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A thermal radiator underlies plasticity to extreme temperatures in the world's largest bird

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Abstract

Organisms inhabiting extreme thermal environments, such as desert birds, have evolved various adaptations to thermoregulate during hot days and cold nights. However, our knowledge of selection acting on thermoregulatory traits and their evolutionary potential is limited, particularly for large organisms experiencing extreme temperature fluctuations. Here we show, using thermal imaging that the featherless neck of the ostrich (*Struthio camelus*) acts as a 'thermal radiator', protecting the head from overheating during hot conditions and conserving heat during cool conditions. We found substantial individual variation in thermal plasticity of the neck to dissipate heat away from the head that was associated with increased egg-laying rates during high ambient temperatures. Combined with low, but significant, heritability estimates of individual thermal profiles, these findings suggest that the ostrich neck functions as an adaptive thermal radiator with evolutionary potential. There were also signatures of past selection, since ostriches originating from more volatile climatic regions and females that incubate during hot daytime conditions exhibited especially high thermal plasticity. Taken together our results indicate that morphological adaptations involved in ostrich thermoregulation, such as the neck, are experiencing ongoing selection and are crucial for successfully reproducing under fluctuating climatic conditions.

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Introduction

Organisms need to manage heat and cold stress to survive and reproduce in variable environments [1]. The universal challenge of coping with thermal stress[2–5] has promoted the evolution of morphological adaptations that regulate temperature[6–8]. For example, the ears of the elephant (*Loxodonta africana*)[9], the extended bill of the Toco Toucan (*Ramphastos toco*)[10,11] and the featherless head patches of the Zebra Finch (*Taeniopygia guttata*)[12] all function to reduce thermal stress. These structures effectively work as 'thermal radiators'[12,13], emitting excess heat to the surrounding environment during hot conditions and reducing heat loss during cold conditions[13]. However, whether such thermal radiators vary among individuals, are heritable and are targets of selection is unclear. Consequently, our understanding of the ecological and evolutionary processes shaping such thermal adaptations is limited[3].

The evolutionary capacity of populations to cope with short-term thermal stressors, such as heatwaves, requires genetic variance in thermoregulatory traits[2,5,14]. Yet empirical findings suggest that genetic variation in thermal and climatic adaptations is often low[15,16]. Body temperatures and thermal adaptations are also often strongly phylogenetically conserved[17,18] and seem to evolve slowly compared to other traits[19]. Williams[20] even questioned if endothermic body temperatures could evolve, suggesting there will be evolutionary stasis in thermal adaptations. The challenges associated with quantifying genetic variance in thermoregulatory traits have hindered progress in assessing whether thermal adaptations can evolve at a sufficient pace to keep up with climate change. This has, in turn, made it difficult to assess the possible elevated extinction risks caused by increasing temperatures[21–25].

An important factor influencing selection for thermoregulation is body size. Large bodies can cause higher thermal inertia, and a slower rate of body temperature change, compared to small

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bodies[1,26]. Thermal inertia can help maintain body temperatures during cold conditions, but can jeopardise survival and reproduction when extreme temperatures cause heat stress[2,4,5,14,23,27]. Large animals might be particularly vulnerable to rapid changes in climatic conditions as their rate of adaptation is predicted to be lower due to their longer generation times and lower population sizes[24,25]. Understanding how large-bodied animals cope with thermal stress in fluctuating and stressful thermal environments therefore requires particular attention.

Here we study thermoregulation and thermal plasticity in the largest surviving bird species in the world: the flightless ostrich (*Struthio camelus*) (Fig. 1). We have recently shown that there is heritable variation in thermal tolerance, and that both heat and cold stress reduce reproductive success in female ostriches[4,27]. However, the specific phenotypic adaptations that underlie these differences in reproductive thermal tolerance are unknown, as well as the possible role of thermal plasticity and genetic variation in plasticity. To link individual variation in thermoregulation with reproductive success, we combined a large-scale thermal imaging dataset (n_{images}=5531, $n_{individuals}$=794) with daily weather records and measures of individual reproductive success in the Klein Karoo, South Africa. This hot and dry area shows some of the highest fluctuations in temperature in Africa and on Earth, with ambient temperatures ranging from -5 to 45°C[4]. Additive genetic variance in thermoregulation was estimated using a nine-generation pedigree. With this data we examined: 1) if there are morphological features that act as thermal radiators that enable heat to be dissipated when hot and conserved when cold; 2) how variation in the efficiency of thermal radiators influence reproductive success, measured as egg-laying rates; 3) levels of genetic variation in thermal radiators within populations, and 4) if the efficiency of thermal radiators differs between populations from environments which have experienced different levels of past climatic fluctuations during their evolutionary history.

