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Bird Movement Predicts Buggy Creek Virus Infection in Insect Vectors

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ABSTRACT

Predicting the spatial foci of zoonotic diseases is a major challenge for epidemiologists and disease ecologists. Migratory birds are often thought to be responsible for introducing some aviozoonotic pathogens such as West Nile and avian influenza viruses to a local area, but most information on how bird movement correlates with virus prevalence is anecdotal or indirect. We report that the prevalence of Buggy Creek virus (BCRV) infection in cimicid swallow bugs (*Oeciacus vicarius*), the principal invertebrate vector for this virus, was directly associated with the likelihood of movement by cliff swallows (*Petrochelidon pyrrhonota*), an amplifying host for the virus, between nesting colonies. The prevalence of BCRV in bugs was also directly correlated with the number of swallows immigrating into a site. Birds that move into a site are often transient individuals that may have more often encountered virus elsewhere. These results indicate that the magnitude and direction of daily bird movement in a local area can accurately predict transmission foci for this virus and provide rare quantitative evidence that birds can play a critical role in the dispersal of certain vector-borne viruses. Key Words: Arbovirus—Bird movement—Buggy Creek virus—Coloniality—*Petrochelidon pyrrhonota*—*Oeciacus vicarius*—Virus ecology. Vector-Borne Zoonotic Dis. 7, 304–314.

INTRODUCTION

A MAJOR QUESTION in the study of zoonotic diseases is the extent to which migratory birds carry and transmit pathogens from one locale to another. Because birds are thought to serve as important amplifying hosts for arthropod-borne viruses (arboviruses) such as eastern and western equine encephalomyelitis and West Nile and for directly transmitted viruses such as avian influenza (Reed et al. 2003, Tracey et al. 2004, McLean 2006, Olsen et al. 2006, Reisen et al. 2006), bird movement over both short- and long-distance scales can potentially introduce these pathogens to host populations previously unexposed. The spread of various bird-associated infectious diseases is often as-

sumed to correlate with the known movement of migratory birds, yet in most cases this assumption is based largely on a few anecdotal observations or on broad inferences taken from a general understanding of bird behavior (Stamm and Newman 1963, Lord and Calisher 1970, Calisher et al. 1971, Bennett et al. 2004, Tracey et al. 2004, Morshed et al. 2005, Gilbert et al. 2006, Melville and Shortridge 2006, Kilpatrick et al. 2006a, Olsen et al. 2006, Orme-Zavaleta et al. 2006, Smith et al. 1996). Furthermore, some evidence indicates that the spread of the putatively bird-dispersed West Nile, eastern equine encephalomyelitis, Venezuelan equine encephalitis, and avian influenza viruses over large geographic areas is a very slow process and that birds move them

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rarely, if at all (Calisher et al. 1971, Dickerman et al. 1980, Rappole and Hubálek 2003, Anonymous 2006, Olsen et al. 2006). There are no studies, to our knowledge, that have explored empirically the direct relationship between bird movement and the prevalence of virus infection at either the local scale or over larger geographic distances. If bird movement is related to virus prevalence in an area, this information may prove useful in predicting where novel virus introductions and potential epidemics may occur, and in understanding the spatial distribution of enzootic arboviruses.

Buggy Creek virus (BCRV) is a bird-associated alphavirus (Togaviridae) that is potentially transmitted from one locale to another by its principal amplifying host, the colonially nesting cliff swallow (*Petrochelidon pyrrhonota*). BCRV is within the western equine encephalomyelitis virus antigenic complex (Hayes et al. 1977, Calisher et al. 1980, 1988). Its principal invertebrate vector is the blood-feeding swallow bug (Hemiptera: Cimicidae: *Oeciacus vicarius*), an ectoparasite of the cliff swallow (Hopla et al. 1993, Brown et al. 2001). This virus is unusual in being one of the few alphaviruses routinely vectored by an insect other than mosquitoes (Strauss and Strauss 1994). Because the wingless swallow bugs are largely sedentary and confined during much of the year to occupied and unoccupied cliff swallow nests (Loye 1985, Brown and Brown 2004a, 2005), the spatial foci for BCRV presence are predictable. This allows comparison of site characteristics such as bird colony size or bug population size to the prevalence of virus (Brown et al. 2001, Moore et al. 2007).

