

Migrate small, sound big: functional constraints on body size promote tracheal elongation in cranes

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Abstract

Organismal traits often represent the outcome of opposing selection pressures. Although social or sexual selection can cause the evolution of traits that constrain function or survival (e.g. ornamental feathers), it is unclear how the strength and direction of selection respond to ecological shifts that increase the severity of the constraint. For example, reduced body size might evolve by natural selection to enhance flight performance in migratory birds, but social or sexual selection favouring large body size may provide a countervailing force. Tracheal elongation is a potential outcome of these opposing pressures because it allows birds to convey an auditory signal of exaggerated body size. We predicted that the evolution of migration in cranes has coincided with a reduction in body size and a concomitant intensification of social or sexual selection for apparent large body size via tracheal elongation. We used a phylogenetic comparative approach to examine the relationships among migration distance, body mass and trachea length in cranes. As predicted, we found that migration distance correlated negatively with body size and positively with proportional trachea length. This result was consistent with our hypothesis that evolutionary reductions in body size led to intensified selection for trachea length. The most likely ultimate causes of intensified positive selection on trachea length are the direct benefits of conveying a large body size in intraspecific contests for mates and territories. We conclude that the strength of social or sexual selection on crane body size is linked to the degree of functional constraint.

Introduction

Trade-offs between reproduction and survival can cause opposing directional selection pressures during different stages of life, resulting in traits that are suboptimal for any specific purpose (Darwin, 1859; Stearns, 1989; Schluter *et al.*, 1991). Sexual selection often opposes natural selection by targeting traits that are critical to function, despite negative consequences for survival (e.g. ornamental feathers; Zahavi, 1975; Andersson, 1994). As optimum trait phenotypes for survival and reproductive functions diverge, opposing selection pres-

ures on that trait are expected to intensify (Schluter *et al.*, 1991). However, empirical evidence from Trinidadian guppies contradicts this theoretical prediction. In guppies, females generally prefer male traits that increase predation vulnerability (e.g. vibrant coloration), but the intensification of predation pressure causes female preference to shift towards phenotypes that decrease vulnerability (Breden & Stoner, 1987; Schwartz & Hendry, 2007). Thus, when ecological conditions amplify constraints on a guppy signalling trait, sexual selection parallels natural selection, without necessarily affecting selection strength.

Sexual selection and social selection (Lyon & Montgomerie, 2012; Tobias *et al.*, 2012) tend to favour large body size, a trait that is generally associated with competitive ability and fecundity in both sexes (Andersson, 1994; Blanckenhorn, 2000). However, evolutionary increases in body size may be precluded by genetic or ontogenetic constraints (Reeve & Fairbairn, 1996;

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Badyaev, 2002) or functional advantages of being small (Blanckenhorn, 2000). A prediction from theoretical models of avian flight energetics is that the evolution of long-distance migration by flapping flight should lead to evolutionary reductions in body size, allowing birds to minimize the duration of migration (Alerstam & Lindström, 1990; Lozano *et al.*, 1996; Hedenström, 2008). Smaller-bodied birds have advantages over larger-bodied conspecifics: (i) they expend less energy per unit body mass in flapping flight at maximum range speed (V_{mr} ; speed that maximizes distance travelled per unit energy), usually the speed of migration (Pennycuick, 1969; Raynor, 1982); (ii) they restore fat reserves more quickly during stopover (Lindström, 1991); and (iii) they have higher maximum fuel-carrying capacity (Lindström, 1991) and correspondingly longer maximum flight ranges (Pennycuick, 1978, 1989; Klaassen, 1996).

If natural selection for small body size to enhance migratory flight performance opposes sexual or social selection for large body size, actual body size should be intermediate (Fisher, 1930). As intermediate body sizes are smaller than the optimal body size for reproduction, sexual or social selection should favour traits that confer the appearance of large body size without increasing body size. Acoustic formant spacing is a vocal characteristic that provides a mechanism for body size exaggeration (Fitch, 1999; Harris *et al.*, 2006; Charlton *et al.*, 2007). The frequency differences between adjacent formants (energy peaks in the frequency spectrum) are inversely related to the length of the sound-producing tube (Fant, 1960; Fitch, 1999). In birds, the trachea is the effective sound-producing tube and its length scales positively with body mass ($\propto m^{0.39}$; Hinds & Calder, 1971). Subtle reductions in formant spacing resulting from a longer trachea can be perceived by birds and mammals, conferring the auditory perception of a larger body size (Fitch, 1999; Fitch & Kelley, 2000; Reby *et al.*, 2005; Charlton *et al.*, 2007, 2011a; Taylor & McComb, 2010). These exaggerated vocal cues are sufficiently advantageous that diverse bird clades have independently evolved tracheal elongation (TE), a bizarre morphology in which the trachea forms loops or coils inside the body (Fitch, 1999).

