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Prevalence of Buggy Creek Virus (Togaviridae: *Alphavirus*) in Insect Vectors Increases Over Time in the Presence of an Invasive Avian Host

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Abstract

Invasive species can disrupt natural disease dynamics by altering pathogen transmission among native hosts and vectors. The relatively recent occupancy of cliff swallow (*Petrochelidon pyrrhonota*) nesting colonies in western Nebraska by introduced European house sparrows (*Passer domesticus*) has led to yearly increases in the prevalence of an endemic arbovirus, Buggy Creek virus (BCRV), in its native swallow bug (*Oeciacus vicarius*) vector at sites containing both the invasive sparrow host and the native swallow host. At sites without the invasive host, no long-term changes in prevalence have occurred. The percentage of BCRV isolates exhibiting cytopathicity in Vero-cell culture assays increased significantly with year at sites with sparrows but not at swallow-only sites, suggesting that the virus is becoming more virulent to vertebrates in the presence of the invasive host. Increased BCRV prevalence in bug vectors at mixed-species colonies may reflect high virus replication rates in house sparrow hosts, resulting in frequent virus transmission between sparrows and swallow bugs. This case represents a rare empirical example of a pathogen effectively switching to an invasive host, documented in the early phases of the host's arrival in a specialized ecosystem and illustrating how an invasive species can promote long-term changes in host–parasite transmission dynamics.

Key Words: Arbovirus—Disease dynamics—Invasive species—Pathogen spillback—Virulence—Virus transmission.

Introduction

A major consequence of an invasive species' entering a new ecosystem is potential disruption of natural host–pathogen dynamics (Prenter et al. 2004). When an invasive species can serve as a host for endemic pathogens it encounters in the new environment, two opposite effects on transmission ecology are possible: (1) the invasive may be a less competent amplifying host, resulting in reduced overall pathogen transmission among all hosts through the widely discussed dilution effect (e.g., Hess and Hayes 1970, Schmidt and Ostfeld 2001, Keesing et al. 2006), or (2) the invasive species may be more competent at amplifying the pathogen or is a better reservoir host, potentially resulting in increased transmission to native hosts or vectors through "spillback" (Kelly et al. 2009). Empirical evidence shows that increased host diversity can reduce parasite or pathogen transmission in some systems (e.g., Telfer et al. 2005, Ezenwa et al. 2006, Swaddle and Calos 2008, Suzán et al. 2009, Carver et al. 2011, O'Brien et al. 2011). However, despite several cases of invasives causing apparent changes in parasite ecology that are seemingly deleterious to native hosts (e.g., Allan et al. 2010, Pisanu et al. 2010; reviewed in Prenter et al. 2004), empirical examples of pathogen spillback are few (Kelly et al. 2009), and cases of native pathogens switching to an invasive species as their primary host are almost unknown.

Disruption of transmission dynamics by an invasive host may also lead to changes in virulence of a parasite or pathogen. While many parasites that colonize new hosts die out, presumably because they have not encountered those hosts before and are not adapted to exploit them (Mitchell and Power 2003, Torchin et al. 2003, Lee et al. 2005), the parasites or pathogens that find a new host suitable may rapidly evolve either higher or lower virulence (Ebert and Bull 2008). How virulence changes may depend on host density, with the extent of virulence varying directly with population density of total hosts and the frequency of opportunities for horizontal transmission (e.g., Lipsitch and Moxon 1997, Messenger et al. 1999, Ewald and De Leo 2002, Lebarbenchon et al. 2010). While eventually the extent of virulence will probably

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stabilize at some equilibrium level, we might expect measurable directional shifts in parasite or pathogen virulence at least in the early stages of ecological invasion by a new host (Ebert and Bull 2008).

