



RESEARCH ARTICLE

## Examination of multiple working hypotheses to address reproductive failure in reintroduced Whooping Cranes

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### ABSTRACT

Understanding multiple challenges that restrict conservation success is a central task of applied ecology, especially when resources are limited and actions are expensive, such as with reintroduction programs. Simultaneous consideration of multiple hypotheses can expedite identification of factors that most limit conservation success. Since 2001, reintroduction of a migratory population of Whooping Cranes (*Grus americana*) has been under way in eastern North America. Hatching success, however, has been extremely low. In our study area, in and near Necedah National Wildlife Refuge in central Wisconsin, USA, we simultaneously tested 3 hypotheses explaining poor hatching success: harassment of incubating birds by black flies (Simuliidae), effects of captivity, and inexperience of breeders. When black flies were experimentally suppressed, hatching probability doubled. Daily nest survival for Whooping Cranes was strongly and negatively related to an index of black fly abundance, particularly of *Simulium annulus*. Daily nest survival was negatively but only weakly related to the number of generations that ancestors of breeding Whooping Cranes had been in captivity and was not related to nesting experience. We also examined whether Whooping Cranes were nesting later to avoid stress from black flies. Phenology shifted earlier with more growing degree days and greater nesting experience and was only weakly related to year. Overall, improved hatching success did not lead to better reproductive success. Although effects of black flies on hatching success can be mitigated through management, such actions would not be adequate to generate satisfactory population growth. Recognition of this limitation was hastened through experimentation.

**Keywords:** *Bti*, captive selection, daily nest survival, experience, *Grus americana*, nest phenology, Simuliidae, *Simulium annulus*

### Examen de múltiples hipótesis de trabajo para determinar el fracaso reproductivo en individuos reintroducidos de *Grus americana*

### RESUMEN

Entender los múltiples desafíos que limitan el éxito de la conservación es una tarea central de la ecología aplicada, especialmente cuando los recursos son limitados y las acciones son caras, como en los programas de reintroducción. La consideración simultánea de múltiples hipótesis puede agilizar la identificación de los factores que más limitan el éxito de la conservación. Desde 2001, se ha puesto en marcha la reintroducción de una población migratoria de *Grus americana* en el este de América del Norte. El éxito de eclosión, sin embargo, ha sido extremadamente bajo. Evaluamos simultáneamente tres hipótesis que explican el bajo éxito de eclosión: acoso a las aves incubando por parte de moscas negras (Simuliidae), efectos de cautiverio y falta de experiencia de las aves reproductivas. Luego de suprimir experimentalmente a las moscas negras, la probabilidad de eclosión se duplicó. La supervivencia diaria del nido de las grullas se relacionó fuerte y negativamente con un índice de abundancia de la mosca negra, particularmente de *Simulium annulus*. La supervivencia diaria del nido se relacionó negativa aunque débilmente con el número de generaciones que los ancestros de las grullas reproductivas habían permanecido en cautiverio y no se relacionó con la experiencia de anidación. También examinamos si las grullas anidaron más tarde para evitar el estrés producido por las moscas negras. La fenología se adelantó con más días de grado de crecimiento y mayor experiencia de anidación, y solo se relacionó débilmente con el año. En general, un mejoramiento del éxito de eclosión no llevó a un mejor éxito reproductivo. Aunque los efectos de las moscas negras en el éxito de eclosión pueden mitigarse a través de su manejo, estas acciones no serían adecuadas para

generar un crecimiento poblacional satisfactorio. El reconocimiento de esta limitación pudo acelerarse a través de la experimentación.

*Palabras clave:* Bti, experiencia, fenología del nido, *Grus americana*, selección cautiva, Simuliidae, *Simulium annulus*, supervivencia diaria del nido

## INTRODUCTION

Reintroducing species is often resource demanding, yet it has become a widespread tool for endangered species management (Seddon et al. 2007, Ewen et al. 2012). Successful reintroduction is generally defined as establishment of a self-sustaining population (Burgman et al. 1993, Converse et al. 2013a, Converse and Armstrong 2016). Therefore, factors that depress demographic performance of reintroduced populations are critical to identify.

A variety of factors can influence the demography of reintroduced populations. Removing threats that originally led to extirpation is a necessary first step in rendering historical habitat capable of supporting a population (Kleiman 1989, Seddon et al. 2007). As the period between extirpation and reintroduction widens, however, the assumption of habitat suitability in the absence of original threats can become more tenuous because of changing environmental conditions. Furthermore, propagules used for reintroduction must come from either captive or extant wild populations, and the genetic composition of the propagules must be appropriate for the target location. Genetic drift and changes in selective pressures, however, can cause the source population to be maladapted for a proposed reintroduction site (Snyder et al. 1996, Ford 2002, Kostow 2004). Even when habitat and population genetics are suitable, learned traditions, such as migratory routes or antipredator responses, might need to be reacquired before final barriers to reintroduction can be removed (Snyder et al. 1996, Griffin et al. 2000, Duff et al. 2001). Thus, environmental, genetic, and behavioral mechanisms can all influence reintroduction outcomes (Armstrong et al. 2002).

Given that multiple factors can inhibit a reintroduction and obscure each other, focused research can be critical to reintroduction success (Armstrong et al. 2002, Seddon et al. 2007, Armstrong and Seddon 2008). For complex ecological questions, formulation and simultaneous consideration of multiple competing hypotheses can advance knowledge in the most efficient manner (Seddon et al. 2007). Furthermore, management in the face of uncertainty is more effective when uncertainty can be articulated, because multiple competing hypotheses of system function (Williams et al. 2007, Nichols and Armstrong 2012, Converse et al. 2013a) and experiments can allow investigators to rapidly evaluate evidence for competing hypotheses (Nichols and Williams 2006). Here, we present results from an experi-

mental and observational study designed to elucidate environmental, genetic, and behavioral factors that might impede reintroduction of Whooping Cranes (*Grus americana*).

The Whooping Crane Eastern Partnership (<http://www.bringbackthecranes.org>, accessed August 29, 2017) began releasing Whooping Cranes in 2001 with the goal of establishing a population in eastern North America (now known as the eastern migratory population [EMP]; Servanty et al. 2014, Urbanek et al. 2014). The core summering population occurs at Necedah National Wildlife Refuge (NNWR) in central Wisconsin, USA, and the first eggs were laid at NNWR in 2005 (Urbanek et al. 2010a). By 2008, many pairs were beginning to nest, but hatching success in the EMP was lower than that in (1) the reintroduced Florida non-migratory population (Spalding et al. 2009) and (2) the wild population (Kuyt 1981). Perhaps more importantly, hatching success in the EMP was substantially lower than the level necessary for a self-sustaining population (Servanty et al. 2014), and nest failure appeared to be synchronous (Urbanek et al. 2010b).

Blood-feeding adult black flies (Simuliidae), specifically *Simulium annulus*, were hypothesized to stress incubating Whooping Cranes in the EMP and force them to abandon their nests (Urbanek et al. 2010b; hereafter “black fly hypothesis”). Runge et al. (2011) evaluated evidence for this and other hypotheses for nesting failure and concluded that testing the black fly hypothesis warranted high priority. Using observational data from 2009 to 2011, King et al. (2013) examined the black fly hypothesis and concluded that numbers of adult *S. annulus* were negatively correlated with nest survival rates. Converse et al. (2013b) considered the same hypothesis, using data from 2009 to 2010, and analyzed data with a daily nest survival model that accounted for the incomplete time series of biting fly abundance indices derived from carbon dioxide traps. A moderately strong negative relationship between *S. annulus* and daily nest survival was found (Converse et al. 2013b). Nevertheless, no analysis had used all available data for 2009–2013, including data from 2 yr (2011–2012) in which all known target black fly populations were manipulated, and a post-treatment control year (2013). Various competing hypotheses for poor reproductive success have been proposed (Converse et al. 2018), including selection for traits that are beneficial in captivity (“captive selection hypothesis”; King et al. 2013), effects of captive rearing on

bird behavior, and effects of bird age and experience (“age and experience hypothesis”).

In addition to understanding the causes of nest failure, potential solutions are also important. King et al. (2013), after concluding that the black fly hypothesis was supported by evidence, suggested that reintroduced Whooping Crane pairs could shift nest initiation dates later, as a facultative response to local stressors. King et al. (2013) observed that some pairs in the population had already delayed nesting between years. These observations lead to the prediction that breeders experienced with black flies would adjust nest initiation dates to avoid black fly emergence (“behavioral response hypothesis”).

Here, we use a combination of experimental data, gained by suppressing populations of black flies, and observational data to evaluate evidence for 6 hypotheses for nest initiation and nest failure. Three hypotheses related to nest failure include (1) the black fly hypothesis (nest abandonment due to harassment by biting flies), (2) the captive selection hypothesis (maladaptive reproductive behavior due to selection for traits that are optimal in captivity but not in the wild), and (3) the age and experience hypothesis (poor reproductive performance by younger and less experienced birds). Three hypotheses related to nest initiation include (4) the behavioral response hypothesis (Whooping Cranes respond to black fly harassment behaviorally by delaying nest initiation), (5) the age and experience hypothesis (date of nest initiation is influenced by age or previous nesting experience but greater experience does not delay initiation), and (6) the environmental hypothesis (Whooping Crane nest initiation is driven by environmental factors such as temperature).