The objective of this study is to explore how the prevalence of BCRV infection in swallow bug vectors is related to the extent of movement by cliff swallows into a colony site. Using mark-recapture in the field and multi-state statistical techniques, we estimated the likelihood that a bird from one colony site moves to another. Multi-state mark-recapture models allow one to estimate the probability that a bird makes a transition from one geographical site (or state) to another in essentially the same statistical way that survival and recapture are typically estimated (Nichols and Kendall 1995, Lebreton and Pradel 2002). We compared the probability of immigration to a site by a bird from elsewhere in the study area to the prevalence of virus in the bug vectors at that site. This enabled us to correlate bird movement on relatively small spatial scales to local virus infection prevalence and provided insight into the role of avian hosts in the distribution of this arbovirus. Bird movement is likely to be particularly important in influencing virus prevalence at a site when the birds that move are more likely to be infected, perhaps because they are transient individuals and, by virtue of their behavior, more exposed to virus (i.e., more likely to be bit by vectors) than are sedentary, resident birds (Lloyd-Smith et al. 2005, Kilpatrick et al. 2006b).

METHODS

Study organisms

BCRV was first isolated in 1980 from swallow bugs collected at a cliff swallow colony along Buggy Creek in Grady County, west central Oklahoma (Hopla et al. 1993). BCRV is very similar to another alphavirus, Fort Morgan virus (FMV), which is also associated with cliff swallows and swallow bugs (Hayes et al. 1977, Calisher et al. 1980, Scott et al. 1984). Based on nucleotide similarities and phylogenetic analyses, BCRV and FMV are strains of the same virus (Pfeffer et al. 2006).

Cliff swallows are highly colonial passerines that breed commonly in western North America from the Pacific coast to the Great Plains and more rarely farther east (Brown and Brown 1995). They build gourd-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. 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 depart by late July. Most birds raise only one brood.

The hematophagous swallow bug (Fig. 1) is an ectoparasite primarily of cliff swallows. Swallow bugs are nest-based parasites that

FIG. 1. Swallow bugs (*Oeciacus vicarius*) clustering at the entrance of an unused cliff swallow nest, apparently in attempts to disperse on the legs or feet of a bird.

overwinter in cliff swallows' nests or in the cracks and crevices of the nesting substrate near the nests. Infestations can reach 2600 bugs per nest, and the bugs affect many aspects of cliff swallow life history (Brown and Brown 1986, 1992, 1996, Chapman and George 1991, Loye and Carroll 1991). Swallow bugs begin to reproduce as soon as they feed 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). Bug populations at an active colony site increase throughout the summer, reaching a peak at approximately the time nestling cliff swallows fledge. The bugs seem to be adapted to withstanding long periods of host absence, in some cases for up to three consecutive years (Smith and Eads 1978, Loye 1985, Loye and Carroll 1991, Rannala 1995). Bugs also parasitize introduced house sparrows (*Passer domesticus*) that occupy nests in some cliff swallow colonies (Hopla et al. 1993, Brown et al. 2001). Swallow bugs disperse between nests within a colony by crawling on the substrate and disperse between colony sites by clinging to the feet and legs of cliff swallows that move from one site to another (Brown and Brown 2004a).

Study site

Our study site is centered at the Cedar Point Biological Station (41°13′ N, 101°39′ W) near Ogallala, in Keith County, along the North and South Platte Rivers, and also includes portions of Deuel, Garden, and Lincoln counties, southwestern Nebraska, USA. Cliff swallows have been studied there since 1982. Approximately 160 cliff swallow colony sites are in our 150 × 50 km study area, and about a third are not used in a given year. In our study area, colony size ranges from two to 6000 nests, with some birds nesting solitarily. Over a 20-year period, mean (± SE) colony size ($n = 1363$) was 363 (± 16) nests. Each colony site tends to be separated from the next nearest by 1–10 km but in a few cases by ≥20 km. The study site is described in detail by Brown and Brown (1996).