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Results

The neck as a thermal radiator

We found evidence for extensive thermal plasticity in both the head and neck, with surface temperatures (T) rising with air temperatures (T_{Head} (credible interval, CI) = 10.1 (9.1, 11.0), pMCMC = 0.001; slope T_{Neck} (CI) = 12.3 (11.2, 13.4), pMCMC = 0.001; Fig. 2A; Tables S1-S2) and declining as air temperatures decreased (T_{Head} (CI) = -6.1 (-7.8, -4.3), pMCMC = 0.001; T_{Neck} (CI) = -6.3 (-8.4, -4.4), pMCMC = 0.001; Fig. 2B; Tables S1-S2). The neck exhibited significantly more thermal plasticity (a steeper slope) compared to the head (Increasing air T_{Neck} vs Increasing air T_{Head} (CI) = 1.5 (1.3, 1.8), pMCMC = 0.001; Decreasing air T_{Neck} vs Decreasing air T_{Head} (CI) = -2.2 (-2.9, -1.4), pMCMC = 0.001, Fig. 2C; Table S3). The greater thermal plasticity of the neck suggests that it may function as a thermal radiator to get rid of excess heat to protect the head and brain. This was supported by neck and head differences being small at benign temperatures (air temperatures > 20°C & < 30°C), where the need for thermoregulation is reduced, but large at low (air temperatures <= 20°C) and high temperatures (air temperatures >= 30°C) (Fig. 2D).

The amount of thermal plasticity differed between males and females (Figs. 1, 2C). Males were less plastic than females, with a slower increase in neck surface temperature at high temperatures (Males vs $females_{Neck}$ (CI) = -1.5 (-2.0, -0.9), pMCMC = 0.001; Fig. 2C; Table S2; Table S1 for similar results for the head). This result was not due to body mass differences between males and females (Table S5; Fig. S1-S2, Table S4 for similar results for the head), making it unlikely that sex differences in thermal plasticity are explained by the larger males having more thermal inertia.

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The importance of the thermal radiator for reproduction

To test if the neck is functionally important as a thermal radiator, we quantified its efficiency in regulating head temperature, measured by the individual temperature difference between the neck and the head (neck-head temperature). We found that neck-head temperature was significantly related to egg-laying rates (Fig. 3A). This buffering effect of the neck temperature on head temperature was positively related to female egg-laying rates when hot, but not when benign ($Hot_{Neck\text{-}Head}$ (CI) = 0.16 (0.01, 0.31); pMCMC = 0.039; Fig. 3A; Table S7).

Neck-head temperature differences also increased significantly when females incubated during hot afternoons, but not cold mornings (Fig. 3B-C). Furthermore, the neck showed a stronger plastic response compared to the head when females were incubating during the hot afternoons (paired t-test: $t_{1,11}$= 2.3, P = 0.041). Monitoring the thermal profile of an incubating female further illustrated that thermal stress increased as incubation progressed (Fig. 3D-E). After 35 minutes, the female halted incubation, moved into shade, and opened her wings. This reduced her surface temperature to the level of non-incubating (standing) females (Fig. 3D). These results suggest that head temperature regulation, via heat dissipation from the neck, has benefits for female egg production as well as for their ability to successfully incubate these eggs (Fig. 3A; Table S7). The differences in thermal plasticity between the sexes may therefore reflect sex-specific adaptations to their different reproductive roles: whereas females incubate during the hot hours of the day, males primarily incubate during the night [28].

Quantitative genetics and the heritability of thermal radiator efficiency

When investigating the potential for the neck as a thermal radiator to evolve, we found that the repeatability of the neck-head temperature among individuals ranged from 0.09 to 0.15 at cold, benign and hot temperatures (Fig. 4, Table S8). Repeatability is expected to be relatively low as thermal profiles can be influenced by many factors, such as air temperature, microhabitat

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and activity prior to measurement (Figs. 1-2). Nevertheless, heritability estimates were significant, ranging from 0.04 to 0.06, in the neck-head temperature (Fig. 4, Table S8, see also Table S9-S10 for separate models of head and neck). These heritability estimates are low, suggestive of limited evolutionary potential, but it should be noted that they are capped by low repeatabilities. With our current data it is difficult to know the exact magnitude of these heritabilities, other than that they are significantly greater than zero.

