Heritable variation in thermoregulation is associated with reproductive success in the world's largest bird

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Abstract

Organisms inhabiting extreme thermal environments, such as desert birds, have evolved spectacular adaptations to thermoregulate during hot and cold conditions. However, our knowledge if and how selection acts on thermoregulation and the potential for evolutionary responses is limited, particularly for large organisms experiencing extreme temperature fluctuations. Here we use thermal imaging to quantify selection and genetic variation in thermoregulation in ostriches (*Struthio camelus*), the world's largest bird species that is regularly exposed to increasingly volatile temperatures. We found that females that are better at regulating their head temperatures had higher egg-laying rates under hotter conditions. Thermoregulatory capacity was both heritable and showed signatures of local adaptation. Females originating from populations exposed to more unpredictable climates were better at regulating their head temperatures in response to temperature fluctuations. Together these results reveal that past and present evolutionary processes have shaped genetic variation in thermoregulatory capacity, which appears to protect critical organs, such as the brain, from extreme temperatures during reproduction.

Impact Summary

Large animals inhabiting extreme thermal environments, such as deserts, are predicted to be particularly vulnerable to the sustained heat waves expected in their future climate. However, previous research on the evolution of thermoregulation has focused on the effect of increasing temperatures on survival in ectotherms. Here we study the ostrich (*Struthio camelus*), the world's largest bird, that inhabits some of the hottest and driest regions on Earth. We show that the ability of females to reproduce during hot conditions is associated with their thermoregulatory capacity. Furthermore, variation in thermoregulation is heritable and related to past climatic conditions: Females from populations originating from parts of Africa with more extreme temperature fluctuations were better able to thermoregulate, indicating local adaptation to different climatic conditions. Together, these results suggest that thermoregulation in this large desert bird has evolved in response to past climatic conditions, remains genetically variable and is currently under selection due to its effect on reproduction.

Introduction

Organisms need to manage heat and cold stress to survive and reproduce in variable climates (Angiletta, 2009). The universal challenge of coping with thermal stress (Hurley *et al.*, 2018; Sales *et al.*, 2018; Walsh *et al.*, 2019; Schou *et al.*, 2021) has promoted the evolution of adaptations to regulate body temperature (Huey *et al.*, 2003; Angiletta, 2009). For example, physiological mechanisms of selective brain cooling in birds (Fuller *et al.*, 2003), sweating in humans (Périard *et al.*, 2015) and morphological traits such as the ears of African Elephants (Weissenböck *et al.*, 2010) and the bills (Tattersall *et al.*, 2009; Symonds & Tattersall, 2010) and featherless skin patches (Szafrańska *et al.*, 2020) in different species of birds can all help to regulate temperature (McCafferty *et al.*, 2011). The different mechanisms by which animals thermoregulate and how these mechanisms could promote survival have been intensely studied. However, much less is known about the heritability of thermoregulatory traits and how such traits affect reproductive success, particularly for organisms living in the regions most affected by climate change (Walsh *et al.*, 2019), such as deserts (Bourne *et al.*, 2020; Schou *et al.*, 2021).

One group of animals expected to be particularly vulnerable to changing temperatures are large endotherms (birds and mammals). Larger bodies have a higher thermal inertia and slower rate of body temperature change compared to small bodies (Bogert, 1949; Angiletta, 2009). Thermal inertia can help maintain body temperatures during cold conditions, but it can also increase physiological stress under sustained heat, jeopardising survival and reproduction (Hurley *et al.*, 2018; Sales *et al.*, 2018; Bourne *et al.*, 2020; Parratt *et al.*, 2021; Schou *et al.*, 2021, 2022). Large animals are also expected to be particularly vulnerable to changing climates because their rate of adaptative evolution is potentially limited by longer generation times and lower population sizes (Cardillo *et al.*, 2005; Atwood *et al.*, 2020). Understanding how large-bodied

animals cope with thermal stress in fluctuating thermal environments therefore requires particular consideration.

The evolutionary potential of populations to adapt to thermal stressors such as heatwaves and cold snaps, depends on genetic variance in traits involved in thermoregulation (Hurley *et al.*, 2018; Sales *et al.*, 2018; Bourne *et al.*, 2020). Current empirical evidence suggests that genetic variation in thermoregulatory traits is often low, constraining species responses to shifting temperature regimes (Hoffmann *et al.*, 2003; Kellermann *et al.*, 2009). For instance, body temperatures and thermal adaptations are typically strongly phylogenetically conserved (Kellermann *et al.*, 2012a, 2012b) and evolve slowly compared to other traits (Moreira *et al.*, 2021). The late evolutionary biologist George C. Williams even questioned if endothermic body temperatures could evolve at all, suggesting that there may be evolutionary stasis in body temperatures and thermal adaptations (Williams, 1992). However, it is unclear if genetic variance in thermoregulatory traits is genuinely low, setting constraints on evolutionary responses, or whether such variation is just difficult to measure, given the challenges in quantifying individual variation in thermoregulatory capacity. This is a genuine empirical challengefor large vertebrates with their small population sizes and associated difficulties in obtaining large enough sample sizes.

Here we study the potential for evolutionary responses in thermoregulation in the world's largest bird, the ostrich (*Struthio camelus*) (Fig. 1). Estimating additive genetic variance in thermoregulation, and its relationship to fitness, requires measuring these traits across hundreds of individuals of known pedigree. To do this we studied a captive population of ostriches in the Klein Karoo region of South Africa, where a large number of individuals have been reared in a semi-natural environment over 25-years to produce a large nine-generation pedigree (Schou *et*

al., 2021, 2022). To quantify thermoregulation, we used thermal imaging (infrared camera technology) as this technique allows the surface temperature of hundreds of individuals to be non-invasively measured (Tattersall & Cadena, 2010). The temperature profiles of 423 adult females were repeatedly measured an average of six times (total images=2744). Such surface temperatures have been shown to be positively correlated with internal body temperatures in several species of birds (Andreasson *et al.*, 2016; Szafrańska *et al.*, 2020; Gauchet *et al.*, 2022) and insects (Tsubaki *et al.*, 2010; Svensson *et al.*, 2020). Thermal imaging has therefore emerged as a powerful technique that has been used to quantify body temperature variation and thermoregulatory capacity across diverse taxa under a variety of natural contexts (Tattersall *et al.*, 2009; Svensson & Waller, 2013; Andreasson *et al.*, 2016; Moore *et al.*, 2019; Svensson *et al.*, 2020; Szafrańska *et al.*, 2020; Gauchet *et al.*, 2022).