Field collections of bugs

In 1999–2005, swallow bugs were collected from the outsides of cliff swallow nests during the birds' summer nesting season (May–July). Bugs were either distributed across the bottom and sides of the nests and below the entrance (where they lay eggs and rest between blood meals), or clustered just inside the tubular entrances of the nests (to presumably facilitate dispersal when a transient bird passes by and makes physical contact with the nest; Fig. 1). We brushed bugs off nests into a wide-mouthed collecting jar. We collected from throughout a colony site (in parts where nests were accessible), but only took from nests where bugs were visible to us (i.e., no nests were collected, and thus no bugs from inside or behind the nests were included). We attempted to collect a minimum of 1000 bugs per site and sampled 10–30 nests (depending on the level of infestation) randomly from throughout all accessible parts of a colony. Active colony sites were sampled once (on one date) during the summer. Bugs were transferred from the collecting jar to plastic bags, transported to the Cedar Point Biological Station, and sorted into pools of 100 individuals while alive. Pools were frozen at −70°C. immediately after sorting. In 1998 only, entire nests (five to six per site) were collected soon after cliff swallow nestlings had fledged, and all bugs present were harvested alive using a Berlese funnel and later sorted into pools of 100 after they had been frozen at −70°C. In all years, a given pool was not restricted necessarily to bugs from a single nest, and some contained bugs of mixed nest origin from within a colony.

Virus isolation

Pools of 100 bugs were macerated by mortar and pestle and suspended in 2.0 mL of BA-1, a growth medium containing M-199 Hank's salts, 1% bovine serum albumin, 0.05M Tris-HCl (pH 7.5), 0.35 g/L sodium bicarbonate, 100 U/mL penicillin, 100 μg/mL streptomycin, 1 μg/mL Fungizone (Gibco-BRL, Gaithersburg, MD). Homogenates were clarified by centrifugation. 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 sodium bicarbonate, 29.2 mg/L 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 3 additional days. A bug pool was considered positive for BCRV if one or more plaques were present after the 5 days incubation, and (in 2004–2005) the harvested virus suspension tested positive for BCRV RNA in a specific reverse transcription–polymerase chain reaction (RT-PCR; Moore et al. 2007). Scoring of plaques and RT-PCR was done blindly with respect to sample origin (colony site).

Mist-netting and capture of birds

In 1998–2005, we periodically mist-netted cliff swallows at colonies throughout the nesting season and used the resulting captures and recaptures to estimate daily movement probability. Nets were placed across culvert entrances and against the sides of bridges to catch birds as they exited their nests, or dropped from the top of a bridge to catch birds below as they flushed out (Brown 1998, Brown and Brown 2004b). We chose colonies to include based on their accessibility to us, ease of netting, and colony size, and they were mostly in the center of our study area within a 35-km radius of the Cedar Point Biological Station. A capture occasion at a colony site equated to a single day, with netting usually done for 3–3.5 hours per day per site, although in some cases netting extended for up to 7 h per day at a site.

The occasions on which birds were caught extended over total time periods of 3–82 days within the season at a given colony site (mean, 31.9).

All birds caught received a numbered U.S. Geological Survey band if not previously marked. The total sample size of birds banded and used in this study, over all years and colonies, was 103,083 distributed among 24–33 colonies per year. Because both adult and juvenile cliff swallows move bugs between colonies (Brown and Brown 1996) and both are fed upon by bugs, for this paper we combined captures from adult birds and juveniles (those having fledged that season). Further details on field methods are given in Brown and Brown (2004b).

Over all years of the study, we sampled bugs for virus at 55 colonies where we also netted the birds present there, and for these sites we were thus able to estimate both prevalence of BCRV infection in bugs and bird movement probability to the site. At all other colonies we netted birds but did not sample bugs, either because the sites were fumigated to remove bugs or because the nests were inaccessible to us. These sites were used in estimating movement probabilities to and from the colonies where we sampled bugs.