Further support for a genetic basis of the neck as thermal radiator was evident from a significant negative genetic correlation (rg) between the intercept (at 20°C) and the slope of the thermal reaction norms to increasing temperatures (rg (CI) = -0.60 (-0.79, -0.27); Table S11). Individuals that under benign conditions (low intercept at 20°C) had relatively little heat loss through their neck compared to their head, emitted more heat through their necks as temperatures increased (steeper positive slopes). In contrast, individuals with a relatively high neck heat loss at benign temperatures (high intercepts at 20°C) exhibited little change with increasing temperatures (shallower slopes ~ 0. Fig. S4).

Thermal plasticity differs between populations from different climatic regions

We compared the efficiency of the neck at regulating head temperatures across three different ostrich populations, all kept at the study site in South Africa (Fig. 5). These populations, South African Blacks (SAB: *Struthio camelus*), Zimbabwean Blues (ZB: *S. c. australis*) and Kenyan Reds (KR: *S. c. massaicus*) differ in their geographic origin and evolved under different climatic regimes (Fig. 5A-B). East African regions, where the KR naturally occurs, is less seasonal, exhibiting lower fluctuations in temperature and precipitation, than the regions where ZB and SAB populations occur (Fig. 5B). Ostriches from these populations also differed in

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morphology, particularly size and shape (Fig. 5C-D, Tables S12-S13), with KR and ZB having longer necks than SAB (Fig. 5C-D).

We investigated whether variation in morphology and climatic conditions across populations corresponded to differences in the thermoregulatory properties of the neck. Morphological differences between the three populations did not explain variation in neck and head thermal plasticity. There were nonetheless pronounced differences between populations in plasticity that matched with the climatic stability of the environments they originated from. The KR, that occurs in more stable natural environments, displayed little plasticity, i.e. showed a constant neck-head temperature when temperatures increased from benign to hot (Fig. 5E). This was not the case for the SAB and ZB that originated from the more seasonal environments of southern Africa. They showed higher thermal plasticity, with the neck-head temperature increasing from benign to hot (Fig. 5E). This suggests that the population differences in adjustments in the neck to stabilize head temperatures have been been shaped by past and current climatic conditions in different regions across Africa.

Discussion

This study shows that the ostrich neck functions as a thermal radiator, dissipating heat away from the head under high temperatures and reducing heat loss under cooler conditions (Fig. 2). The efficiency of this thermal radiator appears to have a genetic basis and promotes reproduction under a greater range of temperatures (Fig. 3-4). Ostriches from populations that experience greater temperature changes were also more efficient at dissipating heat through their necks. These results suggest that the ostrich neck is an efficient thermal radiator with the potential for further adaptation that appears to have partly evolved in response to fluctuating climates (Figs. 4-5).

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Previous mechanistic studies have identified various morphological structures as potential thermal radiators, including bare skin patches[12] and external appendages, such as the ears of elephants[9], the enlarged claws of fiddler crabs[29] and the beaks of birds[10,11,30]. In addition, secondary sexual signaling characters involving melanin or structural iridescent colors can also have cascading effects on body temperature, and be either beneficial in cold environments or to decrease risk of overheating in hot environments[31–33]. While these previous mechanistic studies have provided compelling evidence for the thermoregulatory consequences of various morphological traits, they have not provided direct evidence for their fitness consequences, plasticity, and underlying genetic variation. In this study, the results of sex differences (Fig. 2), population differences (Fig. 5) and significant additive genetic variance in thermal plasticity (Fig. 4) jointly suggest that past and present climatic conditions have shaped the evolution of the ostrich neck as a thermal radiator.