The variable extent of TE among crane species represents an evolutionary response to social or sexual selection for apparent large body size, but the proportional length of the trachea can vary while actual body size is subject to functional constraints related to ecology and flight performance. Hence, a comparative study of crane TE provides a rare opportunity to disentangle functional constraints from sexual selection (Schluter *et al.*, 1991; Jones, 1992; Kotiaho, 2000). In this study, we examined the relationships among migration distance, body mass, sexual size dimorphism (SSD) and trachea length in cranes. We predict that migration distance is a negative predictor of body mass and a

positive predictor of proportional trachea length and SSD. We assume that proportional trachea length provides an index of the strength of social or sexual selection on apparent body size, whereas SSD represents an independent and indirect index of the degree of asymmetry between selection operating on male and female on body size, respectively.

Materials and methods

Data collection

Mean migration distance, body mass and trachea length data for 15 species of crane including 2 subspecies of *Grus canadensis* Linnaeus (1758) were obtained from the literature and fresh carcasses (Table 1). We used the average of male and female body mass values to represent each taxon. We calculated SSD as (male body mass / female body mass) * 100. For two species, *G. nigricollis* Przevalski (1876) and *Balearica regulorum* E. T. Bennett (1834), separate body mass data for males and females were unavailable so we cube-transformed average tarsus length of each sex as a proxy for body size (Freeman & Jackson, 1990). To test Bergmann's rule as an alternative explanation for interspecific variation in body mass, we used the absolute value of the midpoint of breeding latitude using range maps from savingcranes.org.

Trachea measurements

We examined 39 *Grus canadensis tabida* and 22 *Grus canadensis canadensis* specimens that were salvaged or donated by hunters (Table S1). We extracted tracheae by breaking and removing the sternal bone, removing the ossified trachea from inside the keel, cutting the syrinx at the lungs and detaching the trachea at the glottis. We measured unstretched tracheae from the pessulus of the syrinx to the end of the epiglottis of the larynx by tracing string along the length of the trachea then marking and measuring the string to the nearest millimetre. We fluid-preserved all tracheae in 95% EtOH to maintain tracheal ring size (Miller *et al.*, 2007) and deposited them along with skeletons and primary feathers at the Museum of Southwestern Biology (Albuquerque, NM, USA).

Statistical analyses

We hypothesized that (i) migration distance is a negative predictor of body mass, (ii) trachea length scales positively with body mass both within and among species, and (iii) migration distance is a positive predictor of trachea length and SSD after accounting for body mass. We tested these hypotheses using conventional and phylogenetically corrected statistical analyses in R (R Development Core Team, 2013). For all analyses, we

Table 1 Migration distance, body mass, trachea length, sexual size dimorphism (SSD) and midpoint breeding latitude data for crane taxa.

	Migratory distance (km)	Body mass (kg)	Trachea length (cm)	SSD (male size as % female size)	Mean breeding latitude
<i>Balearica regulorum</i>	0*	3.77†	50‡	108§	7°S
<i>Balearica pavonina</i>	0*	3.63*	53.3‡	100*	9°N
<i>Leucogeranus leucogeranus</i>	5313¶	6.19**	NA	116.1**	66°N
<i>Grus nigricollis</i>	694††	6.0†	NA	121.7*	30°N
<i>Grus monachus</i>	3484‡‡	3.78**	118.2‡	112.3**	55°N
<i>Grus grus</i>	3370§§	4.77**	111.2‡	128.5**	58°N
<i>Grus americanus</i>	4008¶¶	6.85*	147‡	114.1*	60°N
<i>Grus japonensis</i>	1474¶¶	8.23**	149.4‡	119.4**	49°N
<i>Anthropoides virgo</i>	3031¶¶	2.42†	53.3‡	101.1*	44°N
<i>Anthropoides paradisea</i>	0*	4.65†	64.6‡	156.3†	28°S
<i>Buzgeranus carunculatus</i>	0*	8.16†	86.6‡	108.2*	12°S
<i>Grus antigone</i>	0*	6.21**	88.1‡	128.2**	22°N
<i>Grus rubicunda</i>	0*	6.25†	106.9‡	112.7†	24°S
<i>Grus vipio</i>	2558¶¶	6.01**	121.8‡	123.6**	48°N
<i>Grus canadensis canadensis</i>	3694***	3.28†††	60.6‡‡‡	114†††	58°N
<i>Grus canadensis tabida</i>	1450§§§	5.27†††	71.9‡‡‡	113.5†††	44°N

Sources of data: *Johnsgard (1983); †Dunning (2007); ‡Gaunt *et al.* (1987); §Walkinshaw (1964); ¶Higuchi *et al.* (2006); **Swengel (1992); ††Qian *et al.* (2009); ‡‡Higuchi *et al.* (1992); §§Ellis *et al.* (1992); ¶¶Kuyt (1992); ***Krapu *et al.* (2011); †††Jones & Witt (2012); ‡‡‡MSB specimens; §§§Drewien *et al.* (1999).