In this study, we examine whether there have been long-term changes in prevalence of a vector-borne virus, as measured in its insect vectors, following the arrival of an invasive host species. We also present more limited data on temporal changes in cytopathicity of this virus, as a potential index of virulence, and we use the results to explore how the invasive host may alter the ecology of this host–parasite system. The pathogen is Buggy Creek virus (BCRV; Togaviridae, *Alphavirus*), an arthropod-borne virus (arbovirus) historically associated exclusively with colonially nesting cliff swallows (*Petrochelidon pyrrhonota*) as its vertebrate host and the ectoparasitic swallow bug (Hemiptera: Cimicidae: *Oeciacus vicarius*) as its exclusive vector (Hopla et al. 1993, Pfeffer et al. 2006, Padhi et al. 2008). The hematophagous bugs reside year-round in the swallows' mud nests, with BCRV overwintering in bugs there (Brown et al. 2010c), and thus the birds' colony sites represent predictable spatial foci where virus can be detected at all times of the year (Brown et al. 2008, 2009).

Within the last 50–150 years, the introduced house sparrow (*Passer domesticus*), a species native to Europe (Robbins 1973), has moved into cliff swallow colonies in many parts of North America, often usurping active swallow nests or occupying abandoned ones (Scott et al. 1984, Brown et al. 2009). In the process, house sparrows have been exposed to swallow bugs and BCRV. Sparrows are highly competent amplifying hosts, being more likely than swallows to be infected and exhibiting higher virus titers and greater mortality (when infected as nestlings) than cliff swallows (O'Brien et al. 2011). The potential disruption of this host–pathogen system by house sparrows provides the opportunity to compare long-term trends in virus prevalence both in the presence (at swallow colony sites containing sparrows) and absence of the invasive host (at sites without sparrows) and to ask in what ways this virus has potentially responded to the arrival of the novel host. We use 11 years of data on virus prevalence in the vectors collected from 1998 to 2008 at our study site in western Nebraska. Because BCRV occurs in two ecologically distinct lineages in the Great Plains (Padhi et al. 2008, Pfeffer et al. 2006, Brown et al. 2009), we also evaluate whether any temporal changes in virus detection could be due to changes in relative abundance of the two lineages.

Materials and Methods

Study organisms and study site

Cliff swallows are highly colonial, migratory passerines that breed across much of western North America (Brown and Brown 1995). They build gourd-shaped mud nests on the sides of cliff faces, inside highway and railroad culverts, and underneath bridges. The nests persist from year-to-year and are frequently reused by cliff swallows for multiple seasons (Brown and Brown 1996). Swallows arrive in our study area in early to mid May and typically raise a single brood, with most nestlings fledging by mid July. Individual colonies are highly synchronous and are quickly vacated by swallows after the nestlings fledge. Nestlings are in the nest for about 26 days before fledging (Brown and Brown 1995).

House sparrows were introduced repeatedly into North America beginning in the 1850s (Robbins 1973) and are now widely dispersed and found mainly in peridomestic settings. Sparrows are semicolonial, often forming aggregations of 2 to 20 nests in proximity. They are sedentary, remaining at or near breeding sites year-round (Anderson 2006). House sparrows are multibrooded, with nesting in our study area beginning in late April and ending in mid August; peak egg laying periods are in mid May, late June, and late July. New broods are started soon after earlier ones fail or fledge. Nestlings fledge at 14–17 days of age (Anderson 2006). Sparrows build their nests inside the cliff swallows' mud structures, filling the swallow nests with straw and feathers.

The swallow bug is a hematophagous nest-based ectoparasite, and as many as 2600 bugs have been found in a single cliff swallow nest (Brown and Brown 1996). Swallow bugs are long-lived and can survive without a blood meal for up to 3 years (Smith and Eads 1978, Rannala 1995). Bugs feed on birds mostly at night and cluster on the outside of active nests during the day after blood feeding. Swallow bugs, like other cimicids, are noxious, and no vertebrates are known to feed on them (Usinger 1966).

BCRV is a single-stranded, positive-sense RNA alphavirus in the western equine encephalomyelitis virus (WEEV) antigenic complex (Hopla et al. 1993, Pfeffer et al. 2006, Padhi et al. 2008). This virus is ecologically distinct from other alphaviruses in that it is transmitted by swallow bugs rather than mosquitoes (Rush et al. 1980, Hopla et al. 1993, Brown et al. 2008). BCRV presumably closely coevolved with cliff swallows and swallow bugs, not having been found in any organism not associated with swallow colonies. The two lineages of BCRV (designated A and B) found in the Great Plains differ by about 6% at the nucleotide level of the E2 gene (Pfeffer et al. 2006, Padhi et al. 2008, Brown et al. 2009).