We focus on the surface temperature of the head, as shielding the brain from extreme temperatures is of crucial importance for animals (Kilgore *et al.*, 1976; Fuller *et al.*, 2003; Beltrán *et al.*, 2021). Combining our thermal image data with daily measurements of reproductive success and pedigree information, we test: (1) if head temperatures are regulated by blood circulating around the surface of the neck (Fig. 1B), and if the neck could thus serve as a so called 'thermal radiator' as shown for other morphological traits in other animal species (Tattersall *et al.*, 2009; Weissenböck *et al.*, 2010; Darnell & Munguia, 2011); (2) if variation in the efficiency of head thermoregulation influences female reproductive success, which is highly sensitive to temperature (Schou *et al.*, 2021, 2022); (3) if there is genetic variation in the efficiency of head thermoregulation; and (4) if populations originating from regions with different climatic regimes have evolved different head thermoregulatory capacities.

Results

Thermoregulation across naturally fluctuating temperatures

The surface temperatures (T) of the head and neck were both positively correlated with air temperature (Fig. 2; Tables S1-2). The rate of temperature change with increasing and decreasing temperatures was, however, significantly higher for the neck compared to the head (Increasing air T_{Neck} vs Increasing air T_{Head} (CI) = 1.6 (1.2, 1.9), pMCMC = 0.001; Decreasing air T_{Neck} vs Decreasing air T_{Head} (CI) = -2.1 (-3.1, -1.2), pMCMC = 0.001, Fig. 2C; Table S3). Consequently, there were large differences between neck and head temperatures at extreme temperatures (low < 20°C and high > 30°C), but at benign air temperatures (20 to 30°C), where the need for thermoregulation is minimal, neck and head temperatures were similar (Fig. 2D, Table S4).

Despite head and neck temperatures differing in their rates of change, we found no direct evidence that the neck actively regulates the surface temperature of the head. There was no accelerating change in surface temperature, as would be expected if blood flow was actively directed to the neck at critical air temperature inflection points (Fig. S1) (Tattersall *et al.*, 2009; Janse van Vuuren *et al.*, 2020). Instead, neck temperatures increased linearly with air temperature (Figs. 2A-B). This suggests that the deviation in head and neck temperature ('relative head temperature') captures the ability of individuals to thermoregulate and protect vital organs, such as the brain, from thermal stress. Furthermore, given the neck does not appear to actively regulate head temperatures, it likely provides useful information as a control on the current exposure of individuals to temperature stress. Such information can be difficult to quantify in free ranging animals due to microclimatic factors and previous behavioural activity.

Reproductive consequences and evolutionary potential of head thermoregulation

To examine if head thermoregulation has evolutionary potential, we investigated whether variation in thermoregulatory capacity, as measured by relative head temperature was heritable and related to reproductive success. We found that females with lower relative head temperatures during hot periods had higher egg-laying rates (Fig. 3A. $Hot_{Head\text{-}Neck}$ (CI) = -0.16 (-0.30,0.00); pMCMC = 0.027; Table S5), which was not the case under more benign temperatures ($Benign_{Head\text{-}Neck}$ (CI) = -0.06 (-0.18,0.05); pMCMC = 0.277; Table S5). This suggests that there is selection for the ability to cool the head during high temperatures due to its effects in promoting reproduction.

We further found that the capacity of females to regulate head temperature was heritable as revealed by positive and significant heritabilities and evolvabilities(Hansen *et al.*, 2011) (h^2 ranged from 0.06 to 0.08. Fig. 3B, Table S6, see also Table S7-S8 for separate models of head and neck). Heritability estimates were similar and significantly different from zero across cold, benign and hot conditions, although measures of additive genetic variances and repeatabilities were slightly higher under cold and hot conditions. Heritabilities are capped by how repeatable measurements are across individuals, which can often be low for labile physiological traits, such as thermal profiles when measured non-invasively under semi-natural settings where few variables are controlled (Figs. 1-2). Our repeatability estimates of the relative head temperature ranged from 0.12 to 0.17 (Fig. 3B, Table S6) which is quite low. It is therefore plausible that our heritability, while significant could underestimate the true heritability of relative head temperature due to the inherent difficulties in quantifying individual variation in thermoregulatory traits.

Thermoregulatory ability differs between populations from different climatic regions

We investigated whether head thermoregulation has evolved in response to past climate conditions by comparing the thermoregulatory ability of females from populations originating from different geographic regions (Fig. 4A-B). At our study site in South Africa, there are three different ostrich populations from different regions of Africa: South African Blacks (SAB: *Struthio camelus*), Zimbabwean Blues (ZB: *S. c. australis*) and Kenyan Reds (KR: *S. c. massaicus*) that have evolved under different climatic regimes (Fig. 4A-C). The climate in the East Africa, where KR naturally occur, is less seasonal, exhibiting lower fluctuations in temperature and precipitation, than the regions where ZB and SAB populations occur (Fig. 4C). Comparing individuals from the three populations that have all been raised in South Africa allowed us to examine population variation in head thermoregulation in a 'common garden' setting.