\log_{10} -transformed body mass (kg) and trachea length (cm) to account for allometric growth. We examined the residuals using ordinary least squares with a normal QQ plot and a Shapiro–Wilk test and found that they were normally distributed and homoscedastic for most analyses. We present one-tailed *P*-values for all analyses because we have directional, *a priori* hypotheses.

We accounted for phylogeny using a time-calibrated tree from Krajewski *et al.* (2010) (Fig. 1, Appendix S1). Because Krajewski *et al.* (2010) did not include subspecies, we applied a date of ~1.5 million years for the split between *G. c. tabida* and *G. c. canadensis* (Fig. 1), based on Rhymer *et al.* (2001). We tested for phylogenetic inertia in each of our four variables by estimating Blomberg's *K* (Blomberg *et al.*, 2003) using the R package *picante* (Kembel *et al.*, 2010). To account for phylogenetic inertia, we conducted our regression analyses using phylogenetic generalized least squares (PGLS) as implemented in *ape* (Paradis *et al.*, 2004). We repeated each analysis using two separate evolutionary models for the phylogenetic covariance structure: (i) Brownian motion and (ii) Ornstein–Uhlenbeck with a single optimum (Felsenstein, 1988). AIC values supported the Brownian motion models in every case, so we only report the results from that model. For the visualization of these phylogenetic statistical patterns, we applied phylogenetically independent contrasts (PICs; Felsenstein, 1985) to each of our variables, using *ape*.

To test hypothesis (1), we examined the relationship between migration distance and body mass with conventional linear regression and PGLS. To rule out Bergmann's rule as an alternative explanation, we used the same methods to test for an effect of breeding latitude

on body mass. To test hypotheses (2) and (3), we used body mass and migration distance as predictors of trachea length in a multiple regression. For visualization, we conducted a regression of trachea length against body mass and plotted the residuals as a function of migration distance. As an alternative to analysing migration distance as a continuous variable, we looked at the presence or absence of migratory habit. We used analysis of covariance (ANCOVA) to test whether trachea length and SSD, respectively, scale differently with body mass in migratory and nonmigratory cranes.

Results

When we accounted for phylogeny using a Brownian motion model, migration distance was a significant negative predictor of body mass (PGLS: $F_{2,12} = 8.08$, $R^2 = 0.366$, scaling coefficient = -6×10^{-5} , $P = 0.006$: Fig. 2a), consistent with hypothesis (1). The PGLS result was even stronger when we added the two crane species for which we lacked trachea length data, for a total of 16 terminal taxa ($F_{2,14} = 9.13$, $R^2 = 0.389$, scaling coefficient = -5×10^{-5} , $P = 0.003$). However, migration distance did not predict body mass in a conventional, nonphylogenetic regression ($F_{1,14} = 0.35$, $R^2 = 0.025$, $P = 0.561$). Breeding latitude was a significant negative predictor of body mass in phylogenetic analyses ($F_{2,14} = 6.33$, $R^2 = 0.272$, scaling coefficient = -0.005 , $P = 0.011$), but not in conventional analyses ($F_{1,14} = 0.08$, $R^2 = 0.006$, $P = 0.390$). This indicates that cranes at higher latitudes evolve reduced body masses, which is opposite of the expectation under Bergmann's rule.