## METHODS

### Study Area

A majority of Whooping Crane nests used in our analysis were established at NNWR (centered on 44.06°N, 90.17°W), where each captive-reared bird was housed before its release. Dispersal of released Whooping Cranes, however, resulted in ~6% of the subsequent territories located up to 357 km outside NNWR (H. Thompson personal communication). The habitat used by Whooping Cranes in central Wisconsin is dominated by large open wetland complexes in the glacial Lake Wisconsin sand plain ecoregion (Omernik et al. 2000). NNWR contains a mixture of open water, emergent herbaceous wetland, and woody wetland that dominates the managed flowages, with dense mixed forest, shrub, and grassland communities dominating the uplands. Habitat conditions for NNWR and surrounding areas were described by Van Schmidt et al. (2014).

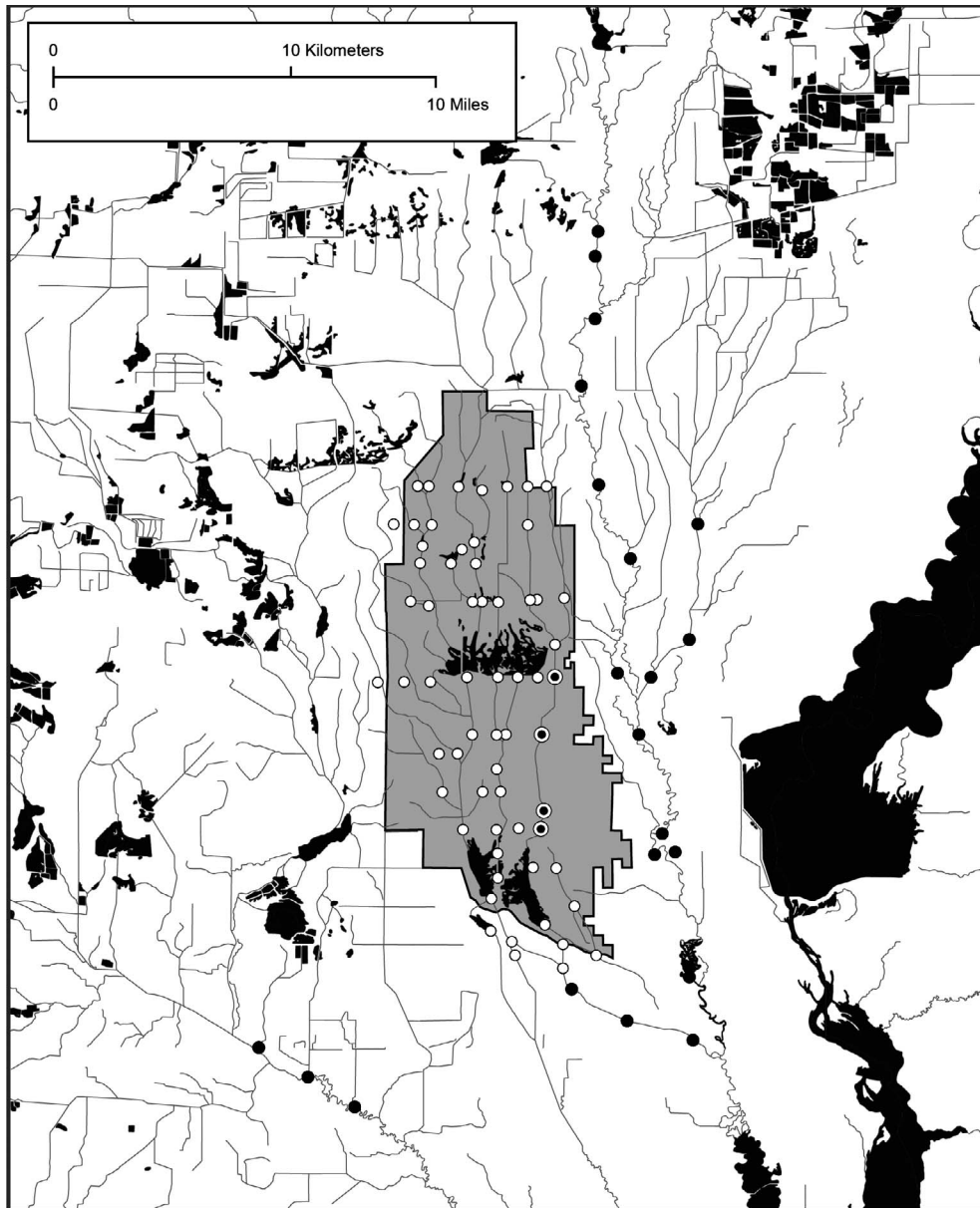
### Whooping Crane Monitoring

All Whooping Cranes with nests in our study were individually marked and monitored (Urbanek et al. 2014). In addition to colored leg bands and USGS bands, each individual carried a combination of satellite tracking devices and VHF radios (Urbanek et al. 2010a). We tracked known Whooping Crane pairs from arrival, usually in early March through the end of nesting (2009–2013), to determine complete nest histories for every potential nesting pair. The number of known pairs that arrived at the Wisconsin breeding grounds, which we expected to nest, were 12 in 2009, 17 in 2010, 20 in 2011, 30 in 2012, and 35 in 2013. These pairs were located by ground or aerial surveys, once every 2 days on average. Whooping Cranes take 55–60 hr from ovulation to oviposition for each egg (Gee and Russman 1996), so locating individuals once every 2 days before a nest was established allowed most nests to be found before the second egg was laid. Twice-daily aerial surveys, augmented by ground tracking, were conducted once the first nest of a season was located. Aerial surveys were conducted in fixed-wing aircraft at ~160 km hr<sup>-1</sup>, from 300 m altitude. Nests were circled until location and status could be determined. Intensive tracking maximized the chance of locating all nests, and few, if any, nest initiations were missed. Pairs with failed nests were monitored to detect renesting events.

Nest fate was defined by 3 possible outcomes: (1) hatched (at least one chick was seen); (2) failed; or (3) incubated >30 days (full-term incubation; Gabel and Mahan 1996) but did not hatch. Such nests might have had dead embryos or infertile eggs for various reasons, some related to our hypotheses (e.g., poor nest attendance related to black flies) and some not (e.g., inexperienced pairs copulate poorly, reducing fertility). A full-term, 30-day incubation (Gabel and Mahan 1996) without hatch was treated as failing on day 30.

### Experimental Black Fly Suppression

At least 26 species of black flies occur in or near NNWR, but only 3 ornithophilic species are attracted to Whooping Cranes: *Simulium annulus*, *S. johannseni*, and *S. meridionale* (King and Adler 2012). In 2009, we sampled all representative stream segments that contained flowing water (57 sites) and were likely to serve as habitat within NNWR for larval black flies of the target species (Figure 1; King and Adler 2012). We also sampled 20 adjoining sites with flowing water within a radius of 10 km from NNWR. A 10 km radius was selected because the greatest dispersal distance known for *S. annulus* is 8 km (Bennett and Fallis 1971). Only sites that had the target species in 2009 were resampled in 2010–2013. *Simulium annulus* and *S. johannseni* inhabit large streams and rivers, typically >10 m wide but as small as 2 m wide, often downstream of a



**FIGURE 1.** Sampling locations for larval black fly populations in and near Necedah National Wildlife Refuge (NNWR), Wisconsin, USA, 2009–2013. Black dots represent locations in streams and ditches where *Simulium annulus* and *S. johannseni* (ornithophilic species that attack Whooping Cranes) were found in all 4 yr; white dots represent locations where they were not found in any year. Bull's-eyes denote sites that had significant populations of *S. annulus* in 2009 but not in 2010–2013. Waterbodies are black while NNWR is gray.

lake outlet (Adler et al. 2004). Our sampling included all lake outlets on the refuge and the few large streams and irrigation ditches, which at bank full were <5 m wide. Our sampling program on the refuge allowed us to sample each segment of flowing water >0.3 m wide such that no stretch of flowing water was left unsampled for >2 km along its length. Ditches with standing water were not sampled; water must be flowing to harbor immature black flies (Adler et al. 2004). In years when we found no immature

black flies where larvae had occurred in 2009, we sampled the habitat by walking ~1.5 km along the stream to ensure that we were not missing the target species. Five years of sampling larval black flies established that the pest species of interest were routinely developing in the larger, non-channeled waterways surrounding NNWR but not within NNWR. The developmental consistency of larval populations in the waterways adjoining NNWR provided a high level of confidence that all sites within this larger



geographic area had been identified. We hand sampled larvae from all available substrate types (i.e. debris, stones, and trailing vegetation) at each site by transferring them with forceps from substrates to vials of 1:3 acetic ethanol for subsequent chromosomal and morphological identification (Adler et al. 2004).

To kill target black fly larvae, we applied *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) to larval habitat by dispensing it from a 20 L carboy at a steady rate while wading bank to bank or walking across bridges (Gray et al. 2011, 2012). We applied larvicide at the label rate of 25 ppm of Vectobac 12AS (lot no. 185-513-N9, Valent BioSciences Corporation, Libertyville, Illinois, USA). *Bti* is specific to larvae of black flies, mosquitoes, and some nonbiting midges (Molloy 1982).

We treated larval black fly populations with *Bti* from 2010 to 2012 and monitored adult black fly populations from 2009 to 2013. Our research design provided a pre-treatment control year (2009) and a post-treatment control year (2013) that bracketed treatment years and allowed us to distinguish an effect caused by black flies from time-related influences such as a gradually maturing Whooping Crane population. Within-year controls were not possible because we could not manipulate black flies at individual nests.

