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THE ROYAL SOCIETY

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Arbovirus infection increases with group size

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Buggy Creek (BCR) virus is an arthropod-borne alphavirus that is naturally transmitted to its vertebrate host the cliff swallow (*Petrochelidon pyrrhonota*) by an invertebrate vector, namely the cimicid swallow bug (*Oeciacus vicarius*). We examined how the prevalence of the virus varied with the group size of both its vector and host. The study was conducted in southwestern Nebraska where cliff swallows breed in colonies ranging from one to 3700 nests and the bug populations at a site vary directly with the cliff swallow colony size. The percentage of cliff swallow nests containing bugs infected with BCR virus increased significantly with colony size at a site in the current year and at the site in the previous year. This result could not be explained by differences in the bug sampling methods, date of sampling, sample size of the bugs, age structure of the bugs or the presence of an alternate host, the house sparrow (*Passer domesticus*). Colony sites that were reused by cliff swallows showed a positive autocorrelation in the percentage of nests with infected bugs between year t and year $t+1$, but the spatial autocorrelation broke down for year $t+2$. The increased prevalence of BCR virus at larger cliff swallow colonies probably reflects the larger bug populations there, which are less likely to decline in size and lead to virus extinction. To the authors' knowledge this is the first demonstration of arbovirus infection increasing with group size and one of the few known predictive ecological relationships between an arbovirus and its vectors/hosts. The results have implications for both understanding the fitness consequences of coloniality for cliff swallows and understanding the temporal and spatial variation in arboviral epidemics.

Keywords: alphavirus; Buggy Creek virus; coloniality; disease transmission; *Oeciacus vicarius*; *Petrochelidon pyrrhonota*

1. INTRODUCTION

The importance of population size in maintaining disease epidemics has long been recognized by theoretical epidemiologists (Dietz 1988; Anderson & May 1992; De Jong *et al.* 1995). Most viruses require a minimum number of susceptible hosts in order to sustain themselves, leading to the concept of a critical community size (Schenzle & Dietz 1987; Keeling & Grenfell 1997; Swinton *et al.* 1998) and promoting the general view that viral epidemics are more likely to occur in large populations. Some human diseases have been shown to increase in larger cities, for example a city's size needs to reach a threshold of 250 000–500 000 people before a measles epidemic can persist for any length of time (Bartlett 1957; Black 1966). However, there are surprisingly few empirical data on the effect of population (or group) size on viral infection rates in other taxa. This is particularly the case for viral pathogens that use biological vectors for spreading among hosts, in part because, in most cases, we lack complete information on virus, vector and host population dynamics.

Most arthropod-borne viruses (arboviruses) are associated with either vectors or hosts that occur in high density at some stage in their life cycle. For example, many arboviruses, including the West Nile flavivirus that was recently introduced into northeastern USA (Lanciotti *et al.* 1999; Rappole *et al.* 2000), are spread over large geographical areas by migratory birds that sometimes concentrate in large groups or are vectored by invertebrates such as mosquitoes that also occur in patchy concentrations. However, studying how vector or host density affects arbovirus infection rates is not usually feasible because groups of hosts or vectors are often transient and not restricted to particular sites that can be systematically sampled.

In this study, we investigate the incidence of an arbovirus infection that is associated with a colonial bird and its nest-based ectoparasite, which is known to be a vector for the virus. The bird colonies serve as foci for arbovirus infection and, as a result, we could measure how infection is affected by the group size of both the hosts and vectors at different sites within a relatively well-defined metapopulation. Our study focused on the colonially breeding cliff swallow (*Petrochelidon pyrrhonota*), a passerine bird of western North America, and its ectoparasite, the swallow bug (Hemiptera: Cimicidae: *Oeciacus vicarius*), both of which are associated with an alphavirus in the western Great Plains. The virus, which is named Buggy Creek (BCR) virus, is one of the few alphaviruses that is vectored by an invertebrate other than mosquitoes (Strauss & Strauss 1994). In this paper we specifically investigate how alphavirus infection of bugs varies among cliff swallow colonies of different sizes. We also examine other potential ecological correlates of virus infection frequency.