There were pronounced differences in head thermoregulation between the populations in response to increasing temperatures that corresponded to the stability of their past climates. The relative head temperatures of KR females that come from a stable thermal environment, changed very little in response to temperature increases (Fig. 4C, Table S11). This indicates similar rates of change in head and neck temperature with air temperature, and therefore only modest thermoregulation in response to increasing temperatures. In contrast, the SAB and ZB populations, that originate from the more variable thermal environments of southern Africa, showed a decrease in relative head temperature as temperatures increased, suggesting a greater capacity to regulate head temperatures (i.e. head temperature did not increase as fast as neck temperature, Fig. 4D). In contrast, thermoregulation during decreasing temperatures was similar across KRs, SABs and ZABs. These population differences in thermoregulatory

214 capacity were not confounded by differences in body size between the three populations (Tables
215 S12-13, Figs. S2-3).
216

217 **Discussion**

218 Our study has revealed that the capacity to regulate head temperature is important for
219 reproductive success under fluctuating temperatures (Fig. 3). The efficiency of
220 thermoregulation appears to have a genetic basis and enables reproduction under a greater range
221 of temperatures (Fig. 3). Ostriches from populations that experience more pronounced
222 temperature fluctuations were also more efficient at regulating head temperatures, reducing
223 head temperatures relative to the neck. These results suggest that thermoregulation of the head
224 has evolved in response to past climates and may evolve in response to future climatic change
225 (Fig. 4).
226

227 Previous research has identified a variety of mechanisms that aid temperature regulation
228 (Kilgore *et al.*, 1976; Fuller *et al.*, 2003; Angiletta, 2009; Tattersall *et al.*, 2009; Symonds &
229 Tattersall, 2010; Weissenböck *et al.*, 2010; Périard *et al.*, 2015). While such studies have
230 provided compelling evidence for the physiological consequences of various thermoregulatory
231 traits, they have not provided direct estimates of their effect on fitness and underlying genetic
232 variation. Such estimates require more quantitative approaches and are necessary to fully
233 understand how thermoregulatory traits influence future evolutionary responses to changing
234 climatic conditions. Our results demonstrate the power of combining measurements of
235 individual reproductive success with thermal profiles with pedigree data from individuals that
236 have been exposed to natural temperature variation in realistic environmental settings. . The
237 differences (Fig. 4) between phenotypically differentiated populations from different climatic
238 regions together with significant additive genetic variance in head thermoregulation within

239 populations (Fig. 3), suggests that selection to reproduce under fluctuating past and present
240 climates has shaped the evolution of the ostrich's remarkable capacity for head cooling under
241 extreme temperatures.
242
243 Decades of research on birds in temperate zones has focused on food availability in altricial
244 birds as a major limiting factor for reproduction (Lack, 1954; Williams, 1966). However, for
245 precocial birds inhabiting tropical and subtropical areas, like the ostrich, temperature stress
246 during reproduction might pose a more severe challenge than food limitation (Bourne *et al.*,
247 2020). Thermoregulation can maintain non-lethal body temperatures (Tattersall *et al.*, 2009;
248 Symonds & Tattersall, 2010; Tattersall & Cadena, 2010; Darnell & Munguia, 2011; McCafferty
249 *et al.*, 2011; Szafrańska *et al.*, 2020), but heat waves can still compromise population viability
250 by decreasing individual survival or reproductive success (Walsh *et al.*, 2019; Bourne *et al.*,
251 2020; Parratt *et al.*, 2021). Recent research from several taxa suggest that climate-mediated
252 local extinctions might already be common (Sinervo *et al.*, 2010; Wiens, 2016), with signs of
253 collapse of some desert bird communities being documented (Riddell *et al.*, 2019). Whether
254 genetic variation in adaptations underlying thermoregulation is sufficient to enable evolutionary
255 responses to increasingly hot and fluctuating conditions remains an open question.
256
257 The low heritability estimates of thermal profiles in this study make it difficult to predict the
258 evolutionary potential of ostrich populations to adapt to future temperature change. Previous
259 research on ectotherms such as *Drosophila* have also found low levels of additive genetic
260 variance and low heritabilities of thermal tolerance, revealing the challenges to obtain precise
261 quantitative genetic estimates (Hoffmann *et al.*, 2003; Kellermann *et al.*, 2009). Variation in
262 body temperatures can also be nonadaptive or maladaptive, a contention supported by recent
263 empirical research (Ghalambor *et al.*, 2015; Radersma *et al.*, 2020; Stamp & Hadfield, 2020;

Svensson *et al.*, 2020). For example, changes in both core body temperature and external body parts have been shown to be related to lower fitness in insects (Svensson *et al.*, 2020), reptiles (Campbell-Staton *et al.*, 2021), and birds (Nilsson *et al.*, 2016). Such maladaptive thermal plasticity may reflect costs of maintaining homeostasis and stable body temperatures under thermally stressful conditions, leading to selection for thermal robustness (Svensson *et al.*, 2020; Campbell-Staton *et al.*, 2021). It is likely that selection for thermal robustness on one side, and costs of maintaining a stable body temperature on the other side, are shaping genetic variance in thermoregulation in both the ostrich and other organisms inhabiting thermally stressful environments like deserts. While challenging, combining studies on the genetics of thermal tolerance with long-term population monitoring of reproduction and survival is key to forecasting the potential damage caused by climate change. This is especially the case for large, sub-tropical endotherms like the ostrich.

Methods

Study site and study populations

The study was conducted at the Oudtshoorn Research Farm in the arid Klein Karoo region of South Africa (GPS: 33° 38' 21.5"S, 22° 15' 17.4"E). Fenced enclosures (N=181) were used to monitor the reproductive success of ostriches that included 156 (~0.25 ha) for male-female pairs (Cloete *et al.*, 2008) and 22 for groups (~0.47 ha). All individuals had access to *ad libitum* food and water. The ostrich individuals in this study belong to three different subspecies, hereafter referred to as populations: 1) the Masai ostrich (*Struthio camelus massaicus*), or Kenyan Red (KR), 2) the Southern African ostrich, (*S. c. australis*), sometimes referred to as the Zimbabwean Blue (ZB) because of its origin in Namibia and Zimbabwe, and 3) the South African Blacks (SAB), that is thought to be of mixed origin, but is genetically very similar to ZB (Davies et al 2012; unpublished data). SAB are also referred to as *S. c. var. domesticus*. Individuals that had less than 85% expected relatedness to one population, as determined by the pedigree (see below), were considered hybrids. Breeding birds were recruited from surviving chicks from previous years, and parentage data were used to compile a 9-generation pedigree with 139 founding individuals. Ethical clearance was obtained from the Western Cape Department of Agriculture (DECRA R12/48).