Estimation of movement probability

An encounter-history file for each marked bird was constructed for each year, containing all capture occasions during that summer pooled into consecutive 2-day intervals from the date netting started in the study area until it ceased. The encounter-history indicated whether each bird was caught during each 2-day interval and at which colony. Because of the large number of colonies in our study area at which birds were sampled and the resulting large number of parameters had we considered movement probabilities for each pair of colonies separately, we used two states (Lebreton and Pradel 2002) for each bird: presence in a focal colony and presence in all other colonies in the study area (all other colonies combined into the same state). This allowed estimation of (*i*) the transition from a focal site to all other colonies and (*ii*) the transition from all

other colonies to the focal site. Transitions were expressed by the movement parameter, Ψ (± 1 SE), which specifically describes the probability of an individual making the given transition during any 2-day interval during the season. Movement in these analyses reflects both the daily travels of transient, non-breeding birds between sites (perhaps while they are assessing where to nest) and the potential dispersal of breeding individuals elsewhere following a successful or unsuccessful nesting attempt.

In preliminary analyses, we fit different multi-state models to the data for two representative colonies (where we sampled bugs) in each year (Table 1). For each colony, the best-fitting model used for maximum-likelihood parameter estimation modeled (*i*) daily survival separately for the focal colony versus all others combined, with survival constant across time at the focal site and varying with "age" (to account for transients) (Pradel et al. 1997) at the others, (*ii*) daily recapture probability separately for the focal site versus all others combined, with recapture varying with time across the season at each, and (*iii*) daily movement into the focal site as time-constant across the nesting season and daily movement out of the focal site varying with time across the season

(model 1; Table 1). Model fit was assessed with the Akaike Information Criterion weight, which indicates a model's likelihood of being the best one among the candidate set and that which provides the least model selection uncertainty (Burnham and Anderson 2002). For example, in Table 1, model 1 was over six times more likely to fit the data than the next-best one (model 2). Model 1 ranked similarly at all other colonies. The models that incorporated age-dependence in survival used two age classes (first-year and all others) to account for the presence of transient birds in estimating survival (Pradel et al. 1997, Brown and Brown 2004b). Program MARK (White and Burnham 1999) was used for model fitting and to generate maximum-likelihood estimates of survival, recapture, and movement probabilities. Because our models specifically estimated daily recapture probability, any differences among the 2-day intervals in the likelihood of re-catching a bird (due to sampling effort or number of sites netted) were accounted for in estimating movement probability. Recapture parameters for a given 2-day interval when we did not net at a focal site were fixed to 0 using the Fix Parameters utility in MARK.

When data sets do not meet the variance assumptions inherent in the binomial distribution

TABLE 1. MULTI-STATE MODELS FIT TO MARK-RECAPTURE DATA FOR CLIFF SWALLOWS TO ESTIMATE WITHIN-SEASON MOVEMENT PROBABILITIES (ψ) TO AND FROM A FOCAL COLONY SITE (STATE F) AND ALL OTHERS IN THE STUDY AREA (STATE O) FOR A REPRESENTATIVE COLONY IN 2005[a]