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2. METHODS

(a) *Study organisms*

(i) *The virus*

BCR virus is a recombinant alphavirus that is part of the western equine encephalitis (WEE)-related virus group (Hayes *et al.* 1977; Calisher *et al.* 1988; Weaver *et al.* 1997). These viruses tend to be vectored by invertebrates (usually mosquitoes) and amplified and spread over large geographical areas by birds. BCR virus was first isolated in the early 1980s from swallow bugs collected at a cliff swallow colony along Buggy Creek in Grady County, west central Oklahoma (Loye & Hopla 1983; Hopla *et al.* 1993). BCR virus is very similar to another alphavirus, Fort Morgan virus (FM), which is also associated with cliff swallows and swallow bugs (Hayes *et al.* 1977; Calisher *et al.* 1980; Scott *et al.* 1984). BCR virus and FM virus are $> 96\%$ identical at the nucleotide level and $> 98\%$ identical at the amino acid level over the entire structural protein coding region (M. Pfeffer and R. Kinney, unpublished data) and, thus, both are probably strains of the same alphavirus. We know relatively little about the ecology of BCR virus other than the fact that it is primarily associated with swallow bugs and cliff swallows; the role of mosquitoes in its transmission, if any, is unknown.

(ii) *Swallow bugs*

The wingless swallow bug is an ectoparasite, primarily of cliff swallows and is found throughout this bird's wide geographical range (Brown & Brown 1995). Swallow bugs are nest-based ectoparasites that overwinter in cliff swallows' nests or in the cracks and crevices of the nesting substrate near the nests. They are exclusively haematophagous, feeding on the birds mostly at night, and they travel on the adult birds relatively rarely (George 1987; Brown & Brown 1996). Infestations can reach 2600 bugs per nest and the bugs have substantive effects on many aspects of cliff swallow life history (Brown & Brown 1986, 1992, 1996; Chapman & George 1991; Loye & Carroll 1991). The swallow bug is a long-lived ectoparasite that begins to reproduce as soon as it feeds in the spring. Eggs are laid in several clutches that hatch over variable lengths of time, ranging from 3–5 days (Loye 1985) to 12–20 days (Myers 1928). Nymphs undergo five instars in an *ca.* 10-week period before maturing and they feed on birds' blood at each instar stage. Bug populations at an active colony site increase throughout the summer, reaching a peak at approximately the time cliff swallows fledge. Usually one complete generation of bugs is produced during a single cliff swallow breeding season. Because swallow bugs are confined to cliff swallows' nests and colony substrates, they only have access to hosts when cliff swallows occupy a colony site or reuse existing nests. The birds do not use all of the colony sites in a given year (Brown & Brown 1996) and the bugs seem to be adapted to withstanding long periods of host absence, in some cases persisting at a site not used by cliff swallows for up to four consecutive years (Smith & Eads 1978; Loye 1985; Loye & Carroll 1991; Rannala 1995). The bugs appear capable of parasitizing introduced house sparrows (*Passer domesticus*) that occupy nests in some cliff swallow colonies (Hopla *et al.* 1993).

(iii) *Cliff swallows*

Cliff swallows are highly colonial passerines that breed throughout most of western North America (Brown & Brown 1995). They build gourd (bottle)-shaped mud nests and attach them to the vertical faces of cliff walls, rock outcrops or artificial sites, such as the eaves of buildings or bridges. Their nests tend to be stacked closely together, often sharing walls, which facilitates the movement of swallow bugs between nests (by crawling). Cliff swallows are migratory, wintering in southern South America and have a relatively short breeding season in North America. They begin to arrive at our study site in late April or early May and most have departed by late July. They generally raise only one brood.

(b) *Study site*

Our study site is centred near Ogallala, in Keith County, along the North and South Platte Rivers and also includes portions of Deuel, Garden and Lincoln counties, southwestern Nebraska. We have studied cliff swallows there since 1982. There are *ca.* 160 cliff swallow colony sites in our 150 km × 50 km study area, with approximately one-third of these not used in a given year. The size of a colony at a site varies widely; in our study area it ranges from two to 3700 nests, with some birds nesting solitarily. Over a 19 year period, the mean ($\pm$s.e.) colony size ($n = 1282$) was 356.5 ($\pm$16.3) nests. A colony site tends to be separated from the next nearest site by 1–10 km, but in a few cases by $\geqslant$20 km. The birds in our study area nest on both natural cliff faces and on artificial structures, such as bridges, buildings and highway culverts. The study site is described in detail in Brown & Brown (1996).