Our study area is a 60×200 km area largely contiguous with the North and South Platte rivers in western Nebraska, centered at the Cedar Point Biological Station (41°12.59′ N, 101°38.97′ W) in Keith County (Brown and Brown 1996), and contains about 170 cliff swallow colony sites. In the summers of 1998–2008, we studied cliff swallows and house sparrows at colonies situated in concrete culverts beneath highways or railroads and on the sides of bridges. We determined, by checking nest contents or visually surveying, the species occupying each colony site. Information on construction dates of bridges or culverts used as nesting sites was provided by the Nebraska Department of Roads or by our knowledge of when a structure was built. For those swallow colony sites used by house sparrows, we determined first date of occupancy by sparrows based on when the birds were first observed there by us during the course of our 29-year research in the study area. Once house sparrows colonized a swallow site, some typically remained there each subsequent year.

Field sampling

Swallow bugs were collected from active cliff swallow nests by brushing them off the outsides of the birds' mud nests into a wide-mouthed collecting jar. Bugs cluster on the sides and bottoms of active nests where they rest in between blood meals. In a few cases, whole nests were collected soon after cliff swallow nestlings fledged and the nest pieces picked through by hand or put into a Berlese funnel to harvest bugs. Methods

of sampling are described more fully in Brown et al. (2001) and Moore et al. (2007). Nests were sampled from throughout a colony site (in all areas accessible to us). We used prevalence data from a total of 50 active cliff swallow colony sites in 1998–2008, with 36 of these sites sampled in multiple (≥ 2) years, and 16 of them sampled in at least 5 different years. Only one of the colony sites could be sampled in all years, because many sites in our study area are not used by cliff swallows every season or for other reasons (e.g., high water) may have been inaccessible for sampling in certain years.

Bugs taken from nests were sorted into pools of 100 while alive and stored at −70°C until analysis. Because BCRV from bugs at inactive nests (including those collected in winter) is less likely to exhibit plaque formation on Vero cell culture (Brown et al. 2010b, 2010c) and because swallow bugs do not cluster on the outsides of house sparrow nests in large numbers (O'Brien et al. 2011), all analyses here were restricted to bug samples from currently or recently active cliff swallow nests collected during the summer months (May–July), 1998–2008.

Laboratory assays

Bug pools were macerated with a mortar and pestle or a Mixer Mill (MM 31) from Qiagen (Valencia, CA) and suspended in 2.0 mL of BA-1 diluent (Brown et al. 2001, Moore et al. 2007). The homogenate of each pool was centrifuged at 11,000 g for 1 min and subsequently stored at −70°C. In 1998–2003, all bug pools were subjected to plaque assay by adding 100 μL of the supernatant in duplicate to a monolayer of Vero cells (African green monkey cells) in a six-well cell culture plate, incubating it for 1 h at 37°C in 5% CO_2, and then overlaying it with 3 mL 0.5% agarose in M-199 medium supplemented with 350 mg/L sodium bicarbonate, 29.2 mg/L L-glutamine, and antibiotics, and returning it to the incubator. A second overlay containing 0.004% neutral red dye was added after 2 days' incubation for plaque observation. Plaques were scored daily for 5 days (Brown et al. 2001).

Beginning in 2004, all bug pools were first screened with a BCRV-specific RT-PCR protocol to identify potential virus-positive samples. Methods of extracting RNA and performing RT-PCR are given in Moore et al. (2007). Samples that were initially positive for BCRV by RT-PCR were subjected to plaque assay, as described above. Only those confirming positive by plaque assay (plaque-forming units ≥ 1) were considered to be positive in the prevalence analyses for this study, making bug data from all years (1998–2008) directly comparable.

