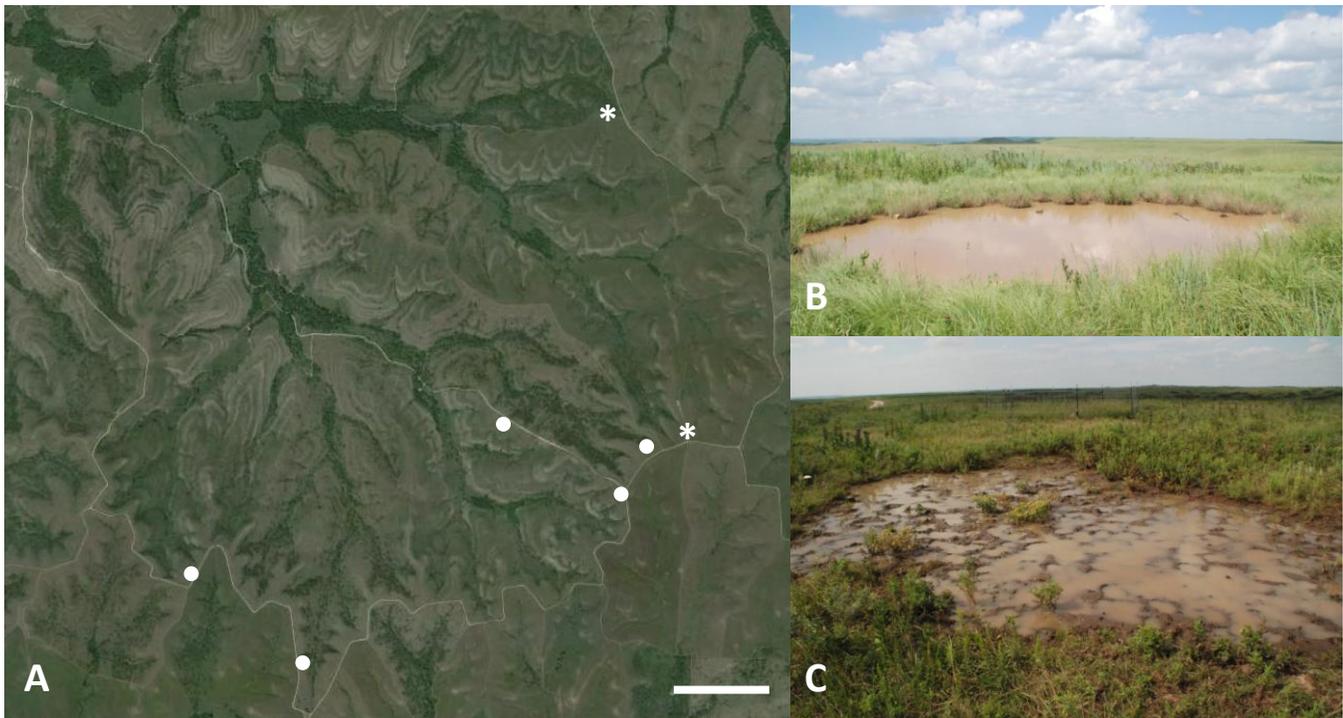
Figure 1. (A) Partial map of Konza Prairie Biological Station (Riley Co., KS) showing spatial relationship of five active wallow sites (circles) and two relict wallows (asterisk). Scale bar = 0.5 km. Google Earth, Google © 2014. (B) Photograph of a relict bison wallow; note the undisturbed nature of the wallow. (C) Photograph of an active bison wallow showing heavy disturbance, as indicated by the abundant hoof prints and disturbed waterline and vegetation.

holding water beginning on August 5, 2013. Many active wallows dried up by the fourth week (August 26), allowing only one to be sampled on that date. One of the relict wallows retained enough moisture for sampling until September 3. For most weeks, only three to five active wallow sites were sampled because active use by bison prevented sampling. The bison moved in large groups and the wallow sites not sampled varied each week. In addition, wider areas of flat, very moist mud was periodically collected, particularly as the water began to recede and when there was no defined shoreline because of soil disturbance by bison. Because of the large surface area and abundant water at one of the active wallow sites, two samples were collected from this location each week.

Mud samples were collected using a trowel. The mud was collected from a swath 1-2 cm deep and 2 cm below, to 3 cm above, the waterline. In the laboratory, 100 ml of mud from each sample was placed in separate 125 × 25 mm plastic Petri dishes and placed in an environmental chamber at 25° C with a 14:10 (L:D) photoperiod. Petri dishes were checked daily for six weeks and emerged adult *Culicoides* were placed in 70% ethanol for later identification using appropriate keys (Holbrook et al. 2000, Blanton and Wirth 1979). Males of the *C. variipennis* group, including *C. sonorensis* and *C. variipennis* (Coquillet), were grouped for presentation, as slide mounting was not performed. Female *C. sonorensis* and *C. variipennis* were identified to species.