Model	$QAIC_c$	$\Delta QAIC_c$	$QAIC_c$ weight	No. estimable parameters
(1) $\phi_{(F\text{-}c,O\text{-}a1t,a2c)}p_{(F\text{-}t,O\text{-}t)}\psi_{(to\ F\text{-}c,\ to\ O\text{-}t)}$[b]	15282.3	0.0	0.86437	74
(2) $\phi_{(a1t,a2c)}p_{(F\text{-}t,O\text{-}t)}\psi_{(to\ F\text{-}c,\ to\ O\text{-}t)}$	15286.0	3.7	0.13541	73
(3) $\phi_{(F\text{-}c,O\text{-}a1t,a2c)}p_{(F\text{-}t,O\text{-}t)}\psi_{(to\ F\text{-}t,\ to\ O\text{-}t)}$	15298.8	16.5	0.00023	77
(4) $\phi_{(F\text{-}c,O\text{-}a1c,a2c)}p_{(F\text{-}t,O\text{-}t)}\psi_{(to\ F\text{-}c,\ to\ O\text{-}t)}$	15388.9	106.7	0.00000	42
(5) $\phi_{(F\text{-}c,O\text{-}c)}p_{(F\text{-}t,O\text{-}t)}\psi_{(to\ F\text{-}c,\ to\ O\text{-}t)}$	15429.8	147.5	0.00000	41
(6) $\phi_{(F\text{-}c,O\text{-}t)}p_{(F\text{-}t,O\text{-}t)}\psi_{(to\ F\text{-}c,\ to\ O\text{-}t)}$	15479.3	197.0	0.00000	101
(7) $\phi_{(F\text{-}c,O\text{-}a1t,a2t)}p_{(F\text{-}t,O\text{-}t)}\psi_{(to\ F\text{-}c,\ to\ O\text{-}t)}$	15499.2	216.9	0.00000	133
(8) $\phi_{(F\text{-}c,O\text{-}a1t,a2c)}p_{(F\text{-}t,O\text{-}t)}\psi_{(to\ F\text{-}c,\ to\ O\text{-}c)}$	15526.6	244.3	0.00000	72
(9) $\phi_{(F\text{-}c,O\text{-}c)}p_{(F\text{-}t,O\text{-}t)}\psi_{(to\ F\text{-}c,\ to\ O\text{-}c)}$	15675.6	393.3	0.00000	39

[a]Survival (ϕ) and recapture (*p*) probabilities were also included in each model. Model fit was similar at all sites, and the best-fitting model (model 1) was used for parameter estimation at each colony.

[b]All parameters were estimated separately for each state except for survival in model 2; time-constancy is denoted in the subscript by *c* and time dependence by *t*; models to correct for transients are denoted by *a1* (the first "age" class, equating to the transients) and *a2* ("age" 2 and older, equating to the residents); movement parameters were those for birds moving to the focal site (*to F*) and moving from the focal site to all others (*to O*).

used in mark-recapture analysis, the data are usually over-dispersed, reflecting lack of independence or some heterogeneity among observations and often brought about by the presence of transients or trap-dependence. Thus, for the data set for each colony, we performed a goodness-of-fit test using program U-CARE (Pradel et al. 2005), and using quasi-likelihood (Burnham and Anderson 2002) adjusted the variance in movement probability estimates by calculating an overdispersion parameter, $\hat{c}$, with the output from U-CARE's Global Multi-Site Test. This method is described further by Pradel et al. (2005). MARK thus provided a quasi-Akaike Information Criterion, adjusted for sample size ($QAIC_c$), used in model weighting.

The estimated number of immigrant birds moving into a colony site per 2-day interval was calculated by multiplying an individual's probability of moving to that site from all others (Ψ) times the total number of birds in all colonies that were included in the data for that year (taken from the estimated colony sizes at those sites). The total number of birds per year used in this estimation varied from 9440 (in 2005) to 15,974 (in 2002). This analysis was performed because annual individual movement likelihood to a site does not directly reflect the total number of immigrant birds at that site unless the source population (all colonies sampled) was the same size in all seasons.

Statistical analysis

The distributions of movement probabilities among colonies and infection prevalences were not normal, and thus to assess the separate effects of several independent variables on virus prevalence at a colony, we ranked all variables and used the rank-transformed values in an analysis of covariance (ANCOVA) (Montgomery 2001). The ANCOVA and nonparametric Spearman rank correlation coefficients were computed using SAS (SAS Institute 1990).

RESULTS

ANCOVA using infection prevalence as the dependent variable ($n = 55$) showed that only bird movement probability to a colony site was a significant predictor of BCRV prevalence in bugs ($F_{1,54} = 77.16$, $p < 0.0001$); non-significant predictors were year ($F_{7,48} = 1.32$, $p = 0.27$), colony size ($F_{1,54} = 0.10$, $p = 0.75$), and colony site (some sites were represented in multiple years; $F_{16,39} = 0.70$, $p = 0.77$). The prevalence of BCRV in swallow bug vectors at a site and the probability of a bird moving from another colony to this site were strongly positively correlated (Fig. 2a). Sites with very low prevalence of BCRV in bugs also had very low likelihoods of cliff swallows moving into that site from elsewhere in the study area; there appeared to be a threshold of about $\geq 40\%$ positive bug pools that was associated with particularly high immigration of birds to a site (Fig. 2a). Because we used time-constant models for estimating movement into a site (Table 1), the movement probabilities reported (Fig. 2a) can be considered to represent "average" values across all time intervals during a season.