Samples from 2004 to 2008 that were initially positive by RT-PCR but negative by plaque assay were re-tested by RT-PCR. In these cases, we re-extracted RNA from the remaining homogenate and performed RT-PCR again (using the same primers). Samples that re-tested positive by RT-PCR the second time were considered to contain noncytopathic, nonvirulent BCRV RNA (Moore et al. 2007, Brown et al. 2010b, 2010c).

A subset of 377 BCRV-positive bug samples were identified to either the A or B virus lineages using the sequencing and phylogenetic methods described by Brown et al. (2008). The samples sequenced were selected to represent a range of different colony sites each year but otherwise were chosen randomly with respect to other ecological characteristics or attributes. Because many colony sites had relatively few isolates sequenced (< 10), analyses of lineage distributions were not done by site; instead, we combined all from each year to examine lineage distribution by year and how that related to overall prevalence by year. Lineage data were expressed as the percentage of lineage A isolates among those sequenced for that year (i.e., number of lineage A isolates/total number of lineage A and B isolates $\times 100$).

Statistical analyses

The response variables in statistical analyses were (1) prevalence of BCRV, expressed as the percentage of all pools tested at a site (or in a given year) that were positive for BCRV, or (2) extent of cytopathicity, expressed as the percentage of RT-PCR positives that exhibited plaque growth. Because predictor variables were both continuous (e.g., colony size) and categorical (e.g., species present), we used analysis of covariance (ANCOVA) to determine the relative effect of each on the response variable. This allowed us to examine both main effects and relevant interactions (e.g., that between year and species present). Nonsignificant interactions ($p > 0.10$) were removed from the final model, but all main effects were retained. We used Proc GLM in SAS (SAS Institute 2004) to perform ANCOVA. Because sample sizes were relatively small for analyses based on year as the sampling unit, we used only rank-order Spearman correlation analyses to examine directional changes across years.

Results

Changes in virus prevalence in vectors

From 1998 to 2008, we tested a total 7421 swallow bug pools collected in summer from cliff swallow nests at active Nebraska colony sites; 1459 (19.7%) were BCRV-positive by plaque assay. When data from all colonies were combined for each year, virus prevalence in bug pools increased significantly over the 11 years at sites containing both house sparrows and cliff swallows ($r_s = 0.88$, $p < 0.001$, $n = 10$ years), whereas the opposite trend (though not significant) occurred at colonies consisting only of cliff swallows ($r_s = -0.31$, $p = 0.35$, $n = 11$; Fig. 1).

This pattern (Fig. 1) was not an artifact of pooling colonies within years: when the data were analyzed separately for the eight colony sites with sparrows that were sampled in at least 5 years, seven showed an increase in virus prevalence with time (positive Spearman rank correlation between prevalence and year) and only one a decrease. Of the eight sites without house sparrows sampled in at least 5 years, three showed a positive correlation and five a negative correlation. The distribution of positive and negative correlations between sites with and without sparrows for this sample of 16 colony sites was significantly different ($\chi^2_1 = 4.3$, $p = 0.039$) and was consistent with the overall data set (Fig. 1).

In analyzing virus prevalence separately by colony, we found no significant effect of colony size but a strong interaction between year and whether a site was mixed-species or swallow-only (Table 1). That year alone was not significant suggests that the temporal pattern was driven largely by the host species occupying a colony (Table 1), consistent with the opposite yearly trends depicted in Figure 1.

Using our subset of virus isolates identified to lineage, there was no significant temporal change in the ratio of lineage A to lineage B isolates during the study. At sites with both house