To evaluate treatment efficacy under field conditions, on April 5, 2010, we conducted a partial treatment of one segment of the Yellow River (Figure 2) that supported target black fly larvae. In 2011 and 2012, suppression efforts were more extensive. We initiated larval mortality assessment 4 hr after *Bti* application by collecting substrates with attached larvae (sticks, leaf packs, and grasses) downstream (300–1,600 m) and upstream (100 m) of the treatment site and returning them to the laboratory where they were placed in aerated tanks. We then determined larval mortality rate in the laboratory for control and treatment samples 24 hr following *Bti* application.

We deployed *Bti* in 2011 (March 31–April 3) and 2012 (March 19–22; Figure 2). The optimal treatment period was determined by examination of larval development, stream temperature, and flow conditions (Gray et al. 2011, 2012), and *Bti* was applied in areas near NNWR that had high larval black fly populations as determined from pre-treatment surveys conducted a few days before treatment. No treatment was applied to water bodies on NNWR because no significant larval populations of the target species were found there (Figure 1). We evaluated larval mortality either as in 2010 or from counts in situ. Operationally, wide-scale black fly suppression presents challenges; in our study, for example, 2 species with different life histories had to be targeted as spring runoff conditions subsided. Each year presented different flow conditions in the rivers, requiring the operators to conduct

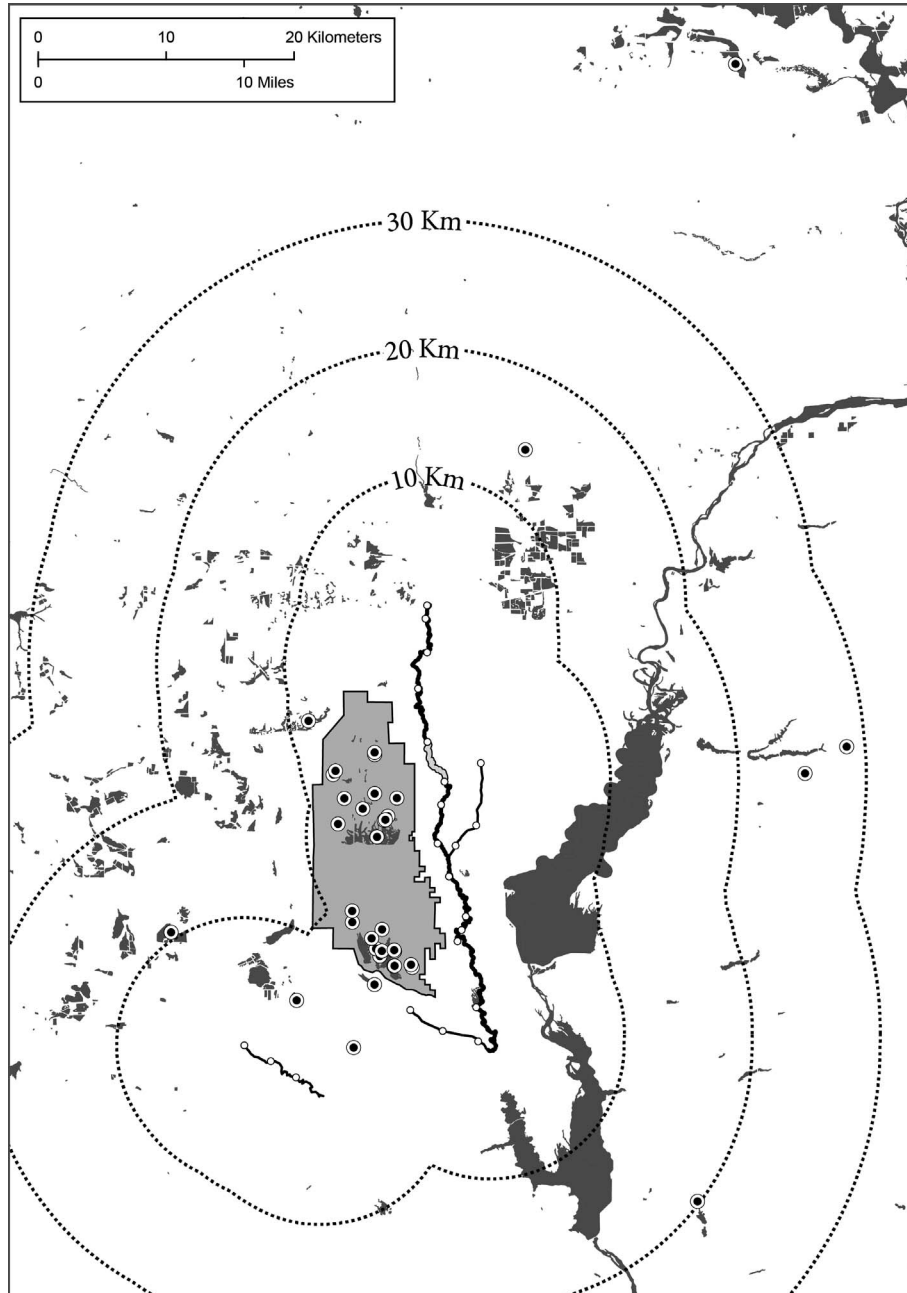
targeted larvicide applications based on the operational parameters and the conditions encountered (Gray and Fusco 2016).

### Adult Black Fly Monitoring

Following the procedure of King and Adler (2012), we deployed Centers for Disease Control and Prevention miniature light traps (with the lights disabled and baited with dry ice as a carbon dioxide source; hereafter “CO<sub>2</sub> traps”), at 7 sites. One trap per site was randomly distributed among Whooping Crane nesting areas on NNWR, stratified between north and south wetland sections of the refuge. In 2009, we sampled traps daily from April 14 to June 19, weather permitting (wind <16 km hr<sup>-1</sup> and no precipitation). In 2010, we sampled 3 of the 7 trap locations and only every ~2 wk during April 15–June 10. In subsequent years, as weather allowed, we sampled at all 7 traps twice weekly during the Whooping Crane nesting season (April 11–June 24, 2011; March 31–June 22, 2012; April 14–July 5, 2013). We activated the traps in early morning and collected the following morning, deploying enough dry ice and battery power to last the 24 hr period. All captured insects were frozen, then placed in 80% ethanol.

We also evaluated the ability of CO<sub>2</sub> traps to provide a useful index of black fly abundance at nests. Monitoring black flies at actual Whooping Crane nests throughout the nesting season posed an unacceptable risk to nests. Instead, in 2012 and 2013, we placed artificial nests within 1 m of 4 of the 7 CO<sub>2</sub> traps. Artificial nests mimicked, to the greatest extent possible, the conditions (particularly the black fly attractants) found at Whooping Crane nests during the entire incubation period. Specifically, local wetland vegetation was shaped on a floating piece of plywood, using dimensions of typical Whooping Crane nests (e.g., diameter = 117 cm; Kuyt 1995). Like King and Adler (2012), we painted plastic Sandhill Crane decoys to mimic Whooping Crane plumage and placed decoys on artificial nests. We used plaster-filled Whooping Crane eggs that had been rubbed on uropygial glands of captive Whooping Cranes. We affixed one glueboard (40 × 65 mm) each to the egg and head of the decoy. To reduce attraction of mammal-feeding black fly species, the observer moved 10 m from the artificial nest and waited 5 min before retrieving the glueboards. Specimen handling followed the methods of King and Adler (2012). We collected these data at each of the 4 artificial nests on 23 days in each of 2 yr.

To determine whether traps provided a useful index of black fly populations at nests, we used linear regression in R 3.1.2 (R Core Team 2012) for counts of *S. annulus*, *S. johannseni*, and *S. meridionale* at artificial nests as a function of counts from adjacent CO<sub>2</sub> traps. We eliminated 2012 from the analysis for *S. johannseni* and *S. meridionale*



**FIGURE 2.** Whooping Crane nest locations in 2009–2013 (bull's-eyes) in relation to black fly treatment spots (white dots) and effective black fly larval treatment areas in our study area in central Wisconsin, USA, 2010–2012. Segments of river treated for all 3 yr (2010–2012) are light gray, those treated for 2 yr (2011–2012) are thick black, and those treated for 1 yr (2012) are thin black. Water bodies are dark gray and Necedah National Wildlife Refuge is light gray. Except where river segments were treated, waterways are not depicted. Concentric rings refer to distances from nearest treatment area.

because so few individuals were captured in that *Bti* treatment year. We transformed count data:  $\ln(\text{count}+1)$ . We first evaluated whether there was an important interaction between trap or year (year only for *S. annulus*) and  $\ln(\text{count}+1)$  from  $\text{CO}_2$  traps. Interpretation was based on whether the 95% confidence intervals (CIs) around the regression coefficients for the interaction terms excluded

zero. On the basis of that result, we developed and ran our final regression models for each species.

We counted all individual blood-feeding adult flies. The flies were identified to family (Culicidae) or to species (all others), primarily by genitalia (Adler et al. 2004). Because of the large numbers of black flies captured in  $\text{CO}_2$  traps (e.g., >70,000 individuals in a single day), we subsampled

collections with >120 black flies by randomly selecting 120–200 specimens to identify. Representative specimens of the various species were deposited as vouchers in the Clemson University Arthropod Collection.