Thermal imaging data

From 2012 to 2018 we took thermal images of ostriches in the enclosures using an infrared thermography camera (H2640, NEC Avio Infrared Technologies). Pictures were taken during October to December, usually from distances between 2m and 25m. We used the software InfRec Analyzer to draw separate polygons within the head and neck of the ostrich in each image, and the average temperature of these polygons were extracted as individual head and neck surface temperatures, respectively. We used the same procedures and same default settings

313 of the software as in our previous work (Svensson & Waller, 2013; Svensson *et al.*, 2020),
314 assuming an emissivity of 1. As a measure of air temperature, we used estimates of hourly
315 temperatures from a weather station positioned 600 m from the field site. We fitted a cubic
316 spline to the hourly temperature estimates of each day using the R-package *mgcv* v.1.8 (Wood,
317 2004), from which we extracted the predicted air temperature at the time-points when thermal
318 images were taken.
319
320 **Datasets and analyses**
321 *Quantifying rate of change in head and neck surface temperatures*
322 From 2012 to 2017, we took 2744 pictures of 423 females from early morning till late afternoon
323 (average images per female = 6.5). This dataset was designed with the aim of having a high
324 number of individuals with repeated sampling within and across years, such that each individual
325 was monitored in different thermal environments, but with little repeated sampling within days
326 (8% of the pictures).
327
328 With this dataset we modelled the change in surface temperatures of the head and neck in
329 response to air temperature using generalized linear mixed models (GLMMs). A previous
330 investigation showed reproductive success of ostriches is highest at a daily maximum
331 temperature of ~20°C (Schou *et al.*, 2021). We therefore defined 20°C as the optimum air
332 temperature for ostriches, and calculated absolute *temperature change* away from this optimum,
333 defining the factor *direction* to denote whether the change in air temperature was due to a
334 decrease or increase from the optimum. To make the intercept of statistical models represent
335 the most benign air temperature, we set 20°C to 0 and calculated deviations above (increases)
336 and below (decreases) this value. The variance of slopes (see below) depends on the scale of
337 the environmental parameter, and we therefore standardized our data by dividing it by the

maximum of the air temperature range, resulting in 1 being the maximum temperature change. Head and neck temperatures were modeled as Gaussian traits in separate models. Models included the fixed effects of temperature change (ranging from 0 to 1) and direction (decreases or increases). The interaction between temperature change and direction was modelled with a common intercept for decreases and increases, as the way temperature change was calculated dictated that the intercepts were identical. We included the fixed effects of population (SAB, ZB, KR or hybrids) and both the linear and quadratic terms of time of day (scaled and centered to a mean of zero and unit variance). We included interactions between population, temperature change and direction.

We accounted for environmental effects that varied across years, such as diet, by including year as a random effect. Photographs were taken across 48 days, and we therefore included date as a random effect. We also added enclosure as a random effect as the enclosures varied in vegetation cover, potentially impacting on the local climatic conditions, and were repeatedly used across years. Temperature change and direction were interacted with individual ID, to allow independent rates of change in surface temperature of each female. This was modelled as a 3x3 unstructured variance-covariance matrix.

GLMMs were run in R v.3.6.0 (R Core Team, 2020) using the Bayesian framework implemented in the R-package MCMCglmm v.2.29 (Hadfield, 2010). For random terms we used the weakly informative inverse-Gamma distribution (scale = 0.001, shape = 0.001, i.e. *V = diag(n), nu =n-1+0.002, with n being the dimension of the matrix*) as priors. Each model was run for 5,100,000 iterations of which the initial 100,000 were discarded and only every 4,000th iteration was used for estimating posterior probabilities. The number of iterations was based on inspection of autocorrelation among posterior samples in preliminary runs. Convergence of the

363 estimates was checked by running the model three times and inspecting the overlap of estimates
364 in trace plots and the level of autocorrelation among posterior samples. Posterior mode and 95%
365 credible intervals are reported for random effects.
366
367 *3.2) Determining the impact of body mass on variation in surface temperatures*
368 To test if differences in the rate of change in surface temperatures with air temperature were
369 caused by differences in body mass, we ran a separate set of models including individual body
370 mass as an additional fixed effect. We had records of body mass for all individuals and when
371 multiple records were available for one individual, we used the record closest to the time of the
372 thermal image. Models for surface temperature of the head and neck were implemented using
373 the model structure described above, but with body mass (scaled and centered to a mean of zero
374 and unit variance) interacted with all the previously described fixed effects.
375
376 *Comparing the neck and the head*
377 We compared the rate of surface temperature change between the neck and head. This was done
378 by running a model that included body part (neck or head) as a fixed effect. The models were
379 implemented using the model structure described above, but with body part interacted with all
380 the previously described fixed effects. We also added image as an additional random effect
381 because head and neck surface temperatures were derived as pairs from the same image.
382
383 *The change in relative head temperature when hot or cold*
384 Results from the analyses above indicated that the head is more robust to changes in air
385 temperature than the neck, potentially indicating the head is protected from temperature
386 fluctuations. However microclimatic factors (i.e. the operative environmental temperature) and
387 the behaviour up until the time of the thermal image will influence estimates of absolute head