(c) *Field collections of bugs*

We collected swallow bugs from cliff swallows' nests in two ways. In 1998, we randomly selected five to seven cliff swallows' nests at each colony site that had been active 1–2 weeks earlier in the season and removed these nests in their entirety from the nesting substrate. Nests were collected in July after all cliff swallow nestlings had fledged. Each nest was individually bagged and taken to the laboratory, where processing commenced within 36 h in all cases. Chunks of dried mud from the nests were placed in a Berlese funnel suspended directly beneath a heat source. Bugs crawled off the mud and dropped through the funnel into a collecting jar. Fine-grain soil and dust from the nest that contained bugs were placed in a Petri dish with filed-off sides in the Berlese funnel. This method harvested virtually 100% of the bugs in each nest. Later visual examination of nest pieces showed that no bugs had escaped collection.

Because of the destructive nature of the nest collection and the time-consuming processing of each nest, we collected bugs directly from the outside surfaces of extant cliff swallows' nests in 1999–2000. We lightly brushed bugs clustered on the bottom and sides of a nest into a wide-mouthed collecting jar held directly below the nest using a paintbrush. We attempted to collect 100 bugs from each nest in this way. In some cases, if we could not obtain 100 bugs from a single nest, we took other bugs from the nests adjacent to it. This seemed justified given the extreme mobility of the bugs and their rapid movement from nest to nest, which was often in response to our disturbance. However, we were still unable to obtain a full 100-bug sample in all cases (see § 3a). This method allowed us to take more nest samples per colony site, usually 20–25 per site unless the colony size was too small to allow this many. We randomly selected nests from all parts of each active colony and, where necessary, sampled from both the early- and late-nesting portions of a colony. Swallow bugs exhibit seasonal differences in their extent of clustering on the outside surfaces of nests, with this behaviour most pronounced in the early nestling stage. Consequently, we were able to collect bugs most efficiently from nests with small

nestlings, and most of our samples in 1999–2000 came from nests at this stage in June and early July.

(d) *Virus isolations and identification*

Viruses from swallow bugs were isolated by a plaque assay in Vero cells (African green monkey kidney cells, American Type Culture Collection CCL-81; Manassa, VA, USA). Pools of 100 bugs were triturated by mortar and pestle and suspended in 2.0 ml of buffer 'B' (M-199 Hanks salts, 1% bovine serum albumin, 350 mg l^{-1} sodium bicarbonate, 100 units ml^{-1} penicillin, 100 mg l^{-1} streptomycin and 1 mg l^{-1} Fungizone in 0.05 M Tris, pH 7.6). Homogenates were clarified by centrifugation (3 min at 14 000 rpm). We added 100 µl of the supernatant in duplicate to a monolayer of Vero cells in a six-well cell culture plate (Corning Costar Corp., Cambridge, MA), incubated it for 1 h at 37 °C in 5% CO_2 and then overlaid it with 3 ml 0.5% agarose in M-199 medium supplemented with 350 mg l^{-1} sodium bicarbonate, 29.2 mg l^{-1} L-glutamine and antibiotics and returned it to the incubator. A second overlay containing 0.004% neutral red dye was added after 2 days' incubation for plaque visualization. Plaques were scored daily for 5 days. Cultures with plaques, which indicate the presence of a virus, were harvested by scraping the cell layer into 1 ml buffer B supplemented with 20% filter-sterilized fetal bovine serum. A 100 µl aliquot of this suspension was used for inoculating 25 cm^2 cell culture flasks (Nunc; http://www.nalgenunc.com/unitech/) with confluent Vero cells held in 8 ml maintenance medium (buffer 'B' lacking both bovine serum albumin and Fungizone) and incubated at 37 °C until a virus-induced cytopathic effect became evident by microscopic inspection (on average between 18 and 24 h).

Viral RNA was extracted from 140 µl of the infectious precleared (5 min at 3500 *g*) supernatant of the second Vero cell passage using the RNeasy extraction kit as recommended by the manufacturer (Qiagen, Hameln, Germany). Five microlitres of the eluated RNA suspension (40 µl) was used as a template in an alphavirus reverse transcription-polymerase chain reaction (RT-PCR) as described by Pfeffer *et al.* (1997). This group-reactive RT-PCR targets a highly conserved region within the *nsP1* gene of the alphavirus genome. In order to identify the alphavirus species, DNA of the respective amplicons was extracted out of agarose gels and the nucleotide sequence determined for randomly selected virus isolates (Pfeffer *et al.* 1998).