The number of colonies analyzed for BCRV each year varied from five to eight, and we found the same relationship in each of the 8 years when each season was analyzed separately (Fig. 2a; Spearman rank correlations of 0.96, 0.90, 0.99, 0.98, 0.72, 0.77, 0.89, and 0.99 for 1998–2005, respectively; $p < 0.05$ for all except 1999 and 2003 where $p = 0.08$ and $p = 0.10$, respectively).

We found no evidence that prevalence of BCRV infection could be explained by co-variation between movement likelihood and colony size: the ANCOVA showed no significant interaction between movement probability and colony size in predicting infection prevalence ($F_{1,54} = 0.65$, $p = 0.65$), and there was not a significant correlation between the probability of a bird immigrating to a site and that site's colony size, over all years combined ($r_s = 0.01$, $p = 0.95$, $n = 55$ colonies).

Bird movement probability into a site was strongly associated with average daily movement probability from that site to all other colonies ($r_s = 0.81$, $p < 0.0001$, $n = 55$ colonies). Consequently, there was also a positive correlation between BCRV infection in bugs at a site and the likelihood of bird emigration from that site (Fig. 2b). Because movement probability from a site to all others was estimated with a model with time-dependent movement (Table 1), for these
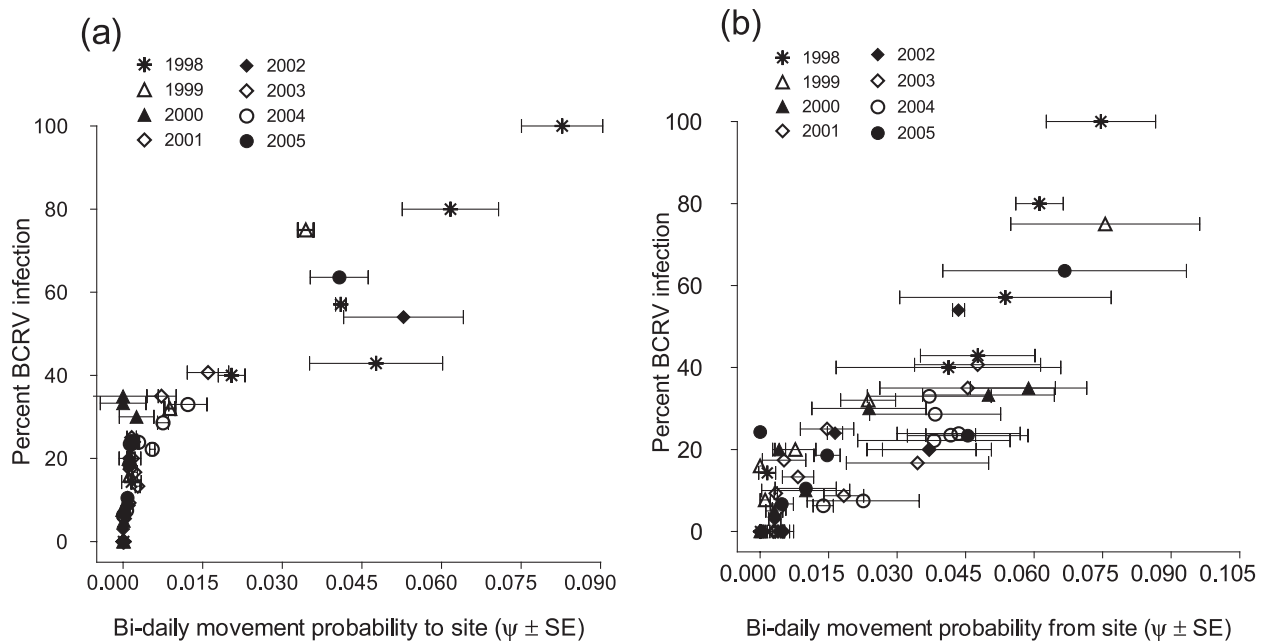
FIG. 2. The percentage of swallow bug pools positive for Buggy Creek virus at a cliff swallow colony site in relation to the probability ($\Psi \pm 1$ SE) that a swallow moves (**a**) to the site from other nesting colonies and (**b**) moves from the site to other colonies in the study area, per two-day interval throughout the summer nesting season by year. Infection of bugs increased significantly with the likelihood of bird immigration to a site ($r_s = 0.95$, $p < 0.0001$, $n = 55$ colonies across all years) and with the likelihood of bird emigration from a site ($r_s = 0.83$, $p < 0.0001$, $n = 55$). Movement probabilities were estimated using the top-ranked model in Table 1 for each site.