### Statistical Analysis: Daily Nest Survival

We were interested in the relative evidence supporting 3 different hypotheses for nest failure: (1) the black fly hypothesis, (2) the captive selection hypothesis, and (3) the age and experience hypothesis. The approach described by Converse et al. (2013b) was used to construct models of daily nest survival and fit the models, using Bayesian Markov chain Monte Carlo (MCMC) methods. Data were nest-encounter histories for 2009–2013, with  $X_{i,t} = 1$  if nest  $i$  was observed alive on day  $t$  and  $X_{i,t} = 0$  if nest  $i$  was not. Ten of 100 nests did not hatch but were incubated beyond 30 days; these were treated as if they failed on day 30.

If the nest was unobserved on a given day, the data were entered as “NA” so that the MCMC sampling routine could predict the nest status on that day; that is, the model accounted for unknown date of nest failure occurring in a multiday interval between observations (Johnson 1979, Dinsmore et al. 2002, Shaffer 2004).

The encounter history data were modeled as

$$X_{i,t+1} | (X_{i,t} = 1) \sim \text{Bernoulli}(S_{i,t}) \quad (1)$$

and

$$X_{i,t+1} | (X_{i,t} = 0) = 0 \quad (2)$$

The nest survival probability  $S_{i,t}$  was modeled using a logit-linear mixed model:

$$\text{logit}(S_{i,t}) = \beta_0[\text{pair}_i] + \beta_1 * \text{re nest}_i + \beta_P * P \quad (3)$$

where the model of survival for nest  $i$  at day  $t$  included a random intercept for nesting pair, to account for the repeated observations of individual nesting pairs, and a fixed effect of reneesting (where re nest was modeled as an indicator variable with value equal to 1 for second or third nests of a pair within a year). Additional fixed effects,  $P$ , were modeled as described below.

For the black fly hypothesis, we considered  $\ln(\text{black fly daily abundance} + 1)$  for a given nest, where the value for a nest was calculated as the weighted average abundance across all traps, with weight equal to the inverse of the squared distance from the trap to the nest. We considered 3 species of black flies: *S. annulus*, *S. johannseni*, and *S. meridionale* (lnAnn, lnJoh, and lnMer, respectively). We also considered all 2-way pairings:  $\ln(\text{species A} + \text{species B} + 1)$ ; thus, lnAnnJoh, lnAnnMer, and lnJohMer.

As described by Converse et al. (2013b), the insect model allowed us to account for the missing daily observations from the CO<sub>2</sub> traps, wherein the predictors

for insect data are themselves modeled in parallel with the survival model, using temporal autoregressive models to account for missing data. For a nest survival model including insect data ( $I_{i,t}$ ), we have

$$\text{logit}(S_{i,t}) = \beta_0[\text{pair}_i] + \beta_1 * \text{re nest}_i + \beta_P * I_{i,t+1} \quad (4)$$

and we model the insect data as

$$I_{i,t+1} \sim \text{Normal}(\mu_{i,t+1}, \tau_{[\text{year}_i]}) \quad (5)$$

where  $\tau_y$  is a year-specific precision term, and

$$\mu_{i,t+1} = \alpha_{[\text{year}_i]} + \rho_{[\text{year}_i]} * (\mu_{i,t} - \alpha_{[\text{year}_i]}) \quad (6)$$

where  $\alpha_{[\text{year}_i]}$  is a year-specific mean and  $\rho_{[\text{year}_i]}$  is a year-specific autoregressive parameter.

To examine the effect of captive selection, we calculated the number of generations to any, and to all, wild ancestors for each nesting bird (including the generation of the bird itself). We defined a “wild” ancestor as an ancestor that was produced from an egg laid in the wild. For example, if a nesting bird had a sire that was bred in captivity from parents that were collected as wild eggs, but a dam that was collected as a wild egg, the value for any wild ancestor = 2 and for all wild ancestors = 3. For each nest, then, we considered the generations to any wild ancestor for both the male and the female nesters (MGenAny and FGenAny, respectively), and generations to all wild ancestors for both the male and female (MGenAll and FGenAll, respectively). We also considered combined metrics: The combined value for any wild ancestor was the minimum of the associated values for the male and female (CGenAny), while the combined metric for all wild ancestors was the maximum of the associated values for the male and the female (CGenAll).

To examine the influence of age and experience, we considered age and 2 different metrics of experience. For age, we considered the age of the male, the age of the female, and the average age of both members of the pair (MaleAge, FemaleAge, and AvgAge, respectively). For experience, we used a binary experience variable (whether a given bird had nested previously) and a cumulative experience variable (how many times a given bird had nested previously) for males, females, and the average of the pair (MaleExp, FemaleExp, AvgExp, MaleCumE, FemaleCumE, and AvgCumE, respectively).

Including all possible combinations for variables associated with each of the hypotheses would have resulted in an overly large model set, so we conducted model selection in 2 steps. First, to select the best variable to represent a given hypothesis, we selected among the suite of proposed variables associated with that hypothesis. Then, we conducted model selection with the variables that were selected at the first step, across hypotheses.

We used the indicator-variable approach to model selection (Kuo and Mallick 1998, Link and Barker 2006, Converse et al. 2013b). Each predictor variable  $m$  was associated with a Bernoulli variable,  $w_m$ . For each sample in the MCMC procedure, the Bernoulli variable was either 0 (and the predictor variable was excluded from the model) or 1 (and it was included). The posterior distribution for the Bernoulli variable is a weight of evidence in favor of including the associated predictor variable in the model. We calculated the Bayes factor (BF) associated with each predictor variable as

$$BF_m = \frac{\frac{w_m | \text{data}}{(1-w_m | \text{data})}}{\frac{w_m}{(1-w_m)}} \quad (7)$$

where the BF for variable  $m$  is an odds ratio calculated from the posterior mean ( $w_m | \text{data}$ ) and the prior ( $w_m$ ) of the Bernoulli indicator variable. We chose the 3 variables (one for the black fly hypothesis, one for the captive selection hypothesis, and one for the age and experience hypothesis) with the highest BFs from the hypothesis-specific analyses for consideration in a second analysis with only those 3 variables (along with the random pair effect and the reneest effect as before). At the second-level analysis, we again used the indicator variable approach to evaluate evidence for the 3 hypotheses of interest, and calculated posterior summary statistics for the regression coefficients conditional on the single model that was sampled most often. In all cases, we standardized predictor variables before inclusion in analyses by subtracting the mean and dividing by the SD of the predictor data.

To avoid bias in model selection, we followed Link and Barker (2006) and Converse et al. (2013b), where the total prior uncertainty of the linear predictor was held constant regardless of the dimensions of the model (i.e. regardless of the values of  $w_m$ ) by scaling the variance of the prior distributions for the regression coefficients. We gave each of the model coefficients a normal prior with mean = 0 and variance =  $V/K$ , where  $K$  is the number of effects entering the model at a particular sample (i.e.  $\sum_{m=1}^M w_m$ ). Each of the Bernoulli-distributed  $w_m$  parameters was given a prior of 0.5. We used standard vague priors for the random pair effect hyperparameters [mean = normal(mean = 0, variance = 1,000), SD = uniform(0, 25)], for the fixed effect of reneesting [normal(mean = 0, variance = 1,000)] and for the variance  $V$  [Gamma(3.29, 7.8)], as described by Link and Barker (2006) and by Converse et al. (2013b).

Finally, to examine the effect of the *Bti* treatment, we also built a simple daily nest survival model that included only the random pair effect, the nesting attempt effect, and the effect of treatment (3 treatment levels: pilot treatment level in 2010; full treatment level in 2011 and 2012; no treatment in 2009 and 2013). We based inference on the

posterior distribution of the regression coefficient for the full (2011–2012) treatment effect.

Computations for nest survival and nest initiation (see below) were performed using JAGS 3.4.0 (Plummer 2003). The package “jagsUI” (Kellner 2015) was used to call JAGS and export results to the R computing environment. We assessed convergence of the MCMC chains based on a Gelman-Rubin statistic <1.1 (Gelman 1996).

### Statistical Analysis: Nest Initiation

We evaluated the behavioral response, age and experience, and environmental hypotheses to understand the timing of nest initiation. In particular, we considered effects of breeding experience, climate (through temperature), and calendar year on date of nest initiation. If birds behaviorally respond to black fly harassment by changing nest initiation date, as predicted by the behavioral response hypothesis, we would predict that nest initiation would shift to later in the year, after black fly emergence was completed. Further, we would predict that more experienced breeders would initiate nests later in the year. Alternatively, if environmental factors (such as temperature) drive nest initiation, this would provide support for the environmental hypothesis. We modeled a continuous effect of calendar year to demonstrate the overall trend in nest initiation (excluding reneests), based on the date each nest was first detected.

Similar to nest survival analysis, we began by considering 6 variables that would best represent the effect of previous breeding experience. The first 3 variables described binary measures of experience, indicating whether the breeding pair for a given nest had any previous nesting attempts—for the male of the pair, the female of the pair, and an average of the male and female of the pair (MaleExp, FemaleExp, and AvgExp, respectively). The additional 3 variables described cumulative measures of experience, indicating the total number of previous nesting attempts, for the male of the pair, the female of the pair, and an average of the male and female of the pair (MaleCumE, FemaleCumE, and AvgCumE, respectively).