The low estimates of heritability we found raises the question if further evolutionary responses to selection for thermal plasticity are possible. Previous studies of thermal adaptation across different organisms have also shown heritability to be low, suggesting that the genetic basis of thermal plasticity may just be difficult to quantify[15,16]. Alternatively, it may be that thermal plasticity is nonadaptive or maladaptive, a contention supported by recent empirical research[34–37]. For example, plasticity in both core body temperature, and the temperatures of external body parts can be maladaptive in both ectotherms, like insects[34] and reptiles[38], and endotherms, like birds[39]. Such maladaptive thermal plasticity may result from the costs of maintaining homeostasis and stable body temperatures under thermally stressful conditions, leading to selection for reduced plasticity and increased thermal canalization[34,38].

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We found that ostriches that experienced the lowest heat loss from the neck at benign temperatures also had the greatest plastic responses to increasing temperatures. This was mirrored by a negative genetic correlation between the slope and the intercept of the thermal reaction norm, revealing that there is additive genetic variation for thermoregulation via the neck, i.e. the presence of genetic variation in thermal plasticity. One interpretation of this result is that individuals operating at their maximum thermoregulatory capacity under benign temperatures have reduced scope for heat dissipation as temperatures increase. This negative genetic correlation is also consistent with a trade-off between the thermal slope and intercept, that is, the ability to buffer against heat stress might conflict with the ability to warm up during cold conditions. Such trade-offs have been discussed for a long time in the thermal adaptation literature, but there is little previous empirical evidence for their existence n endotherms[40–43]. Alternatively, the negative relationship between slope and intercept may simply reflect that the expression of additive genetic variance is higher at benign conditions compared to hot conditions.

Our findings raise general questions about the evolutionary origins of novel thermoregulatory traits beyond the ostrich neck. In particular, has the neck of the ostrich evolved to be long to cope with thermal stress, or is it an example of a so-called ”exaptation”[44], where a pre-existing trait became co-opted for a new purpose? The bills of birds[10,11,30] and sexual signaling traits such as coloration in various invertebrates[29,31,32,34] can also influence thermoregulation, and illustrate how traits with originally non-thermal functions can subsequently be modified and maintained by selection pressures that differ from those that drove original spread of the traits[45]. A classic example of a putative exaptation is the long neck of the giraffe (*Giraffa camelopardalis*), where the original explanation by Darwin was that the neck became extended because of natural selection for foraging efficiency, fueled by interspecific competition[46]. Later

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work questioned this evolutionary origin by revealing that the neck is also important in male-male competition over access to females[46]. In the case of the ostrich, its long neck probably serves multiple functions, including foraging, vigilance and amplification of male mating sounds[28,47] but currently it also functions in thermoregulation. There are signs that other such co-opted thermoregulatory traits are currently rapidly evolving, due to the increasing temperatures of recent and ongoing climate change, consistent with ”Allen’s Rule”[30]. Specifically, the relative length of appendices and bird beaks that function as thermal radiators have increased during recent decades[30]. Given these recent trends in other animals, it is possible that the neck length of the ostrich will increase in the future to improve the ability to get rid of excess heat.

Decades of avian research in the temperate zone has focused on food availability in altricial birds as a major limiting factor for reproduction[48,49]. However, for precocial birds inhabiting tropical and subtropical areas, like the ostrich, temperature stress during reproduction might pose a more severe challenge than food limitation [14]. Morphological traits can maintain non-lethal body temperatures[10–13,29,50], but linking such traits to reproductive success and quantifying their evolutionary potential has proven to be difficult. Recent research from several taxa suggest that climate-mediated local extinctions might already be common[21,22], with signs of collapse of some desert bird communities being documented[51]. Whether genetic variation in adaptations underlying thermal plasticity, such as the ostrich neck, are sufficient to enable rapid and large evolutionary responses to increasingly hot and fluctuating conditions remains an open question. While challenging, combining analyses of 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, especially for vulnerable species such as large, tropical endotherms like the ostrich.

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Methods

1. 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 [52], 11 for solitary males (0.03 ha) 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*), sometimes referred to as the 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).

2. 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 usually taken 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.

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We used the same procedures and same default settings of the software as in our previous work, assuming an emissivity of 1[31,34]. As a measure of ambient temperature, we used estimates of hourly temperatures from a weather station positioned 600 m from the field site. We fitted a cubic spline to the hourly temperature estimates of each day using the R-package *mgcv* v.1.8[53], from which we extracted the predicted ambient temperature at the time-points when thermal images were taken.