analyses we averaged the movement probabilities for a site across all time intervals within the season and presented the result (Fig. 2b) as the mean ± 1 SE.

After converting movement probability to a site into the estimated number of immigrant birds at a colony per 2-day interval, across the season, we found a similarly strong association between the number of immigrant birds and the prevalence of BCRV infection in swallow bugs (Fig. 3). The two sites with the highest BCRV prevalence in bugs attracted 650–900 immigrant swallows per 2 days, compared to almost no birds for the sites with the lowest BCRV prevalence (Fig. 3).

DISCUSSION

These analyses reveal that relative BCRV prevalence in swallow bug vectors at a given site can be predicted by the extent of bird movement into that colony during the summer nesting season. This pattern could not be explained by other variables or co-variation between virus prevalence and colony size. This study is the first to empirically show a relationship between bird movement and prevalence of local virus infection, and the results suggest that as more transient birds move into or pass through a site, more virus will be introduced and infect the insect vectors resident at the colony. The findings suggest that cliff swallows play a major role in determining the geographic distribution and local abundance of BCRV.

Much of the movement of birds between colonies in our study area is by transient individuals that are not nesting or resident anywhere at the time of their moving. Some transients have not yet chosen a nesting site (Brown and Brown 1996), while others are apparent non-breeders assessing sites for future reproduction (Brown 1998). Both groups of birds typically visit multiple colony sites over short time spans, entering unoccupied nests and potentially being exposed to large numbers of clustering bugs at nest entrances (Fig. 1). For example, radio-tracking has revealed that transient individuals will visit as many as seven dif-
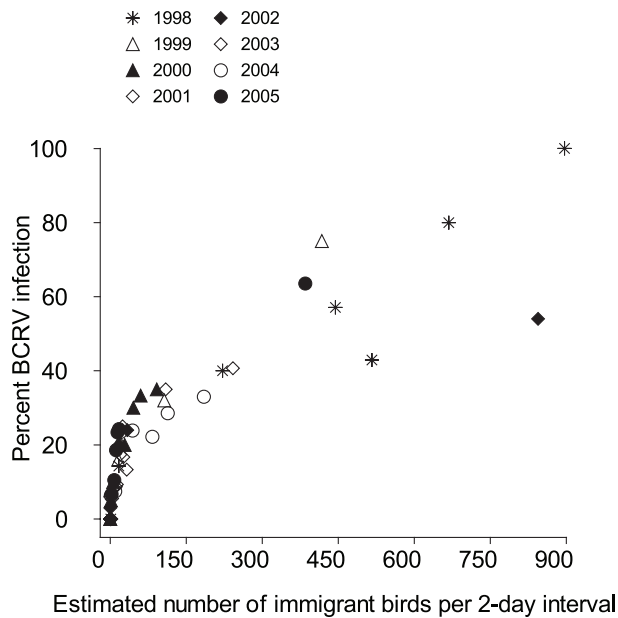
FIG. 3. The percentage of swallow bug pools positive for Buggy Creek virus at a cliff swallow colony site in relation to the estimated number of immigrant birds moving into the site per 2-day interval throughout the summer nesting season by year. Infection of bugs increased significantly with the number of immigrant birds ($r_s =$ 0.95, $p < 0.0001$, $n = 55$ colonies across all years).