We next considered which set of temperature variables would best describe the climate during nesting. Growing degree days (GDD) above 0°C, available from the National Oceanic and Atmospheric Administration, were collected 8 km west-northwest of Necedah, Wisconsin (Station ID: NEHW3 at NNWR). We then summed GDD over various periods within each year: start dates of March 1 or March 15 and end dates of March 26 (earliest nest initiation date), March 31, or April 15 (mean nest initiation date). All combinations of these 2 start dates and 3 end dates gave us 6 temperature variables (GDD1.26, GDD15.26, GDD1.31, GDD15.31, GDD1.46, GDD15.46), where the first number in the variable name is the start date (day 1 = March 1) and the second is the end date (day 46 = April 15).



We assumed that our response variable, nest initiation date, was normally distributed, so we used an identity link function (i.e. linear regression analysis). As for the nest survival analysis, we included nesting pair as a random effect variable and performed model selection in the 2-step manner as described for daily nest survival analysis, using the indicator variable approach for model selection and a prior for  $w_m = 0.5$ . We standardized predictor variables before inclusion in the analysis. We gave each of the coefficients a normal prior with mean = 0 and variance =  $V/K$ , where  $K$  is the number of effects entering the model at a particular sample (i.e.  $\sum_{m=1}^M w_m$ ). We used standard vague priors, including for the total prior variance  $V$  [SD = uniform(0, 25)], for the model error [SD = uniform(0, 25)], and for the random pair effect hyperparameters [mean = normal(mean = 0, variance = 1,000), SD = uniform(0, 25)]. We set priors for  $w_m = 0.5$ .

## RESULTS

### Black Fly Reduction Treatment

In 2009 (control year), larvae of *S. annulus* and *S. johannseni* sampled from NNWR were restricted to a 6.6 km section of drainage ditch (Figure 1) and the population was small compared with other sampled sites. In subsequent years (2010–2013), <10 larvae of the target black fly species were found in ditches that contained flowing water on NNWR. Extensive populations of the 2 principal target black fly species, however, were located in nearby flowing-water habitats beyond NNWR (Figure 1). *Simulium annulus* and *S. johannseni* occupied ~80 km of river habitat, with the greatest population in a 50 km stretch of the Yellow River. We estimated that this section of river produced 2–3 billion larvae of these 2 species each spring, representing the most abundant macro-invertebrates in the river. Although large numbers of adults were collected in CO<sub>2</sub> traps (Table 1), larvae of *S. meridionale* were never found during our sampling program. Adults of this species may have flown in from beyond our sampling area or may have been missed by our sampling protocol. Regardless, presence of adult *S. meridionale* occurred outside of the nesting period of Whooping Cranes (see below).

*Bti* treatment was applied to varying degrees across years but was generally effective at reducing black fly populations. In the pilot year for *Bti* treatment (2010), >99% of mortality in target black fly larvae occurred from the treatment site up to 1.6 km downstream (Figure 2), decreasing thereafter (Supplemental Material Table S1). Larval mortality in samples collected 100 m upstream of the treatment site, used as a control, was negligible (0.6%). Treatment in 2010 affected ~4% of the river habitat (Supplemental Material Table S1), so the overall reduction

of black fly populations in the area of NNWR was probably negligible (Figure 2). In 2011, 51.5 km of habitat for larval black flies were treated (Figure 2). Larval mortality was ~100% within 5 km downstream of each site (Supplemental Material Table S1). Broader suppression in tributaries, as well as in the same 51.5 km stretch of the Yellow River, occurred in 2012 (Figure 2), and larval mortality was >95% as far as 9.2 km downstream. Although larval development of both species is influenced by weather, we found that the hatching pattern of *S. johannseni* was more staggered than that of *S. annulus*. These differences in hatching patterns made it difficult to eliminate both species with a single treatment. In 2011, treatment was effective at removing *S. annulus* but allowed some *S. johannseni* to survive (Table 1). In 2012, rapidly warming temperatures accelerated larval development in both species and some *S. annulus* larvae had already pupated (and could not feed on *Bti*) by the time treatment occurred. By contrast, nearly the entire larval population of *S. johannseni* was exposed to *Bti* treatment and suppressed.

We captured >2 million individual biting flies of a wide variety of taxa in CO<sub>2</sub> traps at NNWR, 2009–2013 (Table 1). Abundance of grouped taxa, even when standardized by correcting for trapping effort, appeared to be unrelated to treatment years except for *S. annulus* and *S. johannseni* (Table 1). The 3 target black fly species formed a high proportion of total trapped individuals in non-full-treatment years and a low proportion of individuals trapped in treatment years. In the second control year, 2013, we captured more than half of all individuals collected in the 4 yr of study. Treatment year 1 (2011) did not suppress *S. johannseni* to the extent that treatment year 2 did. By contrast, treatment year 2 did not remove *S. annulus* to the extent that occurred in 2011.

### CO<sub>2</sub> Trap Black Fly Indices

In our regression model with ln(count+1) from artificial nests as the dependent variable, we found no meaningful interactions between independent variables. Only ln(count+1) from CO<sub>2</sub> traps as the predictor variables were included in our final species-specific regression models. The number of individuals of *S. annulus* captured in CO<sub>2</sub> traps was strongly ( $R^2 = 0.62$ ) and positively (regression coefficient = 0.42, 95% CI: 0.37–0.47) related to the number of individuals captured at artificial nests (Supplemental Material Figure S1). We found a weaker positive relationship for *S. johannseni* ( $R^2 = 0.31$ ; regression coefficient = 0.16, 95% CI: 0.11–0.21; Supplemental Material Figure S1). Our CO<sub>2</sub> traps were not effective predictors of *S. meridionale* at artificial nests; only one *S. meridionale* was ever collected at an artificial nest.

**TABLE 1.** Numbers of biting insects captured during 2009–2013 in our study area in central Wisconsin, USA.

Species of biting fly	Individuals captured in CO <sub>2</sub> traps <sup>a</sup>				
	Control 1 (2009, 53 days)	Test treatment (2010, 5 days) <sup>b</sup>	Treatment 1 (2011, 17 days)	Treatment 2 (2012, 23 days)	Control 2 (2013, 32 days)
<i>Simulium annulus</i>	46,215	2,656	870	42,953	325,899
<i>S. johannseni</i>	233,592	29,246	4,650	196	633,130
<i>S. meridionale</i>	340	12	15,272	21,424	81,100
Additional species of black flies <sup>c</sup>	13,088	402	11,877	2,081	33,617
Mosquitoes (Culicidae)	28,202	199	56,703	134,097	321,048
Horse flies (Tabanidae) <sup>d</sup>	713	65	597	489	269
Biting midges (Ceratopogonidae) <sup>e</sup>	109	8	562	3,553	6,833
Total	322,259	32,588	90,531	204,793	1,401,896
Individuals/trap day	6,080	6,518	5,325	8,904	43,809

<sup>a</sup> Except for 2010, 7 CO<sub>2</sub> traps placed at Necedah National Wildlife Refuge (NNWR) were used.

<sup>b</sup> Only a partial treatment of black fly larval sites was made to experiment with landscape-scale use of *Bti*, and less sampling effort for adults (3 CO<sub>2</sub> traps for 5 days) was conducted at NNWR.

<sup>c</sup> During the 4 yr of study, 20 species were trapped (King and Adler 2012, Whooping Crane Eastern Partnership personal communication): *Cnephia dacotensis* (incidental), *Ectemnia taeniatifrons*, *Greniera denaria*, *Metacnephia saskatchewanana*, *Simulium anatinum*, *S. aureum* group, *S. burgeri*, *S. decorum*, *S. congareenarum*, *S. excisum*, *S. silvestre*, *S. jenningsi*, *Stegopterna mutata*, *Prosimulium arvum*, *P. magnum*, *S. rostratum*, *S. rugglesi*, *S. tribulatum*, *S. vandalicum*, and *S. venustum*. Eight of these species are ornithophilic, but none of them was detected at nests or decoys of Whooping Cranes.

<sup>d</sup> During the 4 yr of study, 11 species were trapped: *Hybomitra illota*, *H. lasiophthalma*, *H. nuda*, *Tabanus sparus*, *Chrysops callidus*, *C. carbonarius*, *C. excitans*, *C. frigidus*, *C. indicus*, *C. sackeni*, and *C. striatus*.

<sup>e</sup> During the 4 yr of study, at least 13 *Culicoides* taxa were trapped: *Culicoides bickleyi*, *C. biguttatus*, *C. crepuscularis*, *C. denticulatus*, *C. haematopotus*, *C. obsoletus*, *C. parapiliferus*, *C. piliferus*, *C. sanguisuga*, *C. spinosus*, *C. stilobezziodes*, *C. travisi*, and *C. venustus*.

### Daily Nest Survival Analysis

Of 108 nests, we included 100 in the nest survival analysis, comprising initial nests and renests from 2009 to 2013. Of the 8 nests that we excluded, 5 were eliminated due to human disturbance that resulted in nest abandonment, 2 were excluded that had fertile eggs placed in the nest to replace infertile eggs, and 1 had an ambiguous fate (Supplemental Material Figure S2). No insect data were available for 2005–2008, so nests from those years were excluded from the analysis.

We used posterior parameter weights at the first level of analysis to choose the 3 variables used to represent the competing hypotheses at the second level of analysis (Table 2): (1) the sum of *S. annulus* and *S. johannseni* (lnAnnJoh) to represent the black fly hypothesis, (2) the generations to any wild ancestor combined across the male and female of the pair (CGenAny) to represent the captive selection hypothesis, and (3) the cumulative number of nesting attempts by the female of the pair (FemaleCumE) to represent the age and experience hypothesis.