surface temperatures. To better estimate the capacity of individuals to regulate head temperature, we compared the temperature of the head to the neck (head-neck surface temperature) to control for differences in operative environmental temperatures. Positive values therefore indicated that the neck was warmer than the head, and negative values that the head was warmer than the neck. To test if the head-neck temperature at high air temperatures differs from benign and cold air temperatures, we grouped air temperature into three categories: Cold (air temperatures <= 20°C, n_{images} = 849), Benign (air temperatures > 20°C & < 30°C, n_{images} = 1069) and Hot (air temperatures >= 30°C, n_{images} = 826). The grouping of air temperature into these three categories was based on the thermal neutral zone of the emu (Maloney, 2008), and to ensure roughly equal replication within the cold and hot categories. These categories do not capture the effects of small deviations in air temperature, and we therefore added a continuous measure of the deviation from the mean air temperature in each category. This variable was constructed by centering and scaling the air temperature records within each air temperature category. We modelled the response of the head-neck temperature (Gaussian) to air temperature category by following the same general approach as above. The major difference was that air temperature category was included as a fixed factor (instead of air temperature change and direction) and interacted with female ID to generate a 3x3 unstructured variance-covariance matrix composed of the cold, benign and hot temperature categories. We also estimated the residual variance separately for each air temperature category.

Effect of neck and head surface temperatures on reproductive success

To test if the capacity to regulate head temperature influences reproductive success we analysed its relationship with rates of egg-laying. We connected thermal measurements of individual females to their egg-laying records. From previous investigations we know that when daily maximum temperature is above or below 20°C ostrich egg laying-rate starts to slowly decrease

two to four days later, possibly because this is the time it takes for the egg to travel down the oviduct (Schou *et al.*, 2021). The maximum temperature was below 20°C in just two of the days during which we monitored thermoregulation, but maximum temperature was above 20°C in 41 days. We therefore monitored whether any eggs were laid two to four days after females were thermal imaged at days reaching more than 20°C. If females with higher neck than head surface temperatures have a higher probability of laying an egg during this time window, this would indicate that the thermoregulatory capacity of the head is indeed important for reproductive success. We are, however, careful with inferring causation, as it is possible that a third confounding factor, such as metabolic rate, may generate an autocorrelation between head regulation and laying rates. To overcome this, we grouped the data into photographs taken during hot and benign times of the days. If an elevated neck to head temperature ratio reflects increased tolerance to heat, then its positive relationship with egg-laying should be most pronounced when hot.

Egg laying rates were analysed using the model structure described above, but with the following modifications: a) the probability of laying (binary, model type: "threshold") was used as the response variable, b) the fixed effects included were head-neck temperature difference (scaled and centered to a mean of zero and unit variance), air temperature category (hot or benign), and population (KR, SAB, ZB or Hybrid), as well as the interaction between head-neck temperature and temperature category, c) as females of two years of age lay fewer eggs than older females (Schou *et al.*, 2021), a factor of age (2 versus >2) was also included as a fixed effect, and d) year, date, enclosure and individual ID were included as random effects. The first 45 days of the breeding season were removed as this is the average time it takes for pairs to acclimate to each other and their enclosure (Schou *et al.*, 2021). We also removed females that laid fewer than ten eggs per year (5% of all females) to avoid including females

from incompatible pairs, and individuals that did not enter the breeding state. Each model was run for 31,500,000 iterations of which the initial 1,500,000 were discarded and only every 10,000th iteration was used for estimating posterior probabilities.

Quantifying the evolutionary potential of relative head temperature

Using the model of the change in head-neck temperature in each air temperature category (see above), we estimated the repeatability (R) of the head-neck temperature under different temperature conditions. This was done using the estimates of permanent individual variances (*pe*) estimated in the variance-covariance matrix of individual ID by air temperature category:

$$(1)\ R = \frac{\sigma^2{}_{pe}}{\sigma^2{}_{pe}+\sigma^2{}_{year}+\sigma^2{}_{enclosure}+\sigma^2{}_{res}}.$$

The individual variance and covariance in head-neck temperatures may originate from both environmental and genetic factors. To partition the among-individual variance that is due to additive genetic effects we added a second 3x3 unstructured variance-covariance matrix of individual ID linked to the pedigree (*a*). With these variance components, we estimated the narrow sense heritability (h^2) of the head-neck temperature in each air temperature category as the proportion of phenotypic variance attributable to additive genetic variance:

$$(2)\ h^2 = \frac{\sigma^2{}_a}{\sigma^2{}_{pe} + \sigma^2{}_a + \sigma^2{}_{year} + \sigma^2{}_{camp}+\sigma^2{}_{res}}$$

We also estimated evolvability (I_A) (Houle, 1992):

$$(3)\ I_A = \frac{\sigma^2{}_a}{trait\ mean^2} * 100$$

One characteristic of evolvability is that it increases very fast as trait mean approaches zero. When we initially used the posterior of the trait mean to estimate evolvability, this caused near infinity estimates of evolvability for some of the samples in the posterior, causing biased estimates of the posterior mode and mean of evolvability. To avoid this, we used the posterior mode of trait mean in the denominator, such that only the uncertainty of additive genetic

462 variance is included in the reported estimate of evolvability. We also ran identical models with
463 head or neck surface temperature as the response variable. The outcome of these analyses is
464 available in the supplementary materials (Tables S7-S8).
465
466 Finally, we modelled head-neck temperature in a random regression model, using the model
467 structure described above. This approach was taken to investigate if the head-neck temperature
468 at the optimum temperature (the intercept) influences the rate of change in head-neck
469 temperatures as air temperatures increase or decrease (the slopes). To test if such a relationship
470 is driven by different families, we added a second 3x3 unstructured variance-covariance matrix
471 of individual ID linked to the pedigree and interacted with temperature change and direction.
472 The genetic variance and co-variance was then used to estimate the genetic correlation between
473 the slopes and intercepts (correlation = $\text{covariance}_{\text{trait1,trait2}}$ / sqrt($\text{var}_{\text{trait1}}$*$\text{var}_{\text{trait2}}$)) (Table S9).
474
475 *Current distribution of ostrich populations*
476 We obtained estimates of current distributions of *S. c. massaicus* (KR) and *S. c. australis* (ZB).
477 This was done by downloading region-based presence/absence data from Avibase
478 (https://avibase.bsc-eoc.org, September, 2020) and plotting these using the R-package
479 "rnaturalearth" v. 0.1.0. To identify climatic differences in their distributions we downloaded
480 19 bioclimatic variables (10min) from WorldClim v. 2 (Fick & Hijmans, 2017). We performed
481 a Principal Component Analyses (PCA) and inspected the loadings of the first four principal
482 components after varimax transformation (Table S10). Based on this inspection we described
483 each principal component by one or two bioclimatic variables to characterize climatic
484 differences.
485
486