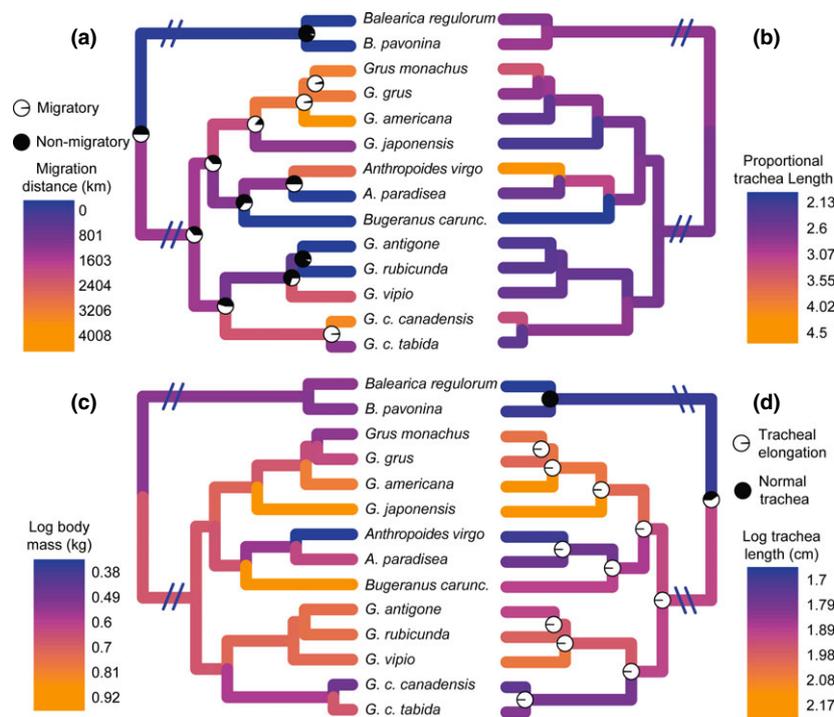


Fig. 1 Phylogenetic relationships among the 14 crane taxa (13 species and one additional subspecies) with branch lengths proportional to time, except where indicated by scale breaks (Rhymer *et al.*, 2001; Krajewski *et al.*, 2010). Branches are colour-coded according to ancestral trait values that were estimated using maximum likelihood in the *ape* package (Paradis *et al.*, 2004). Pie charts at nodes indicate the probability of assignment to alternative binary character states. (a) Branch colours indicate estimated migration distance (km), whereas pie charts indicate migratory habit, which is modelled as a binary variable; (b) proportional trachea length, calculated as log trachea length (cm) divided by log body mass (kg); (c) log body mass (kg); and (d) log trachea length (cm) represented by branch colours, with the presence or absence of tracheal elongation indicated by pie charts. Tracheal elongation is acquired in a single instance near the base of the tree, whereas all other traits exhibit gains and losses (or increases and decreases) during the evolution of cranes.

As predicted by hypothesis (2), body mass was positively correlated with trachea length among species both before and after correcting for phylogeny (Fig. 2b). Conventional ordinary least squares showed that trachea length scaled with body mass^{0.752} ($F_{1,12} = 12.17$, $R^2 = 0.504$, $P = 0.002$, 95% CI: 0.319–1.185), whereas PGLS estimated that scaling coefficient to be 0.441 ($F_{2,12} = 9.76$, $R^2 = 0.436$, $P = 0.003$, 95% CI: 0.176–0.707). Body mass was also strongly associated with trachea length within both *G. c. tabida* ($F_{1,37} = 41.36$, $R^2 = 0.528$, $P < 10^{-6}$, scaling coefficient = 0.750, 95% CI: 0.633–0.867) and *G. c. canadensis* ($F_{1,20} = 11.17$, $R^2 = 0.358$, $P = 0.003$, scaling coefficient = 0.647, 95% CI: 0.453–0.840).

In PGLS, migration distance was a significant positive predictor of trachea length after accounting for body mass (overall $R^2 = 0.610$; migration distance $F_{3,11} = 5.57$, $P = 0.014$; body mass $F_{3,11} = 14.84$, $P < 0.001$), supporting hypothesis (3). We visualized this result by regressing the residuals of trachea length (derived from a PIC regression of trachea length against body mass) against PICs of migration distance ($F_{1,12} = 14.89$, $R^2 = 0.554$, $P = 0.002$, Fig. 2c). In nonphylogenetic

analyses, the residuals of trachea length regressed against body mass were also positively correlated with migration distance ($F_{1,12} = 18.08$, $R^2 = 0.601$, $P < 0.001$).

The positive effect of migratory habit on trachea length after accounting for mass was confirmed in analyses that treated migratory habit as a discrete, two-state variable. Nonphylogenetic ANCOVA revealed significant effects of both body mass ($F_{1,12} = 23.60$, $P < 0.001$) and migratory habit ($F_{1,12} = 12.26$, $P < 0.002$) on trachea length (Fig. 3), and addition of an interaction term did not significantly improve the fit of the model ($P = 0.998$). PGLS confirmed these effects of body mass ($F_{3,11} = 13.08$, $P < 0.001$) and migratory habit (overall $R^2 = 0.567$, $F_{3,11} = 4.51$, $P = 0.027$). There were no significant effects of migration, body mass or trachea length on SSD using phylogenetic or nonphylogenetic analyses.

We found evidence for strong phylogenetic inertia in trachea length and moderate to weak phylogenetic inertia in other variables. Blomberg's K was 1.10 for trachea length, indicating that closely related species were more similar in trachea length than expected

