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SIZE DIFFERENCE IN WHOOPING CRANES REARED FOR TWO REINTRODUCTION METHODS

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Abstract: We investigated a possible size difference in whooping cranes (*Grus americana*) captive-reared for 2 reintroduction methods to establish a migratory population in eastern North America. Cranes reared for ultralight aircraft-led migration (UL) to Florida were significantly larger than cranes reared for direct autumn release (DAR) on the natal area in central Wisconsin. Mean tarsal length was 315.5 ± 0.98 (1 SE) and 308.1 ± 1.87 mm, respectively, for UL and DAR males and 296.9 ± 1.03 and 290.8 ± 2.60 mm, respectively, for UL and DAR females. Because of the different rearing schedules, eggs for the DAR method were generally laid later than eggs for UL. Eggs later in the laying sequence had lower weights and resulted in smaller birds, although this overall effect was small. Size difference did not appear related to genetic factors. Although survival to 5 years after release was not significantly related to size within groups of the same sex and release method, captive-rearing effects such as size on survival and behavior of released birds should be considered in assessment of reintroduction programs.

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Key words: direct autumn release, *Grus americana*, reintroduction methods, size, tarsus, ultralight-led migration, whooping crane.

The whooping crane (*Grus americana*) is an endangered species occurring in a single natural remnant population which nests in and near Wood Buffalo National Park, Northwest Territories and Alberta, Canada, and winters on and near Aransas National Wildlife Refuge (NWR) on the Texas Gulf Coast (Canadian Wildlife Service and U.S. Fish and Wildlife Service 2007). That population was reduced to 15-16 birds in 1942 and by winter 2010-11 had increased to 283 individuals (Stehn and Haralson-Strobel 2014). Recovery of the species may depend on establishment of additional populations. Attempted reintroductions that began in the Rocky Mountains in 1975 (Ellis et al. 1992) and central Florida in 1993 (Folk et al. 2010) were unsuccessful. A third reintroduction using captive-reared juveniles began in central Wisconsin in 2001. The initial reintroduction method consisted of training costume-reared juvenile whooping cranes to follow ultralight aircraft (UL) and then leading the cranes to winter release sites on the Florida Gulf Coast each year through 2010 (Lishman et al. 1997, Duff et al. 2001). A second method, direct autumn release (DAR), was used from 2005 to 2010 and consisted of releasing captive-

reared birds in central Wisconsin in October of each year and allowing them to migrate unassisted. The DAR method was based on earlier studies by Horwich (1989), Urbanek and Bookhout (1992), and Ellis et al. (2001).

The 2 reintroduction methods have been associated with differences in survival and some behaviors after release (Urbanek et al. 2014). The effects of captive propagation on physical characteristics of released birds may also affect post-release success and should be evaluated. Our objective was to further investigate size difference between cranes of the 2 reintroduction methodologies and discern possible implications.

METHODS

Whooping crane juveniles for reintroduction by the UL method were hatched at Patuxent Wildlife Research Center (Patuxent), Laurel, Maryland, from eggs produced in captive propagation facilities at Patuxent; International Crane Foundation (ICF), Baraboo, Wisconsin; Calgary Zoo, Alberta; Audubon Center for Research of Endangered Species, New Orleans, Louisiana; San Antonio Zoo, Texas; and from eggs salvaged from nests on Necedah National Wildlife Refuge (NWR), Wisconsin. Chicks were reared with puppets and costumes to avoid imprinting and habituation to humans (Horwich 1989, Urbanek and Bookhout 1992).

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Chicks were initially trained to follow ultralight trike aircraft (Cosmos, Dijon, France) according to techniques developed by Operation Migration, Blackstock, Ontario (Lishman et al. 1997, Duff et al. 2001) at Patuxent and then transported to Necedah NWR at 35-65 days of age (12 June-15 July). Training continued at isolated facilities on the refuge until the UL migration began in October of each year. Cranes were led to isolated release facilities on Chassahowitzka NWR, Citrus County (HY2001-09) and St. Marks NWR, Wakulla County (HY2008-09), on the Florida Gulf Coast. These assisted migrations began 10-17 October and were completed 30 November-28 January. Cranes were provided food *ad libitum* and protection in a large open-topped pen at each site through the winter and then migrated unassisted northbound on spring migration in late March or early April (Urbanek et al. 2005, 2010).