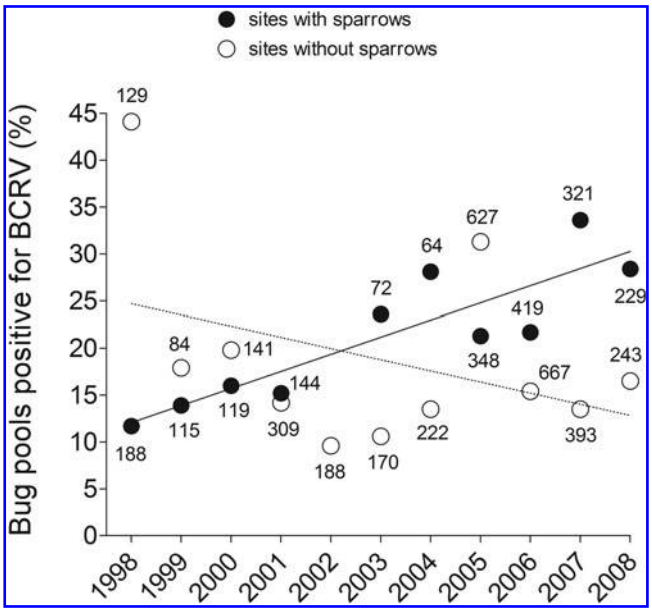

FIG. 1. Percentage of swallow bug pools, collected from cliff swallow nests at active colonies, that were positive for Buggy Creek virus (BCRV) by Vero-cell assay in relation to year of sampling, at colony sites containing both house sparrows and cliff swallows (●, solid line) and at sites with only cliff swallows (○, dotted line). Sample size each year (number of pools tested) is shown by the dots. The number of colonies sampled was 2–15 per year for mixed-species sites and 3–15 per year for swallow-only sites. Lines indicate best-fit least-squares regression.

sparrows and cliff swallows, the percentage of lineage A isolates ranged from 14.3% (in 1998) to 100% (in 2004), with a mean (±standard error, SE) 73.8% (±9.6) per year; the percentage of lineage A isolates did not vary over time at sites with sparrows ($r_s = 0.07$, $p = 0.87$, $n = 8$ years). At swallow-only sites, the percentage of lineage A isolates ranged from 9.1% (in 1999) to 84.6% (in 2002), with a mean 32.9% (±9.5) per year, also with no change over time ($r_s = -0.14$, $p = 0.74$, $n = 8$ years). The percentage of lineage A isolates in a given year was not significantly related to annual virus prevalence at either mixed-species sites ($r_s = 0.19$, $p = 0.64$, $n = 8$ years) or at swallow-only sites ($r_s = -0.07$, $p = 0.86$, $n = 9$ years).

Table 1. Analysis of Covariance to Detect Effects and Interactions of Variables Potentially Affecting Prevalence of Buggy Creek Virus in Swallow Bug Pools at Cliff Swallow Colonies Containing Both Swallows and House Sparrows and Swallows Only ($n = 159$ Colonies)

Variable	F[a]	p
Year	1.39	0.24
Species present[b]	4.86	0.029
Colony size[c]	2.75	0.100
Year × species present	4.87	0.029

[a] *df* for each, 1, 115.
[b] Categorical variable denoting either mixed-species or swallow-only colony.
[c] Number of active cliff swallow nests.

Past use of sites by the invasive host

Among our set of 50 colony sites sampled for virus in bugs, we had information on how long 28 of those had been present in the landscape (i.e., when they were first constructed). For 11 sites with house sparrows, the mean (±SE) year was 1974.6 (±5.0), whereas that for 17 sites with only cliff swallows was 1977.2 (±5.0). The two types of sites did not differ significantly in how long they had been present (Wilcoxon test, $Z = -0.26$, $p = 0.79$). The mean year (±SE) of first occupancy by house sparrows at the 11 sparrow-occupied sites was 1991.4 (±4.1).

Changes in cytopathicity

As an index of changes in BCRV cytopathicity, we examined the percentage of RT-PCR positive bug pools that exhibited plaque formation on Vero cells over the 5-year period during which bug pools were screened by RT-PCR (Fig. 2). The percentage of pools exhibiting cytopathicity increased significantly over time at sites containing both cliff swallows and house sparrows ($r_s = 0.90$, $p = 0.037$, $n = 5$ years), whereas at sites with only cliff swallows there was no significant change during these years ($r_s = -0.10$, $p = 0.87$, $n = 5$; Fig. 2). The linear pattern at the sites with house sparrows was largely driven by the years 2004 and 2008, which were markedly different from each other (Fig. 2).