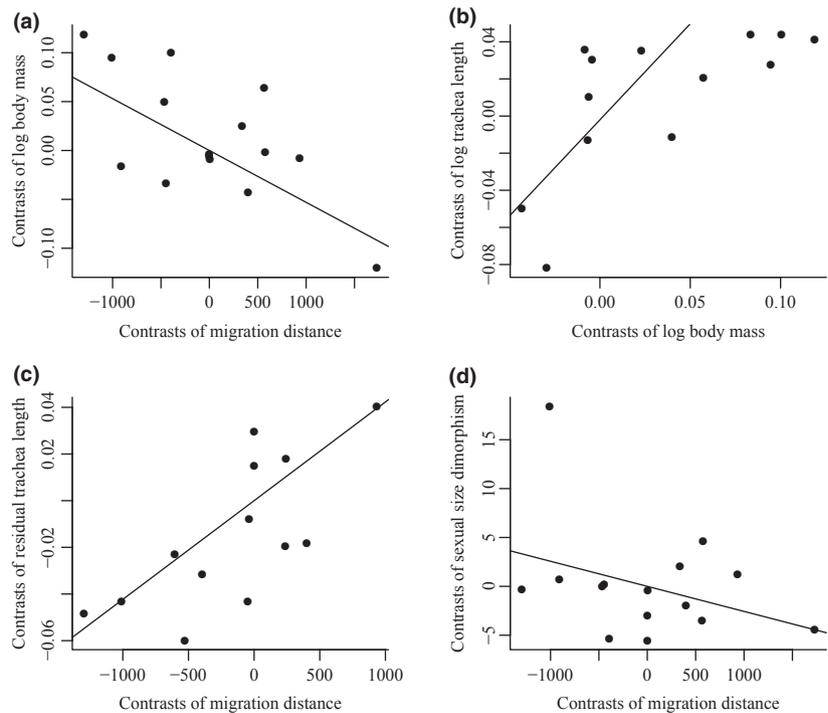


Fig. 2 Phylogenetically independent contrasts of (a) migration distance and log body mass ($n = 15$), (b) log body mass and log trachea length ($n = 13$), (c) migration distance and residuals of trachea length from body mass ($n = 13$) and (d) migration distance and sexual size dimorphism ($n = 15$).

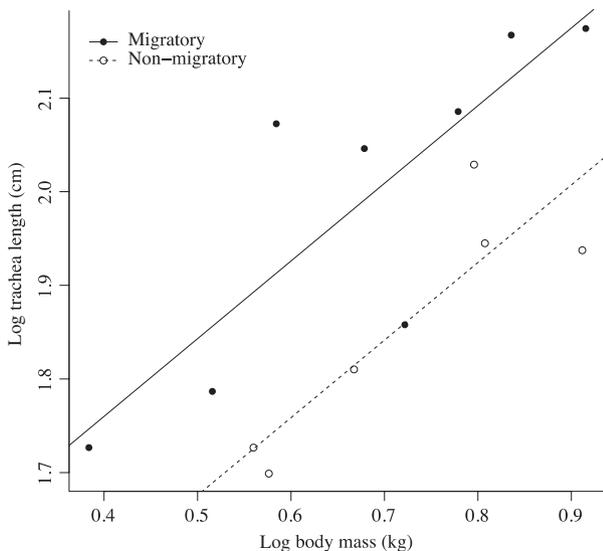


Fig. 3 Scaling of log trachea length as a function of log body mass for migratory (slope = 0.822, y-intercept = 1.434, adjusted $R^2 = 0.635$, $P = 0.006$) and nonmigratory (slope = 0.836, y-intercept = 1.258, adjusted $R^2 = 0.712$, $P = 0.011$) cranes. The intercepts are significantly different ($P < 0.002$), although the slopes are not ($P = 0.998$).

under a model of Brownian motion. This finding, which is typical of life history traits (Blomberg *et al.*, 2003), suggests that there is limited evolutionary lability in trachea length, perhaps due the physical

constraints imposed by the sternum. Blomberg's K indicated substantially more lability in body mass ($K = 0.34$), migration distance ($K = 0.44$), and SSD ($K = 0.31$).

Discussion

Evolution of reduced body size in migratory cranes

We found that body size is negatively correlated with migration distance among crane species after accounting for phylogeny, consistent with flight energetics theory. Although migratory crane taxa are not smaller-bodied than sedentary ones on average ($t_{14} = 0.687$, $P = 0.503$), each migratory taxon tends to be smaller than its closest less migratory relative (Fig. 1). This suggests that the evolution of migration causes an evolutionary size reduction rather than the evolution of a particular size. Ancestral state estimates are consistent with the reversibility of this mechanism, because both body mass and migratory distance fluctuate upward and downward during the evolution of cranes (Fig. 1). We found that cranes at higher latitudes had evolved reduced body mass, contrary to expectations under Bergmann's rule. This suggests that either cranes are not subject to temperature effects on body size or that opposing selection pressures mask the Bergmann's rule pattern.