(e) *Designating colonies and colony sizes*

Cliff swallow colonies were defined as groups of nesting pairs that at least occasionally interacted behaviourally (Brown & Brown 1996). In most cases, a colony was simply all the nests on a given bridge or highway culvert. Colony size is considered here as being the maximum number of cliff swallow nests to have housed one or more eggs. Active nests were counted at some sites by periodically checking the nest contents with a dental mirror and flashlight, whereas the colony size at other sites was estimated by counting the number of nests in active sections of the colony. Full details on the methods of determining colony sizes are given in Brown & Brown (1996). The presence or absence of house sparrows at each site was noted when we visited to collect bugs or in the course of other work at these colonies.

(f) *Estimating the population sizes of swallow bugs*

The estimated swallow bug population sizes at the colony sites were those of Rannala (1995), which were conducted in our study area in 1993 and at many of the same sites used in this study. Briefly, Rannala's (1995) method was a two-step process in which he first established the correlation between the counts of visible bugs on the outsides of a nest and the total number of bugs in the nest as determined by collecting the nest and harvesting the bugs with a Berlese funnel. He then used randomized stratified and cluster sampling for censusing bugs on the outsides of nests throughout each colony. The swallow bug population size in the colony as a whole was estimated using a regression factor relating the censuses to the total bugs in a nest and knowing the total number of active nests (Rannala 1995).

3. RESULTS

(a) *Effects of sample size, date and bug age*

Because we used two sampling methods for swallow bugs and because little was known about the virus infection rates in these bugs prior to our study, we first determined how the number of bugs sampled at a site might have influenced the likelihood of finding pools positive for BCR virus. In 1998, the total number of bugs collected (using the Berlese funnel) at the nine colonies sampled ranged from 564 (mean 112.8 per nest) to 6442 (mean 920.3 per nest) per site. The percentage of nests at a site with detectable virus was not significantly correlated with the total number of bugs sampled at the site ($r=0.06$, $p=0.89$ and $n=9$ colonies) or the mean number of bugs per nest per site ($r=0.18$, $p=0.65$ and $n=9$ colonies). This suggested that our results from 1998 were not artefacts of the differences in the number of bugs present in the nests chosen for sampling.

In some cases in 1999–2000, during which we collected bugs by brushing them off the nests (see § 2c), it was not possible to obtain 100 bugs in each sample. We investigated whether the presence or absence of virus in a nest sample was related to the number of bugs in the sample using a categorical logistic regression (CATMOD procedure in SAS). We also modelled the effects of colony size (see below) and date of sampling. Date of sampling had no significant effect on the presence or absence of virus in a sample ($p=0.46$). However, both the number of bugs ($p=0.006$) and colony size ($p=0.044$) were significant. This suggests that the patterns we report are unlikely to be sampling date artefacts, but that the number of bugs in the range below 100 had an effect on the detectability of virus in a sample. We thus excluded all nests with samples of fewer than 100 bugs and used only samples with exactly 100 bugs in all further analyses for 1999–2000. We pooled the data for 1999 and 2000 because (i) we used the same sampling method each year and (ii) we found no significant annual difference in the overall percentage of nests positive for virus (27.6% of 87 nests in 1999 were positive and 19.3% of 243 nests in 2000 were positive) ($\chi^2_1=2.58$ and $p=0.11$).

Because the detectability of virus might vary among bug age classes, we recorded the number of adults and instars (all third, fourth or fifth) represented in each nest's sample in 2000. We found no effect of bug age: nests with and without virus did not differ significantly in their proportions of adult bugs within the 100 bug sample (Wilcoxon test, $p=0.49$). Thus, our detection of virus was not dependent on the ages of the bugs represented in each sample.

All of the virus isolates identified from the swallow bugs were confirmed to be BCR virus.

(b) *Effects of colony size*

The percentages of nests at a site with bugs positive for virus increased significantly with the size of the cliff swallow colony (figure 1). The r^2 values indicated that *ca.* 40 and 26% of the variation in the percentage of nests with virus could be accounted for by variation in the size of the colony in 1998 and 1999–2000, respectively. The same general pattern was found using both methods of sampling bugs.