Chicks for DAR were hatched at ICF from eggs of the captive propagation facilities and costume-reared. They were transferred to an isolated rearing facility on Necedah NWR at 17-57 days of age. Operation of this field facility was closed 18-30 October, and juveniles were moved for release near older whooping cranes elsewhere on the refuge. Juveniles migrated by following whooping cranes, sandhill cranes (*Grus canadensis*), as a group, or alone. In some of the latter cases, juveniles were retrieved and relocated to other areas containing cranes (Zimorski and Urbanek 2010). All cranes were equipped with individually color-coded leg bands and leg-mounted VHF (conventional [very high frequency]) radiotransmitters (Advanced Telemetry Systems, Isanti, MN) and some (3-8 each year) additionally with PTT (satellite [platform transmitter terminal]) transmitters (Microwave Telemetry, Columbia, MD; North Star Science and Technology, King George, VA; Telonics, Mesa, AZ). Cranes were tracked by a team of 2-4 trackers after release throughout their annual cycle and geographic range.

The first author (RPU) measured length of the left tarsometatarsus (tarsus) (Johnsgard 1983:240) of all cranes at 80-139 (mean = 112, UL) and 75-145 (mean = 101, DAR) days of age. Because ossification of the tarsus is complete by 10 weeks of age (Curro et al. 1996), this measure was representative of adult tarsal length and a stable index of bird size. To reduce handling time during banding, project protocol permitted a single linear measurement. Tarsal length was selected as the primary measure of size because it was the standard linear measurement with the highest correlation coefficient to

other measurements (e.g., culmen and wing chord) in 6 species of cranes in previous work, and it was fixed at completion of growth, unlike weight, which varies seasonally by 30% each year (Swengel 1992).

We obtained sex identification from DNA blood tests (Griffiths et al. 1996), egg weights (with few exceptions within 1 day of laying), egg position in laying sequence, identities of dam and sire, and QG (Queller and Goodnight 1989) and AS (allele-sharing, Blouin et al. 1996) coefficients of inbreeding based on microsatellite DNA profiles (Jones et al. 2002) from captive propagation records.

Because size is sexually dimorphic in cranes, we performed analyses separately for each sex with program R (R Development Core Team 2010). We compared size between reintroduction methods with 2-sample *t*-tests corrected for unequal variance. We examined relationships between egg weight and sequence, and size with egg weight/sequence and inbreeding coefficients with linear regression. We examined effect of common parents on size with paired *t*-tests. We compared survival to 5 years after release within each group consisting of the same sex and method with 2-sample *t*-tests corrected for unequal variance.

RESULTS

Male whooping cranes averaged 20 mm longer in tarsal length than females. Mean tarsal length of males ($n = 113$) was $314.3 \text{ mm} \pm 0.91$ (1 SE) and ranged from 289 to 336 mm; two-thirds (prominent data cluster) of males had a tarsal length of 306-324 mm. Mean tarsal length of females ($n = 97$) was 295.3 ± 1.05 mm and varied from 257 to 318 mm; two-thirds had a tarsal length of 285-305 mm.

Mean tarsal length of males was 315.5 ± 0.98 and 308.1 ± 1.87 mm, respectively, for UL ($n = 94$) and DAR ($n = 19$). The difference of 7.4 mm was significant ($t = -3.53$, $v = 28.8$, $P = 0.001$). Mean tarsal length of females was 296.9 ± 1.03 and 290.8 ± 2.60 mm, respectively, for UL ($n = 71$) and DAR ($n = 26$). This difference of 6.1 mm was also significant ($t = -2.17$, $df = 33.2$, $P = 0.038$).

Tarsal length was correlated with weight within all groups: UL males ($R = 0.26$, $n = 81$, $P = 0.019$), DAR males ($R = 0.48$, $n = 19$, $P = 0.039$), UL females ($R = 0.30$, $n = 54$, $P = 0.029$), and DAR females ($R = 0.56$, $n = 26$, $P = 0.003$).

Egg weight and egg sequence data were available

for 166 individuals. There was a highly significant relationship ($F_{1,164} = 20.08, P < 0.001$), and 10.9% of variation in egg weight was explained by the egg sequence (slope = -2.721 ± 0.607). Egg weight decreased with the increase of sequence of the egg in the laying cycle. There was a tendency ($t = 1.91, df = 73, P = 0.059$) for eggs assigned to DAR (mean = $3.38 \pm 0.24, n = 42$) to be later eggs than those assigned to UL (mean = $2.84 \pm 1.82, n = 159$).

Tarsal length was significantly related to the residuals of the egg weight/egg sequence relationship ($F_{1,164} = 5.65, P = 0.019$), but only 3.3% of variation was explained by these residuals (slope = 0.194 ± 0.082). Tarsal length was longer when the egg weight was larger than expected from its sequence.