Although the period of time samples were monitored for emergence in the lab (six weeks) extended past the time the wallows dried in the field, laboratory holding temperatures were significantly less than those that occur in the wallows and development was likely to be slower. Under laboratory conditions,

C. sonorensis may begin to emerge 14 days after oviposition at 26.5° C (Hunt 1994), and it is not known how long it would take adults to emerge from wallows in the field, although the daytime water temperature in these wallows can be quite high (>34° C) (unpublished data). Timing of emergence from field samples was not determined, but it is likely that *Culicoides* larvae that take longer to develop or those that emerge from eggs deposited later in the lifespan of a wallow would not make it to adulthood.

Culicoides larvae were observed in field samples immediately after collection on August 5, seven days after the wallows filled with water. Samples from the first week (August 5) yielded adult *Culicoides* from the active wallows but not from the relict wallows (Table 1). Subsequently, *Culicoides* were reared from both active and relict wallows although with fewer individuals from relict wallows. All seven wallow sites yielded adult *Culicoides* suggesting that ephemeral sites are quickly colonized. Emergence rates were higher from the active wallows with 2.0±0.64 (SE) midges per 100 ml sample than from relict wallows with 0.67±0.44 (/100 ml), although this was only marginally significant by the t-test with unequal variances (t = 1.71, df = 32, P = 0.0971) (Proc TTEST, SAS Institute 2002). Greater *Culicoides* emergence from the active wallows was due to colonization by *C. sonorensis* and *C. variipennis*; these species only emerged from active wallows (Table 1) and made up 80% of the midges from those wallows. *Culicoides crepuscularis* (Malloch) and *Culicoides haematopotus* Malloch were reared from active and relict wallows. Smaller wallows in the study began drying out within 14-21 days after sampling began and most of the larger active wallows were dry by August 26 (~ 30 d). Relict wallows lasted an additional 7-14 days, but no *Culicoides* were reared from these later samples.

Table 1. *Culicoides* species reared from mud collected at active and relict bison wallows on Konza Prairie Biological Station (Riley Co., KS) during August, 2013.

Species by site type	5 August	11 August	19 August	26 August	Species totals
Active wallows	n=6[a]	n=7	n=10	n=1	
C. sonorensis females	2[b]	4	3	7	16
C. variipennis females	2	2	4	0	8
C. variipennis grp. males[c]	4	5	2	5	16
C. crepuscularis	0	2	4	0	6
C. haematopotus	0	1	1	0	2
Culicoides sp.[d]	0	1	1	0	2
Total *Culicoides*	8	15	15	12	
Relict wallows	n=2	n=2	n=2	n=2	
C. sonorensis females	0	0	0	0	0
C. variipennis females	0	0	0	0	0
C. variipennis grp. males[c]	0	0	0	0	0
C. crepuscularis	0	1	2	0	3
C. haematopotus	0	1	2	0	3
Culicoides sp.[d]	0	0	0	0	0
Total *Culicoides*	0	2	4	0	

[a]n = number of wallows sampled, each sample consisted of 100 ml of mud.
[b]*Culicoides* emergence presented represents pooled total for wallows sampled on that date.
[c]These males were not slide mounted, which is necessary to distinguish male *C. sonorensis* and *C. variipennis*.
[d]Damage to body interfered with morphological identification, limiting identification to genus.

Preferential colonization of the active wallows by *C. sonorensis* and *C. variipennis* was presumably because of the higher animal-waste content; these species have been repeatedly found associated with bodies of water contaminated with animal or human waste (Jones 1959, 1965). What specific aspects of waste-enhanced sites are responsible for this preference are unknown. Possible hypotheses include the composition, abundance, and diversity of bacterial species available as food, as well as differences in salinity, mineral content, and other soil characteristics (Schmidtmann 2000, 2006, 2010). Two other characteristics of active wallows that might make them more attractive to *C. sonorensis* and *C. variipennis* are the proximity to high densities of animals that serve as a food source for adult females or lower shoreline slopes that have been associated with higher populations (Rodriguez and Mullens, 1988).

This study is novel in that it identifies relatively fine-scale habitat associations by *Culicoides* spp. in a natural landscape and it is presumed that the driving factor is the presence of, and habitat modifications by, a large ruminant. *Culicoides sonorensis* populations are associated with waste-enhanced aquatic habitats as breeding sites and most previous research aimed to identify such relationships has been conducted in intensively operated dairy cattle farms, primarily in California (Mullens and Rodriguez 1988, Mayo et al. 2014). While this study also describes an association between *C. sonorensis* and *C. variipennis* to ruminants or ruminant waste, the dynamic nature of the ecosystem and the ephemeral nature of potential larval development sites offer an intriguing comparison to the better known *C. sonorensis*-dairy system of California.

The availability of bison wallows and other ephemeral breeding sites may play an important role in the population dynamics of *Culicoides*. The distribution and abundance of breeding sites for vector species and their proximity to host species is likely to be a key factor in mediating transmission cycles. Unfortunately, we lack an understanding of the fine-scale distribution of vector and ruminant hosts on the landscape during both epidemic and inter-epidemic periods. Enhancing our knowledge of *Culicoides* utilization of putative breeding sites in different physiographic regions of the U.S.A., as well as understanding relationships between environmental conditions, vector populations, and infection prevalence in ruminants, are fundamental to advancing our understanding of EHDV and BTV transmission.

Acknowledgments

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