The speed of migration by flapping flight decreases in proportion to mass^{-0.19} as a result of the disproportionate increase in the energetic cost of flapping flight with

increasing body mass (Hedenström, 2008). Conversely, body mass scales positively with efficiency and migratory velocity when using the slower, soaring mode of flight (Pennycuick, 1989; Alerstam & Lindström, 1990; Hedenström, 2008) because larger birds can use their disproportionate energy savings from soaring to reduce refueling time during stopover (Klaassen, 1996). Cranes employ a mixed flight strategy, opportunistically soaring over land when solar radiation is sufficient to generate thermals and flapping to maintain their migratory velocity under other conditions (Pennycuick, 1978; Pennycuick *et al.*, 1979; Johnsgard, 1983; Tacha *et al.*, 1992; Sapir *et al.*, 2011). We conclude that efficiency in the flapping component of crane migration is the key functional constraint that led to body size reduction (or gain) following the evolution (or loss) of migration.

Evolution of longer tracheae in migratory cranes

Migratory crane taxa appear to have decreased in body size and increased in proportional trachea length since diverging from their less migratory relatives, and both mechanisms appear to be reversible (Fig. 1, Fig. 2a,c, Fig. 3). However, we found no significant relationship between migration distance or migratory class and SSD (Fig. 2d). In cranes, sexual selection is likely bidirectional with respect to sex, as both males and females are territorial and provide parental care (Johnsgard, 1983).

The increased tracheal elongation in migratory taxa indicates that either (i) selection for some physiological benefit conferred by a longer trachea became stronger, (ii) physiological or developmental constraints on trachea length were relaxed, or (iii) selection for apparent large body size became stronger. The increased dead air space of a long trachea might confer a fitness advantage to migratory taxa by allowing large birds to maintain appropriate concentrations of CO₂ in the posterior air sacs while synchronizing breathing to their slow wing beat (Schmidt-Nielsen, 1972). However, if tracheal elongation were generally advantageous to large migratory species, it would be difficult to explain why it is rare and often associated with sexually dimorphic, non-migratory taxa (Fitch, 1999). Furthermore, we find no plausible mechanism by which migration would relax functional constraints on trachea length; therefore, we conclude that selection for apparent large body size likely intensified in these lineages.

The evolutionary reduction in body size in migratory cranes may have increased the incentive for signal receivers to heed signals of large body size in intraspecific interactions. The optimum body size for reproductive function (e.g. egg production, predator defence) is almost certainly larger than actual body size, which is subject to myriad energetic and biomechanical constraints (Pauly, 1997). This difference between the actual size and the optimum size for reproductive

function would have increased following the evolution of migration as a result of new functional constraints impinging on body size. This may have triggered stronger expression of mate preferences for large body size, leading to new extremes of tracheal elongation. Alternatively, the intensification of selection for large apparent body size in migratory species may be driven by large winter aggregations that increased the stringency of mate choice by increasing the encounter rate with potential mates (Koenig & Albano, 1986). This mechanism is plausible if winter aggregations increase the opportunities for pair formation or social conflict, amplifying the strength of selection associated with actual and apparent size. However, we do not find this explanation parsimonious because it implies that the concomitant reduction in body size and increase in tracheal length are unrelated events.

Implications for mechanisms of tracheal elongation

Species or individuals that have proportionately longer tracheae emit signals that exaggerate body size (Fitch, 1999; Charlton *et al.*, 2007). Our data showed strong relationships between trachea length and body mass within and among crane species, demonstrating that these signals also provide honest information about body size (Fig. 2b). Thus, slight exaggerations of vocal signals that arise from natural variation within populations can proliferate with minimal diminution of the expected utility to the receiver. Recurring fixation of slight exaggerations can gradually ratchet the social conventions governing the relationship between formant spacing and body size while increasing the length of the trachea towards its physical limits. In some mammal species, the larynx is lowered to increase vocal tract length, but structural constraints imposed by the thorax and sternum serve to maintain honesty of formant spacing as vocal signals of body size (Reby & McComb, 2003; Harris *et al.*, 2006; Sanvito *et al.*, 2007; Charlton *et al.*, 2011b). Similarly, tracheal elongation in cranes is structurally constrained by the bony sternum, which likely contributes to the persistent honesty of crane formant spacing.

What specific selective pressures are likely to have driven the successive elongation of tracheae in crane species? Traits whose primary functions are physical and physiological rather than perceptual are less likely to be subject to evolution by arbitrary, aesthetically driven mate preferences (Prum, 2012). Thus, arbitrary mate preferences (Fisher, 1930; Lande, 1981; Prum, 2010) and indirect benefits of large body size (e.g. 'good genes'; Zahavi, 1975) provide unlikely explanations for greater tracheal elongation in migratory taxa. Although we cannot rule out that body mass or trachea length may be improved as signals of genetic quality when their possessors are migratory, we think that this is unlikely to be a general cause of runaway evolution of