The second level of analysis resulted in calculated posterior inclusion probabilities = 0.940 for lnAnnJoh (BF = 15.667), 0.700 for CGenAny (BF = 2.333), and 0.363 for FemaleCumE (BF = 0.570). Jeffreys (1961) proposed, as a rule of thumb, that a BF between 2 and 3 indicates weak support, a BF between 3 and 12 indicates moderate support, and a BF >12 indicates strong support. Thus, we interpret these results as providing strong support for the black fly hypothesis, weak support for the captive selection

hypothesis, and essentially no support for the age and experience hypothesis.

We calculated regression coefficients conditional on the top-ranked model (lnAnnJoh + CGenAny). For the variable lnAnnJoh, the coefficient was −0.466 (95% Bayesian credible interval [BCI]: −0.800 to −0.147; i.e. greater numbers of black flies were associated with lower nest survival. For the variable CGenAny, the coefficient was −0.303 (95% BCI: −0.552 to −0.043; i.e. greater numbers of generations removed from a wild ancestor were associated with lower nest survival).

We also built a model including only the treatment effect on daily nest survival. We modeled a separate effect of the small-scale treatment in 2010 from the full treatment effect in 2011 and 2012. The coefficient in 2010 for the treatment effect was 0.167 (95% BCI: −0.577 to 0.963), and in 2011–2012 it was 0.452 (95% BCI: −0.030 to 0.940). On the real scale, that translates into a daily survival probability for first nests equal to 0.935 (95% BCI: 0.912–0.955) in non-treatment years (2009 and 2013), and 0.958 (95% BCI: 0.942–0.971) in full-treatment years (2011–2012). This results in probabilities of surviving a 30-day incubation period equal to 0.143 (95% BCI: 0.063–0.252) and 0.283 (95% BCI: 0.166–0.418) for non-treatment and full-treatment years, respectively.

### Nest-Initiation Analysis

We included 109 nests in the analysis of nest initiation date (all first nests 2005–2013; Supplemental Material Figures

**TABLE 2.** Posterior inclusion probabilities (weights) and Bayes factor (see Equation 7) from multiple analyses of daily nest survival in our study area in central Wisconsin, USA. Analysis was conducted in 2 rounds. In round 1, a single variable from each hypothesis category was selected, based on highest weight; in round 2, these 3 variables were combined into a single analysis. Hypothesis categories and variables are defined in the text.

Hypothesis category	Variable	Weight, round 1	BF, round 1	Weight, round 2	BF, round 2
Captive selection	MGenAny	0.252	0.337	–	–
	MGenAll	0.227	0.294	–	–
	FGenAny	0.236	0.309	–	–
	FFenAll	0.228	0.295	–	–
	CGenAny	0.726	2.65	0.7	2.333
	CGenAll	0.247	0.328	–	–
Age and experience	Age.M	0.296	0.42	–	–
	Age.F	0.365	0.575	–	–
	Age.Avg	0.357	0.555	–	–
	Exp.M	0.337	0.508	–	–
	Exp.F	0.342	0.52	–	–
	Exp.Av	0.364	0.572	–	–
	CumAtt.M	0.376	0.603	–	–
	CumAtt.F	0.546	1.203	0.363	0.57
	CumAtt.Av	0.436	0.773	–	–
	InAnn	0.812	4.319	–	–
Black flies	InJoh	0.447	0.808	–	–
	InMer	0.496	0.984	–	–
	InAnnJoh	0.866	6.463	0.94	15.667
	InAnnMer	0.636	1.747	–	–
	InJohMer	0.424	0.736	–	–

S2 and S3). The median date of nest initiation was April 16 (ordinal day 106; [Supplemental Material Figures S2 and S4](#)) over this period.

At the first level of analysis, we chose the binary experience variable averaged over the male and female of the pair (AvgExp) to represent the effect of previous breeding experience, and growing degree days from March 15 to April 15 (GDD15.46) to represent the effect of environmental factors on nest initiation date. We included these variables along with calendar year at the second level of analysis.

We found strong support for including both GDD15.46 and AvgExp but only weak support for including year (BF = 65.09, 97.68, and 2.60, respectively; Table 3). Although median nest initiation dates appeared to increase with advancing year (Figure 3), we suspected that the effect of year was driven by environmental conditions. We ran a post hoc analysis with only year and AvgExp. That analysis resulted in a much higher BF for year (148.95), indicating that much of the effect that would be attributed to year in an analysis without GDD15.46 was better attributed to GDD15.46 (as in our initial analysis). Furthermore, we found a reasonably strong negative correlation between GDD15.46 and year ( $r = -0.63$ ), indicating a decrease in GDD15.46 over the years of the study.

The proportion of experienced nesting pairs increased over the period of our analysis ([Supplemental Material Figure S4](#)). Cranes with previous nesting experience nested earlier (estimated coefficient =  $-3.17$ ; 95% BCI:  $-4.85$  to

$-1.48$ ; [Supplemental Material Figure S3](#)) and earlier nesting occurred when GDD15.46 was higher (estimated coefficient =  $-2.93$ ; 95% BCI:  $-4.65$  to  $-1.19$ ). These regression coefficients were calculated on the basis of the normalized predictors. On the real scale, this translates to a decrease of 7.39 days (95% CI: 3.46–11.28) in the date of nest initiation for an experienced pair compared to an inexperienced pair ([Supplemental Material Figure S3](#), and a decrease of 9.11 days (95% CI: 3.71–14.46) in the date of nest initiation for the warmest compared to the coldest spring recorded over the period of our analysis (2005–2013).

## DISCUSSION

Reintroductions are often high-stakes management actions. For some threatened species, reintroductions might be the sole means of achieving recovery (Converse and Armstrong 2016) but tend to be expensive and prone to failure (Griffith et al. 1989). Quickly identifying the factors that threaten success of a reintroduction is crucial to improving outcomes (Runge et al. 2011). For endangered Whooping Cranes, poor nesting success in the reintroduced EMP threatens the success of the reintroduction, which in turn reduces overall species recovery (Canadian Wildlife Service and U.S. Fish and Wildlife Service 2005).

We found strong evidence for the black fly hypothesis first proposed by Urbanek et al. (2010b), strengthened by showing that CO<sub>2</sub> trap captures predicted black fly

**TABLE 3.** Results of models of nest initiation date (estimated on the basis of the date the nest was first detected) for initial nests within a year in the eastern migratory population of Whooping Cranes in our study area in central Wisconsin, USA. Results include the posterior weights for each of the variables from the model selection procedure, including the first round and the second round of model selection. Also recorded is the Bayes factor (BF) associated with the posterior weights, the coefficient for the relevant predictor (year, linear experience, and growing degree days between days 15 and 46 [GDD15.46], respectively), with the 95% confidence interval of the coefficient in parentheses.

Model	Weight, round 1	BF, round 1	Weight, round 2	BF, round 2	Coefficient
Year	–		0.7223 <sup>a</sup>	2.6	2.11 (0.180–4.075)
FemaleExp	0.5553	1.25	–	–	
MaleExp	0.5077	1.03	–	–	
AvgExp	0.6085	1.55	0.9899 <sup>a</sup>	97.68	–3.17 (–4.85 to –1.48)
FemaleCumE	0.4000	0.67	–	–	
MaleCumE	0.4858	0.94	–	–	
AvgCumE	0.4460	0.81	–	–	
GDD1.26	0.4335	0.77	–	–	
GDD15.26	0.4577	0.84	–	–	
GDD1.31	0.4207	0.73	–	–	
GDD15.31	0.4066	0.69	–	–	
GDD1.46	0.5883	1.43	–	–	
GDD15.46	0.7655	3.26	0.9849	65.09	–2.93 (–4.65 to –1.19)

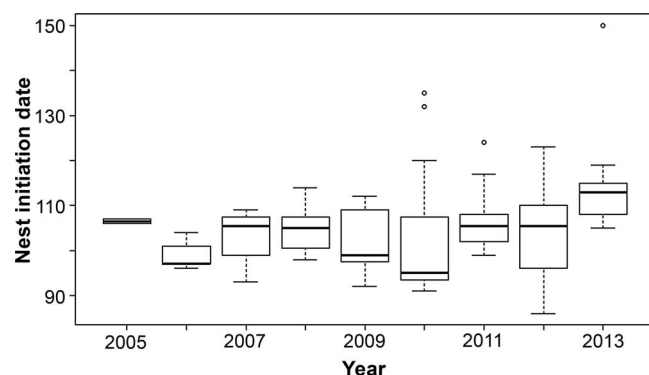
<sup>a</sup> If GDD15.46 was excluded from the second-round modeling, the weights for Year and AvgExp were 0.9933 and 0.9919, respectively.

abundance at artificial nests. Nest failure in other bird species has been associated with black flies (McIntyre and Barr 1997, Bukaciński and Bukacińska 2000, Solheim et al. 2013), but we know of no case in which nest failure has been as extensive, or as sustained, or as causally linked as in this population of Whooping Cranes.