Because swallow bugs overwinter at a colony site, the bugs present in the current year also reflect the conditions at the site the previous year(s), to some degree. We examined whether colony size at a site during the previous season was related to the percentage of nests with virus in the current season. In this analysis, we used a colony size of zero for sites not occupied by cliff swallows during the previous year. We found a similar effect of the previous year's colony size: the percentage of nests with virus increased with the previous year's colony size in both 1998 ($r=0.60$, $p=0.09$ and $n=9$ colonies) and 1999–2000 ($r=0.44$, $p=0.027$ and $n=25$ colonies), and the combined probability was significant ($\chi^2_4=12.04$ and $p=0.017$).

(c) *Spatial autocorrelation in virus prevalence*

There was a significant positive correlation between the percentage of nests with virus at a site in year t and year $t+1$ (figure 2) for sites that we sampled that were active in successive years. However, this spatial autocorrelation broke down over longer time-intervals because there was no significant correlation between year t and year $t+2$ (figure 2). This is most probably because the colony size itself at a site tends to vary from year to year. Note that this analysis (figure 2) is only for sites that were active in both time-periods because those were the sites from which we could collect bugs.

(d) *Bug population size in relation to colony size*

The estimated total number of swallow bugs at a colony site increased significantly with the size of the cliff swallow colony ($r=0.60$, $p<0.001$ and $n=30$ colonies) using Rannala's (1995) estimates of bug population sizes. This analysis included inactive sites, which were assigned a colony size of zero nests. The estimated bug population sizes ranged from 30 to 141 000 bugs per colony site (Rannala 1995). Thus, the size of a cliff swallow colony can be considered a relative index of the bug population size at a site. This result is consistent with other analyses showing higher levels of per capita swallow bug parasitism of cliff swallows in larger colonies (Brown & Brown 1986, 1996).

(e) *Effects of house sparrows*

Because BCR virus has been isolated from the sera of house sparrows occupying cliff swallow colonies, we examined whether the presence of sparrows at a site influenced the prevalence of the virus. Colony sites containing active house sparrow nests had a significantly higher overall rate of virus infection in the bugs of cliff swallow nests. The sites with sparrows had 31.7% positive nests