Discussion

The recent invasion of North American cliff swallow colonies by European house sparrows provides a natural comparison of pathogen dynamics in the presence and absence of the invasive host (O'Brien et al. 2011). The increase in BCRV

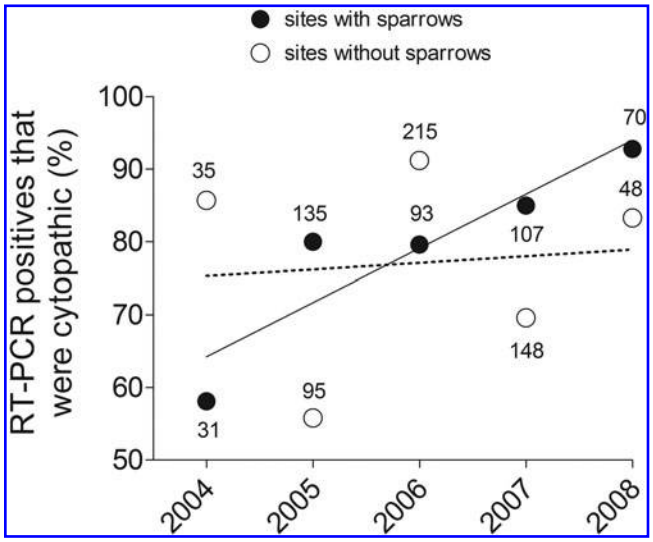

FIG. 2. Percentage of swallow bug pools, collected from cliff swallow nests at active colonies, detected as positive for BCRV RNA by RT-PCR that exhibited cytopathic plaque growth on Vero cells in relation to year at colony sites containing both house sparrows and cliff swallows (●, solid line) and at sites with only cliff swallows (○, dotted line). Sample size (number of confirmed RT-PCR positive samples) is shown by the dots. Lines indicate best-fit least-squares regression.

prevalence in swallow bug vectors over time in the presence of house sparrows (but not in their absence) strongly implicates the sparrow as contributory to this temporal change. We observed the same pattern when colony sites were analyzed separately, indicating that this effect of house sparrows applied widely across different sites. That these infected vectors were all collected from cliff swallow nests suggests increased virus spillback to vectors at sites with house sparrows and that exposure of cliff swallows to BCRV may increase the longer sparrows occupy a site. This example is a rare illustration that a native pathogen can sometimes switch to an invasive host relatively quickly.

Temporal changes in pathogen prevalence

The first house sparrows probably invaded cliff swallow colonies in western Nebraska in about 1940, based on the sparrow's spread across North America (Robbins 1973) and when artificial swallow nesting structures (e.g., concrete bridges) near towns first appeared (Brown and Brown 1996). However, most sparrow use of swallow colonies in the study area has been more recent. Most of our study sites have been present only since the mid-1970s and used by sparrows for about 16–17 years, on average. The 11-year period of this study thus overlaps much of the time that house sparrows have been present at these particular colony sites. Because the majority of house sparrows in our study area do not nest in cliff swallow colonies, instead using cavities and crevices around human habitations, and because of frequent turnover among the sparrows occupying swallow colonies from year to year (O'Brien 2009), it is unlikely that the house sparrow population in the study area at large has had much exposure or evolutionary history with BCRV. Thus, each year that sparrows occupy a swallow site represents a relatively new exposure of them to this virus. This has therefore allowed us to document the evolutionarily early stages of pathogen response to this invasive host.

The linear increase in BCRV prevalence in swallow bugs over an 11-year period at sites with house sparrows (Fig. 1) is unlike the population trajectories reported recently for ecologically similar arboviruses. For example, WEEV and St. Louis encephalitis virus (SLEV) in western North America have declined in prevalence (as measured in vertebrate hosts) over wide areas since the 1960s and especially in the last 25 years for unknown reasons (Reeves 1990, Forrester et al. 2008, Reisen et al. 2008). Others, such as eastern equine encephalomyelitis virus, SLEV in eastern North America, and tick-borne encephalitis virus in Russia, exhibit periodic epidemics separated by interepidemic intervals of variable length but with no systematic long-term increase or decrease (Day 2001, Hachiya et al. 2007, Kornberg 2009). BCRV differs from most of these arboviruses in being historically restricted to a simple ecosystem (cliff swallows, their nests, and swallow bugs), and as a specialist this pathogen might react more strongly than other arboviruses to the addition of an invasive host species to its environment.