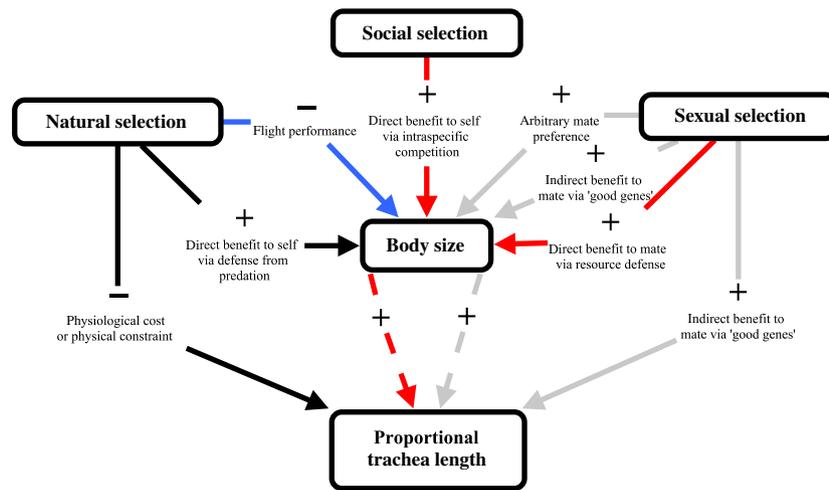


Fig. 4 Potential mechanisms of selection leading to tracheal elongation in cranes. Red lines represent the mechanisms that are most likely to be responsible for the evolution of tracheal elongation in cranes. Gray lines are alternative mechanisms that we consider to be less likely. The dashed lines indicate indirect selection on proportional trachea length as a result of direct selection on body size. Our analyses suggest that migration causes natural selection on flight performance to intensify (blue solid line), reducing body size and concomitantly increasing the strength of the sexual and social selection on body size (red solid lines) and indirectly on trachea length (red dashed lines).

tracheal elongation because it implies that these characters were favoured by conspecifics in precisely the situation where they would have been disadvantageous for migratory performance (Kirkpatrick, 1986). It is more parsimonious to interpret tracheal elongation as a consequence of the direct benefits of large size, either to the signaler or its mate, in intraspecific contests (Fig. 4). These direct benefits may result from both sexes engaging in territorial defence among family groups during and after the breeding season (Johnsgard, 1983). Large body size may yield additional direct benefits due to its utility in fending off known nest predators such as accipitrids, canids, mustelids and corvids, against which the crane's beak is a potentially deadly weapon (Nesbitt & Archibald, 1981; Tacha *et al.*, 1992; Lewis, 1995; Littlefield, 1995).

Conclusions

This phylogenetic comparative analysis of cranes provides empirical support for a negative correlation between body size and migration distance, as predicted from previous work on the energetics of flapping flight. In addition to evolving smaller bodies, migratory crane species evolved more extreme tracheal elongation. These findings indicate that sexual or social selection favouring large body size intensified following an increase in the functional constraints on body size exerted by the energetic costs of migration. The ultimate causes of this selection are probably the direct benefits of large body size to the signaler in intraspecific contests or to its mate in territory defence and predator defence. Our findings imply that opposing selection

pressures on body size during different parts of the annual cycle can catalyse sexual or social selection on related signalling traits, incentivizing runaway evolution of exaggerated phenotypes, driving evolution into novel morphospace.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 List of *Grus canadensis canadensis* and *Grus canadensis tabida* specimens used for trachea and body size measurements.

Appendix S1 A Newick format tree of Gruidae from Krajewski *et al.* (2010).

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Table S1. List of *G. c. canadensis* and *G. c. tabida* specimens used for trachea and body size measurements. Specimens are housed in the Division of Birds in the Museum of Southwestern Biology (MSB). Weblinks to Arctos database specimen records (including additional ancillary specimen data) are embedded in each catalog number.

Specimen	Trachea length (mm)	Body mass (kg)
http://arctos.database.museum/guid/MSB:Bird:29457	686	5.147
http://arctos.database.museum/guid/MSB:Bird:29458	645	5.285
http://arctos.database.museum/guid/MSB:Bird:29491	662	3.559
http://arctos.database.museum/guid/MSB:Bird:29501	666	5.064
http://arctos.database.museum/guid/MSB:Bird:29503	611	4.560
http://arctos.database.museum/guid/MSB:Bird:29605	721	4.992
http://arctos.database.museum/guid/MSB:Bird:29606	788	5.807
http://arctos.database.museum/guid/MSB:Bird:29607	710	5.256
http://arctos.database.museum/guid/MSB:Bird:29608	768	6.121
http://arctos.database.museum/guid/MSB:Bird:29610	695	5.155
http://arctos.database.museum/guid/MSB:Bird:29611	677	3.150
http://arctos.database.museum/guid/MSB:Bird:29612	601	3.169
http://arctos.database.museum/guid/MSB:Bird:29614	522	3.033
http://arctos.database.museum/guid/MSB:Bird:29615	668	4.705
http://arctos.database.museum/guid/MSB:Bird:29616	614	5.165
http://arctos.database.museum/guid/MSB:Bird:29617	492	2.857
http://arctos.database.museum/guid/MSB:Bird:29618	724	4.854
http://arctos.database.museum/guid/MSB:Bird:29619	785	6.046
http://arctos.database.museum/guid/MSB:Bird:29620	800	6.106
http://arctos.database.museum/guid/MSB:Bird:29621	749	5.825
http://arctos.database.museum/guid/MSB:Bird:29622	784	5.691
http://arctos.database.museum/guid/MSB:Bird:29623	709	5.334
http://arctos.database.museum/guid/MSB:Bird:29624	636	3.586
http://arctos.database.museum/guid/MSB:Bird:29625	580	3.172
http://arctos.database.museum/guid/MSB:Bird:29626	634	3.498
http://arctos.database.museum/guid/MSB:Bird:29629	802	5.770
http://arctos.database.museum/guid/MSB:Bird:29630	749	5.814
http://arctos.database.museum/guid/MSB:Bird:29631	820	5.273