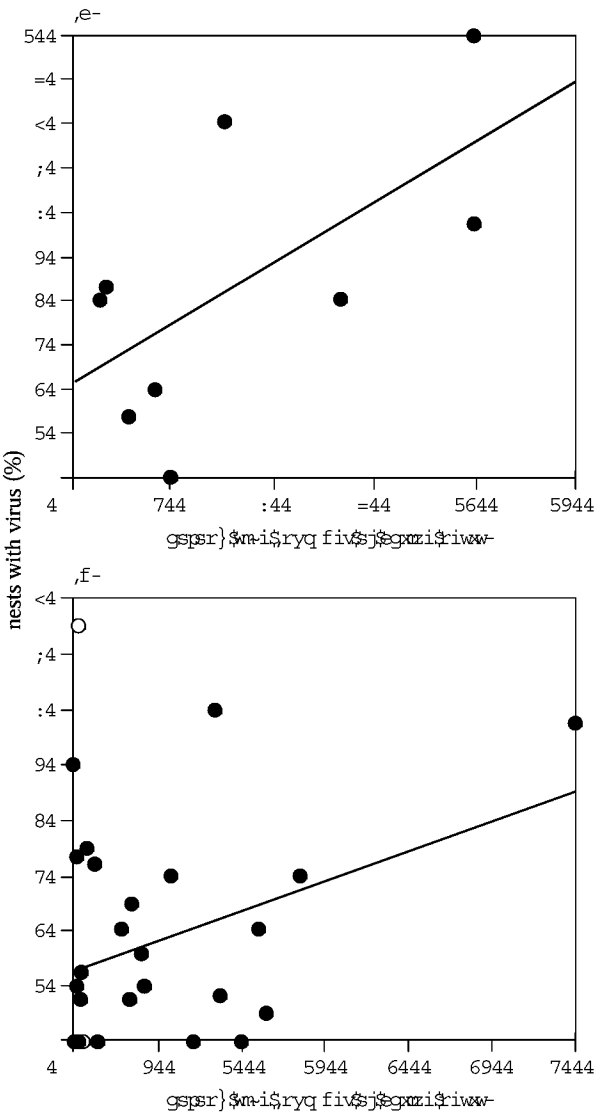

Figure 1. Percentage of nests with swallow bug pools that were positive for BCR virus in relation to cliff swallow colony size in the current year in (*a*) 1998 and (*b*) 1999–2000. The methods for sampling bugs differed in (*a*) and (*b*). The percentage of nests with detectable virus in bugs increased with colony size in 1998 ($r=0.64$, $r^2=0.40$, $p=0.066$ and $n=9$ colonies) and in 1999–2000 ($r=0.51$, $r^2=0.26$, $p=0.009$ and $n=25$ colonies) and the combined probability was highly significant ($\chi^2_4=14.86$ and $p=0.005$). The lines represent best-fit linear least-squares regression. A similar pattern was seen with colony size in the previous year (see § 3b). The colony denoted by an open circle in (*b*) was believed to be anomalous in that it was the only site where active house sparrow nests outnumbered those of cliff swallows.

($n=126$ nests) as compared with 15.2% positive nests ($n=204$ nests) at sites with no sparrows ($\chi^2_1=12.63$ and $p<0.001$).

If sparrows were routinely associated with larger cliff swallow colonies, the apparent effect of colony size (figure 1) might instead mostly reflect the presence of sparrows. However, we found no significant difference between the mean (±s.e.) colony sizes of sites that had sparrows (446.8±93.3 nests and $n=28$) and sites that did not

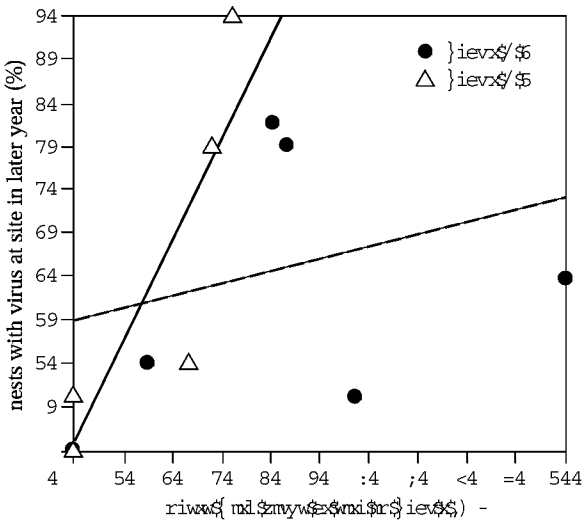

Figure 2. Spatial autocorrelation in the percentage of nests with swallow bug pools that were positive for BCR virus per cliff swallow colony site in year t versus years $t+1$ and $t+2$. There was a significant autocorrelation in year $t+1$ ($r_s=0.98$, $p=0.005$ and $n=5$ colonies), whereas there was no significant autocorrelation in year $t+2$ ($r_s=0.20$, $p=0.67$ and $n=7$ colonies). The lines represent best-fit linear least-squares regression (solid line for year $t+1$ and dashed line for year $t+2$).

(415.4 ± 114.6 nests and $n=36$) (Wilcoxon test, $p=0.21$) in 2000. We used 2000 as a representative year, but the pattern was the same in other years. Similarly, we found no significant relationship between colony size and the presence or absence of house sparrows at a site using a logistic regression (CATMOD procedure in SAS) for the 64 colonies in 2000 ($p=0.84$). Thus, the effect of cliff swallow colony size on virus prevalence (figure 1) is unlikely to be an artefact of a hidden relationship between the presence of sparrows and colony size.

4. DISCUSSION

Infection of swallow bugs by the BCR alphavirus increased with cliff swallow colony size. This result persisted during the 3 years of the study, was robust to differences in the bug sampling methods and could not be statistically explained by the date of collection, sample size of the bugs, age structure of the bugs or the presence of house sparrows. To the authors' knowledge, this is the first demonstration of infection by a single arbovirus increasing with the group size of both its vectors and hosts. The only similar work on viruses is Hochberg's (1991) interspecific comparison using several kinds of viruses, in which he found that gregarious species of butterflies were more resistant than were solitary species, suggesting a greater history of exposure for those feeding in groups. In addition, Davies *et al.* (1991) found that malarial protozoan parasites tended to be transmitted more often in larger primate groups.