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Author Contribution

Conceptualization: E.I.S, C.K.S., M.F.S.; Data curation: E.I.S., C.K.C., M.F.S., A.E., Z.B., S.C.; Formal analysis: M.F.S.; Funding acquisition: C.K.C, E.I.S., M.F.S, S.C.; Investigation: E.I.S., C.K.C., J.M., J.W., M.F.S., S.C., Z.B; Methodology: E.I.S., C.K.C., M.F.S.; Project administration: E.I.S., C.K.C., M.F.S.; Writing- original draft E.I.S, C.K.C., M.F.S.; Writing – reviewing and editing: A.E., CKC, E.I.S., J.M., J.W., M.F.S, S.C., Z.B.

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Data Accessibility

All data extracted from thermal images are available at https://osf.io/fu2wx/ and will be uploaded in an open digital repository such as Dryad Digital Repository (https://datadryad.org/stash) upon acceptance on this manuscript. The remaining data used support the findings of this study are available from the Western Cape Department of Agriculture in South Africa (WCDA). Restrictions apply to the use of some of these data, which are thus not publicly available. These data are however available from the WCDA upon reasonable request. Code for analyses is available on Github: www.github.com/abumadsen/thermal-images-ostrich/tree/main

Competing interests

Authors declare that they have no competing interests.

Supporting Information

Tables S1-S15

Figures S1-S8

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Figures and captions

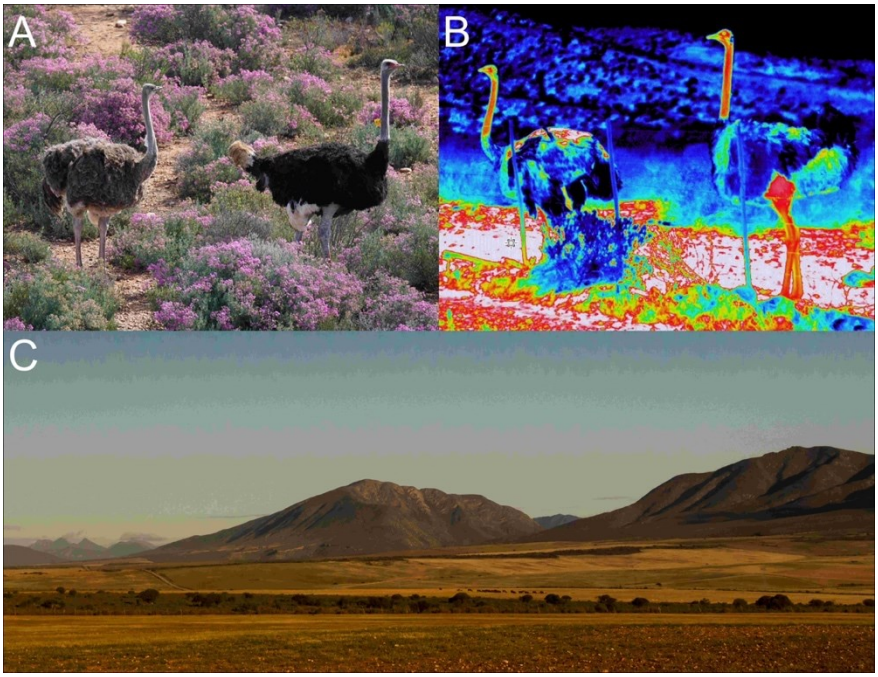

Fig. 1. The ostrich inhabits thermally challenging desert environments such as the Karoo in South Africa. A. The ostrich (*Struthio camelus*) is the world's largest living bird with a feathered body and featherless neck and head. *Left*: female. *Right:* male (photograph by C. K. Cornwallis in Karoo National Park, Western Cape Province of South Africa). **B.** Thermal image of a female (left) and male (right) ostrich in our study population at Oudtshoorn (photograph by E. I. Svensson). Note how the long neck is hot and emits excess heat (red, warm colour). **C.** The dry and tree-less semi-desert environment around the study site is characterized by extensive temperature fluctuations ranging from -5°C to 45°C, causing extreme thermal stress (Schou *et al.*, 2021) (photograph by E. I. Svensson).