Our trap data indicate that *Bti* treatment removed the majority of flies, although comfort behaviors by incubating Whooping Cranes in 2011 suggest that black fly control efforts were not complete that year (King et al. 2015). Overall, our CO<sub>2</sub> trap counts provided a useful index of conditions at Whooping Crane nests in 2012 and 2013, in contrast to King and Adler's (2012) finding of no

relationship between black fly adults captured at CO<sub>2</sub> traps and black flies found at either Whooping Crane nests or artificial nests. King and Adler (2012) did not use dummy Whooping Crane eggs in measurements at artificial nests, although they did use Whooping Crane wings on their decoys. Uropygial gland secretions that we used on eggs (and that could be present on wings) are known to attract black flies (Fallis and Smith 1964, Weinandt et al. 2012). To reduce the risk of abandonment, King and Adler (2012) measured black fly abundance only once at Whooping Crane nests, 1–2 days after nest hatch or abandonment, whereas our measurements occurred throughout the incubation period without disturbance of nests.

When we individually evaluated the 2 black fly species that represented our predictor *lnAnnJoh*, we found substantially more support for an effect of *S. annulus*, compared with *S. johannseni*, on daily nest survival. If it was important at all, *S. johannseni* did not drive nest failure to the degree that *S. annulus* did (Table 2; weight for *lnAnn* vs *lnJoh*; as well as Figure 4A, 4D). Data from artificial nests also suggested that *S. annulus* had a greater affinity for artificial nests than *S. johannseni* (i.e. regression coefficient *S. annulus* greater than regression coefficient *S. johannseni*), which was similar to the finding of King and Adler (2012). Given that some nests survived in the presence of *S. annulus* (Figure 4C), however, there appears to be a threshold of tolerance for black flies by nesting Whooping Cranes. Even though our system was open with respect to black fly movement, our treatment with *Bti* dramatically reduced target adult black fly populations within the study area and increased nest survival, which



**FIGURE 3.** Box plots of estimated initiation for all initial Whooping Crane nests in our study area in central Wisconsin, USA, plotted by year. Black horizontal bar within boxes is the median, and boxes extend to upper 75% and lower 25% of the data. Whiskers above and below boxes describe the remaining quartiles, with outliers (3 or 2 times the upper or lower quartile) indicated by circles.



establishes a causal link between nest survival and black flies. In addition, the effect of the treatment on *S. annulus* and *S. johannseni* was restricted to the full treatment years of 2011 and 2012 (Table 1).

We found only weak evidence for the captive selection hypothesis. The relationship between the number of generations that a nesting pair's ancestors were in captivity and daily nest survival was in the direction predicted by the hypothesis. As the number of generations that a nesting pair's ancestors were in captivity increased, nest survival decreased, but the variation in the number of generations in captivity was relatively small. The variable CGenAny took a value of either 2 or 3 for all nests (calculated as the minimum of the value of the male and female of the pair), which suggests that captive selection reduces postrelease fitness relatively quickly (i.e. within a few generations) if it exists. Only one of our potential predictor variables for the captive selection hypothesis, however, had any support, and this, along with the relatively weak support for CGenAny, suggests caution in interpreting our results. A more definitive evaluation would be accomplished by releases of birds hatched from eggs collected at nests in the remnant Aransas-Wood Buffalo Population of Whooping Cranes. Previous egg collections were used to establish the captive flock without harming the remnant population (Link et al. 2003, Boyce et al. 2005). Further, we recognize that our evaluation of captive selection was restricted by the covariates and model structures considered. When more generations are available, future investigators could consider alternate covariates to represent captive selection effects (e.g., those arising from genomic analyses) and more complex model structures such as nonlinear effects.

Strong captive selection can rapidly change the genetic makeup of a population (Lynch and O'Hely 2001) and has been documented in steelhead trout (*Oncorhynchus mykiss*; Kostow 2004, Araki et al. 2007) and deer mice (*Peromyscus leucopus*; Lacy et al. 2013), but those species are characterized by high genetic diversity and high fecundity, which can lead to substantial genetic change in a few generations (Christie et al. 2012). Following a genetic bottleneck from which  $\leq 16$  individuals survived to represent the current world population (Canadian Wildlife Service and U.S. Fish and Wildlife Service 2005), the Whooping Crane genome is not likely diverse, and individuals reproduce slowly (Urbanek and Lewis 2015), so captive selection that occurred in 1 or 2 generations would not be expected.

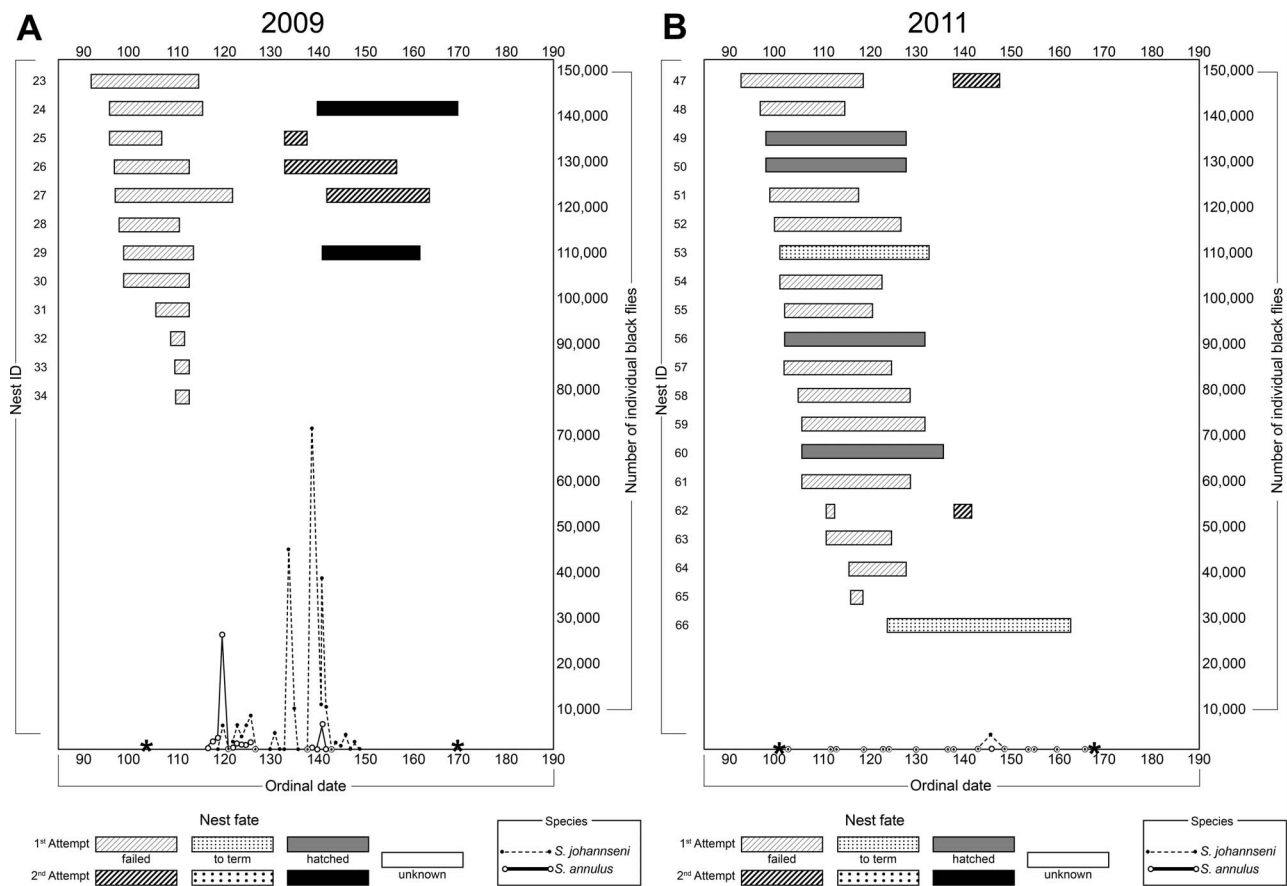
We found no support for the age and experience hypothesis. For example, the pattern of synchronized nest failure observed in 2009 was repeated in 2013, when black fly abundance rebounded post-*Bti* treatment, despite more experienced breeders being present in 2013 (Supplemental Material Figure S4). In wild Sandhill Cranes (*Antigone canadensis*), experience or age was positively related to

reproductive success in the first years following pairing but plateaued after that (Tacha et al. 1989, Nesbitt 1992) unless mate switches occurred (Hayes 2015). A positive relationship between age and reproductive success has also been found in geese (Finney and Cooke 1978, Raveling 1981). Despite the lack of a relationship between age and nest survival, experience does seem to enhance some aspects of Whooping Crane reproduction. Whooping Cranes initiated nests earlier as they became more experienced. Nesting earlier in the season increases the chance of renesting if the first nest is lost, improving the probability of overall reproductive success for a pair.

We did not find evidence for the behavioral response hypothesis; Whooping Cranes did not respond to black flies by delaying nest initiation. Although nest initiation dates were positively related to calendar year in both our study and that of King et al. (2013), this relationship was better explained by temperature, as suggested by the environmental hypothesis. Nesting experience also influenced nest initiation date, but the estimated effect was in the direction opposite that predicted by the behavioral response hypothesis. Experienced Whooping Cranes initiated nests earlier, following a pattern typical across birds (Lack 1968:297–299, Finney and Cooke 1978). Only first-generation (captive-reared and released) Whooping Cranes are involved in our analysis, so we cannot eliminate the possibility that natural selection might shift nest initiation dates in the future.