Inbreeding data were available for 155 individuals. There was no effect of inbreeding coefficients AS ($F_{1,153} = 0.2753, P = 0.601$) or QG ($F_{1,153} = 0.0576, P = 0.811$) on length of tarsus.

Of 29 known sires and 31 dams which contributed progeny to the reintroduction, 9 sires and 11 dams were parents of chicks in both UL and DAR. The UL juveniles were significantly larger than DAR juveniles originating from eggs laid by the same females (Table 1). The same relationship was discernable for male parents only for female chicks when 1 unusually large DAR female chick was removed from analysis.

There was no significant difference in tarsal length between birds surviving and not surviving from release to age 5 years: UL males ($t = 0.77, df = 70, P = 0.444$), DAR males ($t = 1.41, df = 14, P = 0.181$), UL females ($t = -1.12, df = 53, P = 0.267$), and DAR females ($t = -1.07, df = 18, P = 0.300$). Although not significant, mean survival for both reintroduction methods was greater for smaller than larger males and for larger than smaller females.

DISCUSSION

Tarsal length was significantly greater (2.4% for males and 2.1% for females) in UL than DAR juveniles. The range of tarsal length of whooping cranes is limited, i.e., about two-thirds of total project birds were within an 18-mm range for males and within a 20-mm range for females. Differences in tarsal length between the 2 reintroduction groups amounted to 42% and 31% of these ranges for males and females, respectively. A small linear difference in tarsal length corresponds to a much larger 3-dimensional difference in overall size, i.e., weight and volume, of the whooping crane. However, the relationship between a long bone measurement and weight is not generally very predictable for whooping cranes because weight is also related to many other factors such as nutritional condition and season.

Since additional time was needed to train birds to follow ultralight aircraft, eggs laid in the first half of the breeding season were usually assigned to the UL project. The earliest eggs laid at ICF were sometimes shipped to Patuxent for the UL project. However, most eggs at ICF, where laying phenology was later than at Patuxent because of the more northern latitude, were assigned to the DAR project. Because of the different rearing schedules for UL and DAR, DAR tended to receive later eggs. Eggs laid later in laying sequence have lower weights and result in smaller birds, although this overall effect was small.

No inbreeding effect was detected to account for difference in size between the 2 groups. Some of the chicks in both UL and DAR had common parents. The small sample size and unequal weighting (means of means) limited comparisons. Even so, UL chicks were still found to be significantly larger than DAR

Table 1. Mean difference in tarsal length of chicks of common whooping crane sires and dams reintroduced by ultralight-led migration (UL) and direct autumn release (DAR) techniques, eastern migratory whooping crane reintroduction, 2001-2010.

Parent	Sex of chicks	Parents (n)	Mean tarsal length difference (mm)	SE	t	P ^a	df	UL chicks (n)	DAR chicks (n)
Sires	male	5	1.03	2.69	0.38	0.360	4	10	15
Sires	female	5	0.96	4.70	0.20	0.425	4	13	16
Sires ^b	female	4 ^b	5.44	1.81	3.00	0.029*	3	12	15
Dams	male	7	3.35	1.24	2.70	0.018*	6	30	14
Dams	female	7	5.72	2.18	2.62	0.020*	6	18	19

^a One-tailed test (UL > DAR). *Significant at $P = 0.05$.

^b Excluding 1 outlier (unusually large DAR female with 318-mm tarsus)

chicks produced by the same dams. Genetics was not, therefore, responsible for the size difference.

Costume-rearing protocols, although generally similar for UL and DAR, had many subtle differences involved with facilities, staff, general health, feeding, and exercise regimes which may have contributed to the size difference in these 2 groups of birds. While later egg sequence explains some of the smaller size of DAR birds, interaction between food and exercise likely had greater effects.

The DAR cranes exhibited lower survival (65.7%) than UL cranes (85.1%) during their first year after release (Urbanek et al. 2014). This was to be expected because DAR juveniles were released to perform their first autumn migration unassisted. The UL birds were not released until after they had completed their first migration to Florida, and then they were gentle-released with intensive protection through their first winter. Direct or hard releases typically have lower survival than soft or gentle releases (Nagendran et al. 1996).

Size might affect survival. For example, larger size could reduce susceptibility to predation, especially of females. However, larger size of males might increase susceptibility to power line collision. Because of large differences in reintroduction protocols, including many confounding variables, the effects of size on post-release survival were only tested within groups of the same sex and method and found to be insignificant. However, effects of smaller size on behavior and survival of DAR cranes after release could not be directly tested. The use of later eggs and resulting smaller chicks may have post-release effects which remain to be identified and understood in order to fully evaluate and implement successful reintroduction programs.

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