Several lines of evidence indicate that BCRV affects house sparrows more negatively and elicits a greater viremic response in them than in the native cliff swallow host (O'Brien et al. 2011). Nestling house sparrows are about eight times more likely to be infected than nestling cliff swallows, more sparrow infections elicit viremia and at higher levels than in swallows, and nestling sparrows suffer high virus-induced mortality (O'Brien et al. 2010, 2011). Nestling house sparrows, however, can remain alive for several days with high viremia (O'Brien 2009), leading to their potentially infecting many bugs. The temporal increase in virus prevalence in the bug vectors at sites with house sparrows suggests that over time more house sparrows are becoming infected and/or are exhibiting longer or higher periods of infectiousness to bugs.

One potential explanation for the increased BCRV prevalence at sites with house sparrows is that the virus is evolving a greater virulence in this invasive host, possibly owing to the sparrow's recent exposure and its lack of immunological history with this group of New World alphaviruses. Independent of changes in sparrow population size, greater virulence could cause higher rates of host infection and higher levels of viremia in nestlings, leading to greater numbers of bugs being infected. A change in virulence is supported indirectly by the rapid increase from 2004 to 2008 in the percentage of virus-positive bug pools that were cytopathic on vertebrate cells. Cytopathicity of BCRV samples is known to vary with time of year and bird occupancy status of a colony site: RT-PCR positives in winter and at sites with no birds present in summer exhibit little to no plaque growth (Brown et al. 2010b, 2010c). However, the cytopathicity data reported here all came from active sites in summer, so the confounding effects of season and occupancy status do not apply. Further, cytopathicity for BCRV does not reflect simply virus titer, as cytopathicity is unrelated to average virus titer for BCRV (Brown et al. 2009).

A rapid change in virulence is characteristic of the early phases of pathogen exposure to a novel, suitable host (Ebert and Bull 2008), which is consistent with the relatively brief time that house sparrows have been hosts for BCRV and the fact that they are highly competent amplifying hosts. While virulence has been predicted to potentially respond this way when a pathogen encounters a new host, empirical examples are largely limited to the evolution of virulence in the myxoma virus in introduced rabbits of Australia (De Leo et al. 2002). Interestingly, studies of temporal change in virulence of a closely related arbovirus, WEEV, have either found no evidence for long-term virulence changes (Forrester et al. 2008, Reisen et al. 2008, Zhang et al. 2011) or an indication of a decline in virulence over time (Logue et al. 2009). In contrast to BCRV, however, WEEV utilizes many different host species (Reeves 1990) and has not undertaken the same widespread degree of shift to a novel host as BCRV.

The increased virus prevalence in bugs cannot be explained by yearly increases in average cliff swallow colony size of the sites sampled (BCRV prevalence in bugs can vary with swallow colony size) (Brown et al. 2001, Moore et al. 2007), because swallow colony size (in either the presence or absence of house sparrows) was not significantly associated with virus prevalence in this data set. We have no historical information on how the numbers of house sparrows at these sites may have changed over time; if they have increased, this could lead to more bugs being exposed to BCRV. Over the 3-year period from 2007 to 2009, there was no increase in sparrow usage of sites in the study area, and numbers declined between 2007 and 2008 (V. O'Brien and C. Brown, unpublished data).

BCRV lineage A is more closely associated with colony sites containing house sparrows, probably because sparrows amplify virus of this lineage more effectively than that of lineage

B (Brown et al. 2009, 2010a). Lineage B is more associated with the bug vectors, perhaps sustained by vertical transmission in bugs, and lineage B virus is less likely to be cytopathic on Vero cells (Brown et al. 2009). However, we found no evidence that the increase in virus prevalence at sites with house sparrows was directly attributable to the replacement of BCRV lineage B by the more cytopathic lineage A over time. Overall virus prevalence in a given year was unrelated to the relative proportions of the two lineages that season.