ferent colonies, and perhaps as many as 20, over periods ranging from 8 to 23 days (Brown and Brown 1996). Other individuals have visited colonies as far as 23 km apart on the same day (C. Brown and M. Brown, unpublished data). In contrast, resident birds at a colony rarely if ever move between colonies while nesting and are exposed primarily to the bugs and virus in their own nest. The multiple sites visited and the geographic spread of daily movement by transients likely increases their exposure to BCRV because they encounter more bugs, and these individuals may serve as superspreaders (Lloyd-Smith et al. 2005). These transients appear to represent a relatively large fraction of the population in the study area, as demonstrated by survival models with an effect of transients often being the best fit for colonies in an earlier study of daily survival (Brown and Brown 2004b).

Assuming that transient birds are more exposed to virus than individuals in the population at large, the association of BCRV prevalence with cliff swallow movement can result when (*i*) some arriving viremic birds at a site

explore and possibly roost in nests and infect local bugs that feed on them, and/or (*ii*) birds carrying dispersing infected bugs on their legs (Brown and Brown 2004a) pass through a nesting colony and deposit the infected bugs there. Both scenarios may occur, and at present we do not know which is more common. The increased number of immigrant birds (superspreaders) simply increases the likelihood of viremic individuals and/or ones with infected bugs on their legs being represented among the immigrants. Furthermore, the co-variation between immigration and emigration suggests that many cliff swallows within the study area circulate back and forth between sites that are relatively highly infected with BCRV. Thus, sites that receive large numbers of immigrants get many of them from colonies that are also highly infected, contributing to the higher likelihood of a viremic swallow or one with an infected bug being among the immigrants at a given site. Although the estimated number of immigrant birds per two-day interval at the highly infected sites seems very large, the numbers estimated are consistent with two other estimates of the number of transient birds in this population, each done with a different statistical methodology (Rannala 1995, Brown and Brown 2004a).

The bird movement estimated in this study occurred over a relatively small spatial scale: the greatest distance between any pair of colony sites among those included here in any year was 90 km. Whether the observed relationship between the prevalence of BCRV infection in bug vectors and bird movement to a site would also hold for larger spatial scales is unknown. If BCRV viremia is short-lasting, infected birds might not have time to travel long distances before clearing the infection, a situation that may also apply to West Nile virus and other viruses and limit their spread by birds (Reeves 1974, Rappole and Hubálek 2003, Olsen et al. 2006, Owen et al. 2006). We at present know nothing about the duration of BCRV viremias in cliff swallows, but house sparrows can remain viremic with BCRV for 1–3 days after infection (Huyvaert et al. 2008). Mark-recapture work has shown that cliff swallows can travel between colonies that are at least 59 km apart during a 3-day interval (Brown and

Brown 1996) and between colonies up to 23 km apart during a single day, illustrating the potential for viremic individuals to transmit virus among different sites on a relatively local scale.

A positive association between BCRV in vectors and bird movement to a site might happen if more BCRV occurs at large colonies that are more likely to be perennially used (Brown et al. 2001), and if more immigrants are attracted to such colonies because of their size or history. However, the analysis presented here shows that colony size has no effect on BCRV infection prevalence when controlling for other factors and that only immigration to a site statistically predicted the prevalence of BCRV in bugs. This was further supported by the lack of a relationship between colony size and probability of bird movement to a site. Earlier work showed a colony-size effect on BCRV infection in bugs (Brown et al. 2001), but in the present study that effect disappeared when controlling for immigration and when using additional years of data. Colony density and bug population size also cannot be potential explanatory factors, because colony size is highly positively correlated with both, and conclusions about colony-size effects (or lack thereof) thus apply equally to colony density or bug population size (Brown and Brown 1996, Brown et al. 2001).

This study indicates that within-season movement of bird hosts in a local area seems to predict the transmission foci for BCRV, at least among vectors, and that movement of transient birds that more often encounter virus may help generate the spatial heterogeneity in virus prevalence characteristic of BCRV (Brown et al. 2001, Moore et al. 2007) and perhaps other viruses associated with birds. These results also illustrate the insight into patterns of virus transmission that are possible by using systematic mark-recapture and multi-state methods for analyzing movement probabilities.

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