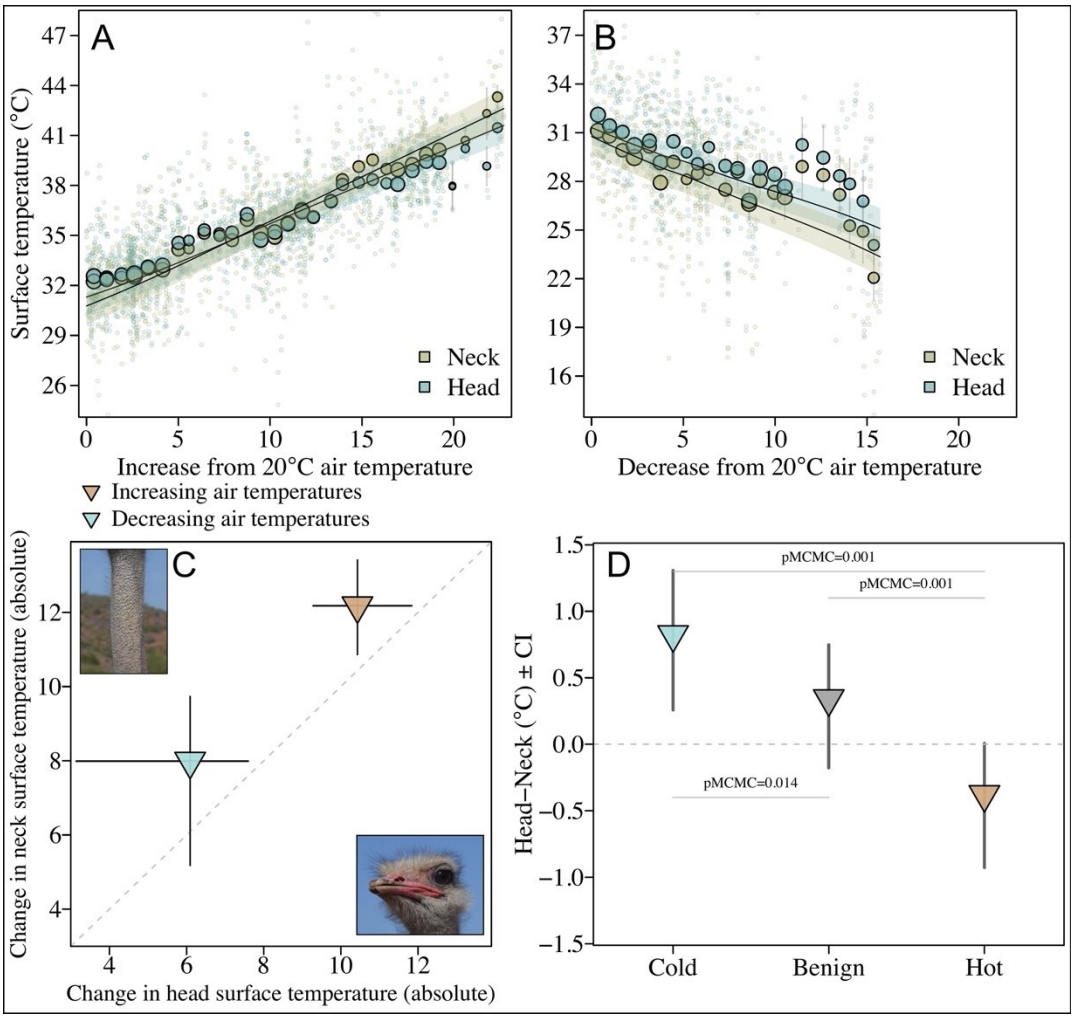

Fig. 2. Neck temperatures show how ostriches thermoregulate their head temperatures. A) When air temperatures increase above 20°C, the surface temperatures of the head increase slower than the neck (n_{images} = 1895, Tables S1-S2). **B)** Similarly, when air temperatures decrease below 20°C the reduction in surface temperatures are greatest in the neck (n_{images} = 849). Three extreme datapoints in A were removed for graphical purposes (see Fig. S4). The larger points are averages with standard errors binned according to temperature. Point size illustrates relative number of individuals: smallest point = 2 and largest point = 71. **(C)** The rate of surface temperature change was steeper for the neck compared to the head (Table S3). This difference in rates of temperature increase between the two body parts was consistent across both decreasing and increasing temperatures ($n_{individuals}$ = 423). **(D)** The difference in rates of temperature increase between the head and neck led to higher discrepancy between neck to head surface temperatures during hot conditions (air temperatures >= 30°C, n_{images} = 826) and cold (air temperatures <= 20°C, n_{images} = 849) compared to benign (air temperatures > 20°C & < 30°C, n_{images} = 1069) conditions (Table S4).