Virus spillback to the native host

The increased BCRV prevalence (and possibly virulence) over time at sites with house sparrows suggests potential pathogen spillback to cliff swallows, given that these data (Figs. 1 and 2) were taken from bugs collected from cliff swallow nests. As more bugs become infected by feeding on viremic nestling house sparrows, the number of infected vectors contacting cliff swallows increases. The increased contact with infected bugs at sites with house sparrows likely is the cause of the higher proportion of cliff swallows being currently infected with BCRV in those colonies, relative to single-species sites (O'Brien et al. 2011).

Swallow bugs move extensively between nests within colonies (Rannala 1995, Brown and Brown 1996), and they prefer cliff swallows as hosts, as illustrated by simultaneous counts of bugs on swallow nests versus sparrow nests at mixed-species sites (O'Brien et al. 2011). However, house sparrows begin nesting earlier in the spring than do cliff swallows at our study sites, and their successive nesting attempts are collectively more continuously distributed in time at a colony, making their nestlings available as hosts to bugs before the hatching of cliff swallows. House sparrows also breed later in the summer, often raising at least one brood after all cliff swallows have departed from a colony. Thus, sparrow nesting phenology prolongs the period during a season when bugs are exposed to virus. Given that these long-lived insects also maintain BCRV over the winter (Brown et al. 2010c), their greater exposure to virus both within a season and between seasons probably accounts for the higher virus prevalence in them at sites occupied by house sparrows. The longevity of bugs (up to 3 years, even in the absence of hosts) (Smith and Eads 1978, Rannala 1995) may contribute, in part, to the temporal increase in virus prevalence, as sites continually accumulate infected bugs. House sparrows perennially use most sites and rarely skip years (unlike cliff swallows) (Brown and Brown 1996), meaning that BCRV is less likely to periodically disappear from a sparrow site (relative to swallow-only sites) through lack of suitable amplifying hosts and thus is less likely to require re-introduction by infected birds or bugs in order to sustain itself.

Temporal changes in competence of hosts for BCRV?

We do not have data on temporal changes, if any, in BCRV prevalence in vertebrate hosts (house sparrows or cliff swallows), as our study of these species was over a 3-year period only (O'Brien et al. 2011). However, data collected on the Fort Morgan strain of BCRV in eastern Colorado, about 215 km from our study site, in the 1970s (Hayes et al. 1977, Scott et al. 1984) revealed virus infection prevalence in nestling house sparrows (8.1%) only about half that we observed in Nebraska during this study (14.1%) (O'Brien et al. 2011). The opposite pattern was seen in cliff swallows: 7.2% of nestling swallows were positive in the 1970s (Hayes et al. 1977, Scott et al. 1984), compared to only 1.8% in the 2000s (O'Brien et al. 2011). These results should not be over-interpreted, given that the data from the 1970s came from a single colony site. However, the pattern supports the hypothesis that in the time since the house sparrow's arrival in cliff swallow colonies, its high competency as an amplifying host has selected for virus variants (of either lineage) that are better adapted to replicating in sparrows than in cliff swallows and that these genotypes are outcompeting ones adapted to cliff swallows. This could explain in part the temporal increase in the number of bugs infected in the presence of sparrows (Fig. 1).

If BCRV is undergoing an evolutionary shift to replicating primarily in the invasive sparrow host and cliff swallows are becoming less likely to be infected, as current data suggest (O'Brien et al. 2011), the increasing exposure of swallows to infected bugs at sites with sparrows (Fig. 1) paradoxically may not result in long-term pathogen spillback to the native swallow host. Increased exposure has no net effect on cliff swallows if the virus has switched to house sparrows. While there is some evidence for slightly increased prevalence of BCRV in swallows at sites with sparrows compared to sites without sparrows (O'Brien et al. 2011) that is consistent with spillback, if the reduction in BCRV prevalence in cliff swallows from the 1970s to the 2000s is real, it suggests that this pathogen may be on a trajectory to switch entirely to its new sparrow host and that it will become irrelevant to swallows. This system provides an unusual opportunity in contemporary time to observe rapid evolutionary changes in pathogen ecology in response to an invasive species.

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No competing financial interests exist.

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