The relative importance of swallow bugs versus cliff swallows in producing this result (e.g. figure 1) is unclear and confounded by each other. Because the number of swallow bugs at a site is correlated with cliff swallow colony size, the population sizes of either organism might reach critical thresholds for sustaining BCR virus infection. Both the bug and the bird are closely associated with BCR virus and its sibling strain FM virus (Hayes *et al.* 1977; Rush *et al.* 1980; Scott *et al.* 1984; Hopla *et al.* 1993). For example, the results presented here show that relatively high percentages of swallow bugs are infected and preliminary data on the presence of BCR virus antibody in cliff swallows show at least 9% of adults with detectable antibody titres ($n=245$ birds) (C. R. Brown, M. B. Brown, N. Komar and S. B. Quick, unpublished data). Nestling cliff swallows are known to become viraemic when fed upon by infected bugs (Scott *et al.* 1984).

However, it seems probable that swallow bugs account for the increase in BCR virus infection in larger groups. This is because (i) the sedentary bugs are present at colony sites throughout the year, unlike the migratory cliff swallows that are in residence for only 8–10 weeks in the summer and (ii) the significant effect of the previous year's colony size is more probably manifested in bugs, in which the current population size at a site will be determined in part by the numbers present during the previous summer. We do not know the capacity of bugs for maintaining infection over the winter, but it seems likely that they can serve as overwintering reservoirs for the virus given the relatively high infection rates during the summer. If so, larger bug populations at a site, deriving in part from past years of use of that colony site, may promote BCR virus persistence simply by a decreased risk of virus extinction through stochastic loss of all infected individuals. This is suggested by the six colonies in which we found no virus infection in any of the nests: besides being relatively small colonies in the year of sampling (figure 1), four (66.7%) had been vacant for at least 2 years prior to the year of sampling. In contrast, among sites with detectable virus, only one out of 19 (5.3%) had been vacant for at least 2 years prior to the year of sampling. Similarly, there was a trend for infection rates to increase in the second season among sites sampled in two consecutive years, as illustrated by the positive slope for year $t+1$ in figure 2 and probably reflecting continuous occupancy by cliff swallows and a greater probability of bug survival between years. Thus, the longer a site is continuously used the less likely it seems that the virus will disappear entirely. This would particularly be the case if, once infected, a given bug maintains its infection for life. Because the average longevity of bugs will be greater when a site is continuously used, a higher total number of infected bugs should result. Vector longevity has been shown to be one of the most important factors in maintaining mosquito-borne bunyavirus infections (Scott *et al.* 1983).

As bug populations at a site increase, the virus may also be maintained and/or the infection rates increased through greater horizontal and vertical transmission of the virus between bugs. We know little about bug population dynamics, but if bug reproduction is positively density dependent, transovarial virus transmission could increase the number of infected individuals in the youngest age classes in larger colonies simply through their higher reproductive rates. Evidence for naturally occurring transovarial transmission has been found for

the closely related WEE alphavirus and other arboviruses in mosquitoes (Turrell 1988; Fulhorst *et al.* 1994; Miller *et al.* 2000). Higher rates of transmission in larger populations may also occur within the adult age classes. Swallow bugs, like other cimicids, exhibit traumatic insemination in which the male punctures the body wall of the female and injects sperm into her abdomen outside of the reproductive tract (Usinger 1966). If virus is transmitted between individuals in this way and if individuals mate more often when they have access to more potential mates in larger populations, this will contribute to the increased levels of BCR virus in larger colonies. Venereal transmission is known in mosquitoes infected with other arboviruses (Turrell 1988).

What role do cliff swallows play in producing the pattern of higher virus prevalence in larger colonies? One possibility is that their relatively ephemeral presence, as compared with the sedentary bugs, has little effect on BCR virus and that cliff swallow colony size is simply a direct index of the bug population size at a site (which is what really matters). However, we know that cliff swallows exhibit viraemia when fed upon by bugs (Scott *et al.* 1984) and, thus, that they may serve as an amplifying host for transmitting active virus back to uninfected bugs that feed on them. Bugs probably find blood meals more quickly and feed on a greater total number of hosts in larger colonies where more swallow hosts are available or are packed in higher density (Brown & Brown 1996). Larger colonies may also be statistically more likely to contain viraemic birds and, consequently, the rates of transmission to uninfected bugs will be greater than at small sites. This effect will be magnified if viraemic birds are more likely to settle in large colonies. We know, for example, that cliff swallows with higher levels of parasitism by fleas (*Ceratophyllus celsus*) tend to settle in larger colonies (Brown & Brown 1996, 1999).