http://arctos.database.museum/guid/MSB:Bird:29632	692	5.140
http://arctos.database.museum/guid/MSB:Bird:29633	614	3.222
http://arctos.database.museum/guid/MSB:Bird:29634	567	3.480
http://arctos.database.museum/guid/MSB:Bird:29635	674	3.318
http://arctos.database.museum/guid/MSB:Bird:29637	570	3.106
http://arctos.database.museum/guid/MSB:Bird:29638	500	2.981
http://arctos.database.museum/guid/MSB:Bird:29639	635	3.393
http://arctos.database.museum/guid/MSB:Bird:29640	609	3.305
http://arctos.database.museum/guid/MSB:Bird:29641	730	5.222
http://arctos.database.museum/guid/MSB:Bird:29642	739	5.195
http://arctos.database.museum/guid/MSB:Bird:29643	779	6.127
http://arctos.database.museum/guid/MSB:Bird:29644	604	3.403
http://arctos.database.museum/guid/MSB:Bird:29645	714	5.132
http://arctos.database.museum/guid/MSB:Bird:29646	579	3.340
http://arctos.database.museum/guid/MSB:Bird:29647	653	4.237
http://arctos.database.museum/guid/MSB:Bird:29648	663	4.034
http://arctos.database.museum/guid/MSB:Bird:29649	761	5.622
http://arctos.database.museum/guid/MSB:Bird:29652	698	5.126
http://arctos.database.museum/guid/MSB:Bird:29653	619	4.588
http://arctos.database.museum/guid/MSB:Bird:29654	758	5.392
http://arctos.database.museum/guid/MSB:Bird:29655	731	5.905
http://arctos.database.museum/guid/MSB:Bird:29657	554	2.990
http://arctos.database.museum/guid/MSB:Bird:29659	674	3.523
http://arctos.database.museum/guid/MSB:Bird:29660	682	5.210
http://arctos.database.museum/guid/MSB:Bird:29661	725	5.755
http://arctos.database.museum/guid/MSB:Bird:29664	682	5.299
http://arctos.database.museum/guid/MSB:Bird:29666	778	5.238
http://arctos.database.museum/guid/MSB:Bird:29667	644	4.890
http://arctos.database.museum/guid/MSB:Bird:29886	625	2.755
http://arctos.database.museum/guid/MSB:Bird:40371	681	5.235
http://arctos.database.museum/guid/MSB:Bird:40372	726	5.896
http://arctos.database.museum/guid/MSB:Bird:40373	717	5.141
http://arctos.database.museum/guid/MSB:Bird:40374	681	5.233

Appendix S1. A Newick format tree of Gruidae from Krajewski *et al.* (2010). Branch lengths are in millions of years. Krajewski *et al.* (2010) calculated divergence dates in parallel analyses using a standard deviation of 0.5, 1.0, and 1.5 on the lognormal priors of the age calibration points based on crane fossil records. We used branch lengths derived from 0.5 standard deviation because it produced a highly resolved topology, however still with a polytomy comprising nodes 12.5 and 11. We used the median value of the 95% highest posterior density interval as our branch length for node 12.5 to resolve the tree.

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(((((G. c. canadensis:1.5,G. c. tabida:1.5):8.5,(G. vipio:4.7,(G. rubicunda:3.8,G. antigone:3.8):0.9):5.3):2.5,((B. carunculatus:8.5,(A. paradisea:4.5,A. virgo:4.5):4.0):2.5,(G. japonensis:7.6,(G. americana:3.7,(G. grus:2.8,(G. monachus:1.3,L. leucogeranus:1.3):1.5):0.9):3.9):3.4):1.5):0.5,14:13.0):18.0,(B. pavonina:3.5,B. regulorum:3.5):27.5);
```