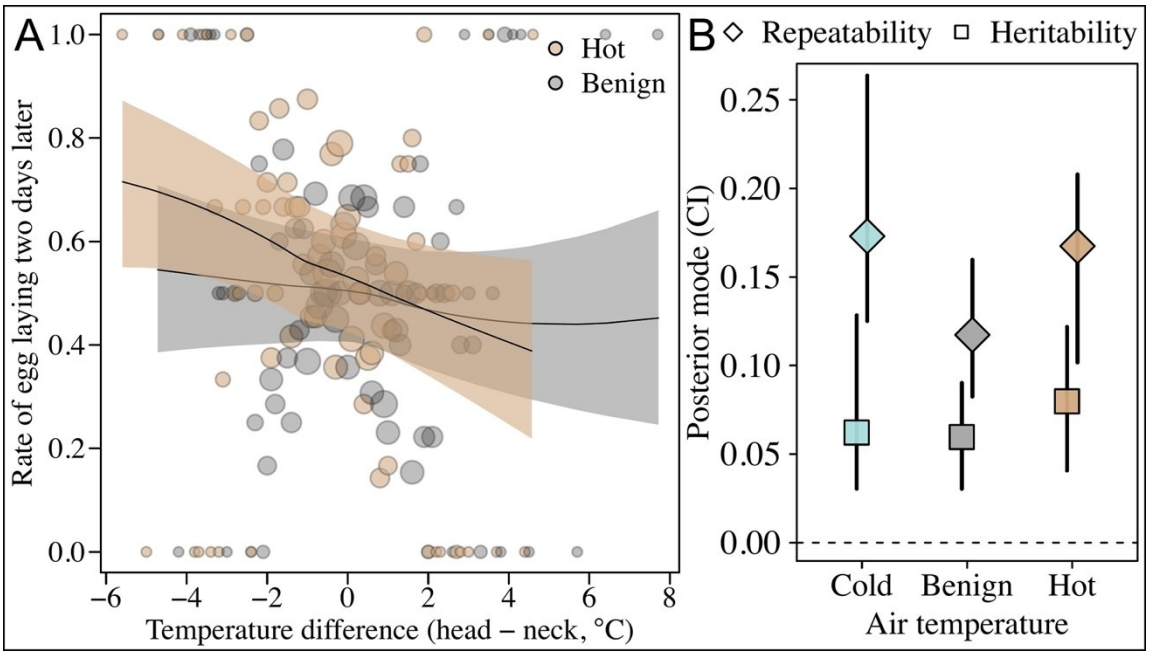

Fig. 3. The evolutionary potential for head temperature regulation. The capacity of individuals to regulate their head temperature was estimated as the difference between head and neck temperatures ('relative head temperature') in relation to air temperature. **A)** On hot days (daily maximum > 20°C, n_{days} = 35), when temperatures were highest (air temperatures >= 30°C, n_{images} = 471, $n_{females}$ = 228) an increased capacity to regulate head temperature (i.e. a lower head than neck temperature) was associated with a higher egg-laying rate two days later. There is no such association when examining thermoregulation at times when temperatures were benign of the hot days (air temperatures = 20-30°C, n_{images} = 615, $n_{females}$ = 220) (Table S5). Maximum temperatures below 20°C were recorded only on two days, preventing us from similar analyses on importance of thermoregulation during the cold times of cold days. **B)** Repeatability and heritability of relative head temperature were estimated at different air temperatures measured at the time when thermal images were taken (cold < 20°C, benign = 20-30°C and hot >= 30°C, Table S6). Note that the heritability of head temperatures under cold conditions could be estimated because there were cold periods (typically mornings) on days where temperatures exceeded 20°C. This contrasts to selection analyses where temperature per day had to be used because of the two day time lag between egg formation and egg laying.

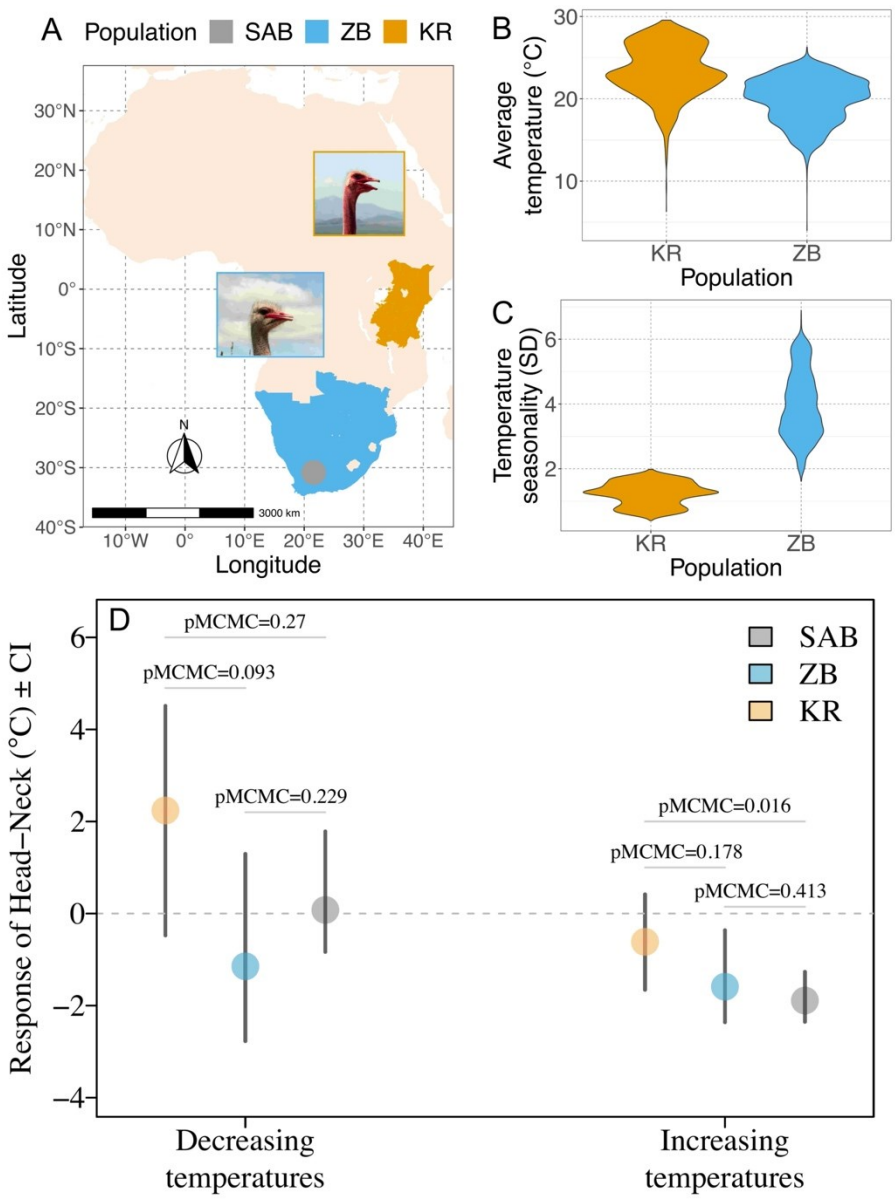

Fig. 4. Ostrich populations originating from more variable climates exhibit larger decreases in relative head temperatures when hot. **(A-C)** Kenyan Reds (KR) inhabit eastern Africa that is less seasonal and has lower temperature fluctuations compared to Southern Africa where Zimbabwean Blues (ZB) and South African Blacks (SAB) occur. Distribution ranges in A were estimated from regional presence/absence data from Avibase (https://avibase.bsc-eoc.org) and climatic data was obtained from WorldClim (Fick & Hijmans, 2017). Average temperature and seasonality were the main bioclimatic variables of PC1 (43%) and PC2 (29%) in a PCA of 19 bioclimatic variables (Fig. S5 & Table S10). **(D)** For both the SAB ($n_{individuals}$ = 264) and ZB ($n_{individuals}$ = 36) populations the relative head temperatures (head-neck) was reduced when temperatures increased, but this was not the case for KR ($n_{individuals}$ = 23) (Table S11, see also Figs. S6-7 which includes hybrid individuals).