The greater overall virus infection rate of swallows' nests in colonies with active house sparrow nests relative to sites without house sparrows suggests that the sparrows may also contribute to the maintenance and transmission of BCR virus. Swallow bugs will use house sparrows as alternative hosts in the absence of cliff swallows (C. R. Brown and M. B. Brown, unpublished data), and this additional host resource may promote bug survival and, thus, annual virus persistence, particularly whenever cliff swallows do not reoccupy a site. Sparrows are non-migratory and use the cliff swallows' nests for roosting even during the non-breeding season and, thus, they may play a direct role in virus amplification during the times of the year when cliff swallows are absent. BCR virus and related alphaviruses have been isolated from house sparrows (Hayes *et al.* 1977; Monath *et al.* 1980; Rush *et al.* 1980; Scott *et al.* 1984; Hopla *et al.* 1993). However, our results indicate that the presence of house sparrows is unrelated to cliff swallow colony size and, therefore, that house sparrows are unlikely to be responsible for the observed increase in virus infection with colony size, at least during the swallows' breeding season.

Most viruses require a critical population size of hosts in order to sustain infections (Schenzle & Dietz 1987; Anderson & May 1992; De Jong *et al.* 1995; Keeling & Grenfell 1997). Such a threshold can exist given various assumptions about the between-group immigration of infected hosts or vectors, the degree of spatial heterogeneity in infection rates and the extent of population subdivision (May & Anderson 1984; Andreasen & Christiansen 1989; Haraguchi & Sasaki 2000). We will not know whether a critical colony size threshold occurs for the cliff swallows themselves until we collect more data on the prevalence of antibody in birds from a variety of sites. However, our data for the swallow bugs do not suggest any threshold colony size below which BCR virus cannot persist (figure 1). For example, some relatively small colonies had relatively high virus infection rates among the bugs in them. This may be because bugs, like mosquitoes and other arbovirus vectors, are generally not negatively affected by the virus (Turrell 1988) and do not show immune responses that lead to the removal of infected individuals from the population of susceptible carriers. Invertebrate vectors such as swallow bugs may instead show viral infection dynamics more like those of macroparasite–host systems, in which permanent infections or reinfections of an individual are possible. In this case, we would expect the virus infection of bugs to be affected principally by the total bug population size in the same way that macroparasitism varies with host density. Macroparasites generally increase with host group size (e.g. Poulin 1991*a*,*b*; Hoogland 1995; Brown & Brown 1996, 2001).

Studies of how BCR virus directly affects cliff swallows are in progress, but the results reported here suggest a potential virus-related cost of coloniality for these birds. Swallows can expect greater exposure to this arbovirus in larger colonies, with 50–100% of nests in the largest colonies containing infected bugs. If this results in a higher likelihood of the birds themselves being infected with BCR virus and if the virus is in any way deleterious to the swallows, the fitness of birds living in large colonies will be reduced. This would be yet another negative consequence of heavier swallow bug parasitism in larger cliff swallow colonies (Brown & Brown 1986, 1996; Chapman & George 1991; Loye & Carroll 1991).

Disease epidemics that are caused by arboviruses (e.g. equine encephalitis or West Nile viruses) are often spatially patchy, with this usually being attributed to unknown aspects of environmental heterogeneity in the distribution of potential vectors and hosts (Hayes 1989; Reisen & Monath 1989; Tsai & Mitchell 1989). Another predominant characteristic of arbovirus epizootics is that they tend to be temporally unpredictable and the foci for these outbreaks in many cases are hard to identify owing to the vagile nature of birds (the common hosts) and mosquitoes (the common vectors). The results presented here are, to the authors' knowledge, the first for an arbovirus that show a temporally and spatially predictive ecological relationship between the virus and its vector/host. It is thus possible to anticipate that 'BCR virus epidemics' are more likely at larger cliff swallow colonies. While the ability of BCR virus to escape the swallow bug/cliff swallow community via a 'bridging' vector such as mosquitoes and, thus, potentially infect humans or livestock is unknown, the results presented here have general biomedical implications in that they show that group size indeed influences arbovirus infection rates. We suggest that systematic studies of other arboviruses that are associated with social hosts or vectors may show the same pattern.

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