Facultative behavioral modifications to facilitate breeding have been recorded in other avian species. Reed Buntings (*Emberiza schoeniclus*), for example, modified their song quality to adjust to anthropocentric background noise that interfered with song displays (Gross et al. 2010). Modifying timing of reproduction, however, requires more than simple behavioral modification in response to stimulation by one environmental cue (Hahn et al. 1997). In Whooping Cranes, a combination of photoperiod, climate, and habitat condition determines reproductive phenology of captive (Gee and Russman 1996, Mirande et al. 1996) and wild (Spalding et al. 2009, Fitzpatrick et al. 2018, present study) individuals, which is similar to patterns in temperate-breeding passerines more generally (Farner 1986). For example, nest initiation by Whooping Cranes ranges from February in Florida (Spalding et al. 2009) to early May in Canada (Kuyt 1995), a range likely controlled by photoperiod and temperature (Fitzpatrick et al. 2018, present study). Yet black fly populations emerge after most Whooping Crane nests have been initiated (Urbanek et al. 2010b), so behavioral modification to avoid black flies would require nesting Whooping Cranes to ignore powerful *zeitgebers* of photoperiod and temperature to delay nesting.

In addition to establishing a causal relationship between abundance of *S. annulus* and daily nest survival, our



**FIGURE 4.** Whooping Crane nest fate compared to abundance of 2 black fly species in our study area in central Wisconsin, USA, in 4 yr: (A) 2009, (B) 2011, (C) 2012, and (D) 2013. Black fly numbers are shown on the right y-axis; nest numbers (non-overlapping among years) are shown on the left y-axis. Ordinal date is shown on the x-axis, and asterisks mark the beginning and end of the sampling period for that year. For 2009 only, black flies were sampled every day, weather permitting, so the only absence data that are listed are for ordinal dates located just before or after black flies were detected in samples. For 2011–2013, all sample days are depicted.

experiment identified additional challenges in this reintroduction effort that were previously masked by poor nesting success. Specifically, experimental reduction of black fly populations resulted in improved daily nest survival and hatching, but few of the resulting chicks survived to fledge 2–3 mo later (Supplemental Material Table S2). Poor fledging success cannot be linked to *S. annulus* or *S. johannseni*, because these species were largely absent by the time chicks hatched (Figure 4). Harassment of young chicks by *S. meridionale* is possible but is unlikely to be the sole cause of poor fledging success because chick mortality occurs over a 10 wk period, longer than adult *S. meridionale* are present (Whooping Crane Eastern Partnership personal communication). Other environmental issues (e.g., predation or poor nutrition) may depress fledging success, or problems might be related to the birds themselves. One potential cause is captive selection, which has not been evaluated for fledging success in this population. Champagnon et al.

(2012) argued that genetically based, predator avoidance behavior in birds could be influenced by captive selection. Although some complicated behaviors have been taught to birds by human caretakers (e.g., establishing migratory routes; Duff et al. 2001) or learned from older conspecifics (Mueller et al. 2013, Teitelbaum et al. 2016), other learned behaviors, such as predator responses, may not be relayed adequately, resulting in poor postrelease reproductive performance (Bremner-Harrison et al. 2004).

Management actions currently under way will allow us to evaluate new and revised hypotheses. Whooping Cranes have been released since 2011 in Wisconsin wetlands with similar structure to those at NNWR but where ornithophilic black flies that feed on Whooping Cranes are absent and where predator populations and wetland productivity differ from those of NNWR (Van Schmidt et al. 2014). These birds will eventually allow a more thorough evaluation of hypotheses regarding environmental factors. In addition, captive Whooping Crane-reared chicks (as

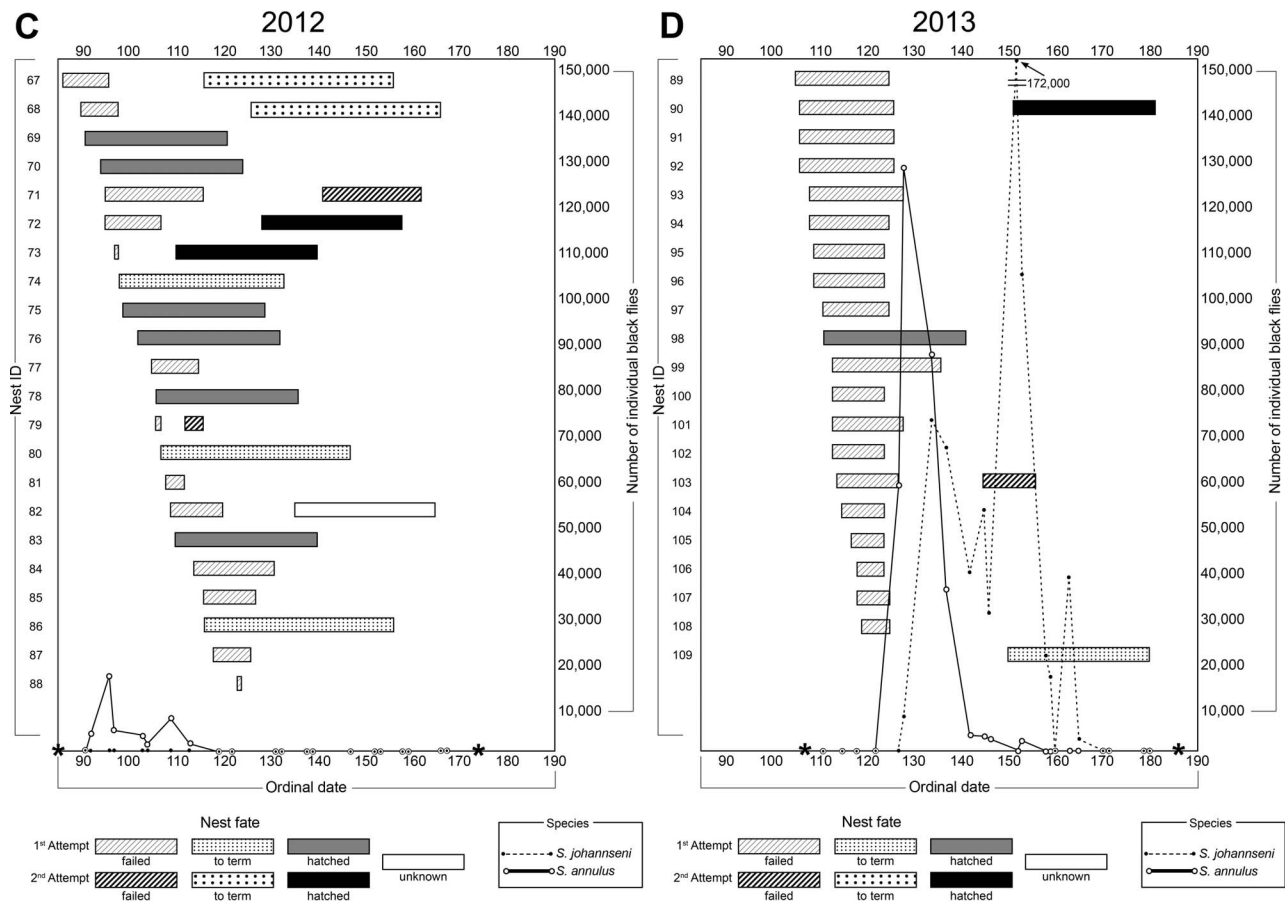


FIGURE 4. Continued.

opposed to captive human-reared chicks) have been released in the population since 2013, and these birds might allow evaluation of the hypothesis that rearing and release methods are inadequate to impart behaviors that birds need to succeed postrelease. Results for these management actions are not yet available because Whooping Cranes take several years to reach maturity (Urbanek and Lewis 2015). Several-year lags in learning and maturity by Whooping Cranes emphasize the importance of designing experiments efficiently as well as effectively.

Black flies represent an environmental cause of nest failure in Whooping Cranes that can be mitigated by (1) management of black fly populations, (2) collecting eggs from initial nests and forcing renesting, or (3) managed and natural natal dispersal to places where black flies that attack Whooping Cranes are not abundant. Even so, it appears that dealing with the effect of black flies alone will not solve the problem of poor reproduction in this Whooping Crane population. Instead, the problem is likely to be a product of multiple factors, including interactions between environmental factors and either genetic or learned characteristics of the released birds. Identifying

and responding appropriately to the causes of reproductive failure will require iterative articulation, evaluation, and modification of multiple competing hypotheses.

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**Ethics statement:** Research effort with Whooping Cranes in the eastern migratory population was conducted through permit TEO48806-1 issued to ICF from the USFWS for endangered species; permit 22339 issued to J.A.B. from USGS for banding; and permit SRL-SCR-001 issued to J.A.B. from the State of Wisconsin for scientific use. Permits for *Bti* treatment were issued to Elmer Gray by the State of Wisconsin 2010–2012 (permit nos. WCR-10-10-29, WCR-11-01-29, WCR-12-29-14). Nest surveys were conducted and insects were captured at NNWR through Special Use Permits issued by NNWR to ICF 2009–2013. All applicable ethical guidelines for the use of birds in research have been followed, including those presented in the Ornithological Council's "Guidelines to the Use of Wild Birds in Research" (Fair et al. 2010).

**Author contributions:** J.A.B., P.H.A., E.G., A.L., A.G., and S.J.C. designed the experiment. J.A.B., P.H.A., E.G., A.L., and A.G. collected data or applied larvicide. S.J.C. conducted the primary data analysis. J.A.B. was the primary author of the paper, with substantial contributions by S.J.C. and P.H.A. All methods were designed and developed by J.A.B., P.H.A., E.G., A.G., and A.L.

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