3. Datasets and analyses

3.1) Quantifying thermal plasticity of head and neck surface temperatures

From 2012 to 2017, we took 5586 pictures of 794 individuals from early morning till late afternoon, giving on average of seven pictures per individual. This dataset was designed with the aim of having a high number of individuals with repeated sampling within and across years, such that each individual was monitored in different thermal environments, but with little repeated sampling within days (8% of the pictures).

With this dataset we modelled the thermal plasticity in surface temperatures of the head and neck in response to air temperature using generalized linear mixed models (GLMMs). A previous investigation showed reproductive success of ostriches is highest at a daily maximum temperature of ~20°C [54]. We therefore defined 20°C as the optimum temperature for ostriches, and calculated absolute *temperature change* away from this optimum, defining the factor *direction* to denote whether the change in temperature was due to a decrease or increase from the optimum. To make the intercept of statistical models represent the most benign temperature, we set 20°C to 0 and calculated deviations above (increases) and below (decreases) this value. The variance of slopes (see below) depends on the scale of the environmental parameter, and we therefore standardized our data by dividing it by the maximum of the temperature range,

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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), sex (male or female) 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, as well as between sex, 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 individual. This was modelled as a 3x3 unstructured variance-covariance matrix.

GLMMs were run in R v.3.6.0[55] using the Bayesian framework implemented in the R-package MCMCglmm v.2.29[56]. 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 estimates was checked by

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running the model three times and inspecting the overlap of estimates in trace plots and the level of autocorrelation among posterior samples. Posterior mode and 95% credible intervals are reported for random effects.

3.2) Determining the impact of body mass on thermal plasticity

To test if differences in thermal plasticity were caused by differences in body mass, we ran a separate set of models including individual body mass as an additional fixed effect. We had records of body mass for 792 individuals and when multiple records were available for one individual we used the record closest to the time of the thermal image. Models for surface temperature of the head and neck were implemented using the model structure described in **Methods 3.1**, but with body mass (scaled and centered to a mean of zero and unit variance) interacted with all the previously described fixed effects.

3.3) Comparing thermal plasticity of the neck with the head

We compared the level of thermal plasticity in surface temperatures between the neck and head. This was done by running a model that included body part (neck or head) as a fixed effect. The models were implemented using the model structure described in **Methods 3.1**, but with body part interacted with all the previously described fixed effects. We also added image as an additional random effect because head and neck surface temperatures were derived as pairs from the same image.

3.4) Investigating the neck as a thermal window

Results from previous analyses (**methods 3.1**) indicated that the neck functions as a thermal window emitting excess heat during hot periods and conserved heat during cold periods. To investigate this further, we defined the efficiency of the thermal window as the difference

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between neck and head surface temperatures. Positive values therefore indicated that the neck was warmer than the head and negative values when the head was warmer than the neck. To test if the neck-head 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} = 1683), Benign (air temperatures > 20°C & < 30°C, n_{images} = 2152) and Hot (air temperatures >= 30°C, n_{images} = 1696). The grouping of air temperature into these three categories was based on the thermal neutral zone of the emu [57], 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 ambient 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 neck-head temperature (Gaussian) to air temperature category by following the same general approach in **Methods 3.1**. 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.

3.5) Effect of neck and head surface temperatures on reproductive success

To test if the efficiency of the neck as a thermal window influences reproductive success we analyzed 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 exceeds 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[54]. We therefore monitored whether any eggs were laid two to four days after females

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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 neck 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 heat emitted via the neck and laying rates. To overcome this, we grouped the data into photographs taken during hot and benign times of the days (**methods 3.4**). 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 in **Methods 3.1**, 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 neck-head temperature difference (scaled and centered to a mean of zero and unit variance), ambient air temperature category (hot or benign), and population (KR, SAB, ZB or Hybrid), as well as the interaction between neck-head temperature and temperature category, c) as females of two years of age lay fewer eggs than older females [54], 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[54]. We also removed females that laid fewer than ten eggs per year 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.

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3.6 Thermoregulation during incubation

In 2017, we obtained thermal images of six females incubating in the morning (<12.00 am) when air temperatures are typically lower, and of 11 individuals incubating in the afternoon (>12.00 am) when the air temperatures were substantially higher. To test if their surface temperatures increased during incubation during each of these two time periods, we used paired t-tests to compare the surface temperatures of each of these individuals with the mean surface temperatures of two standing individuals, one photographed shortly before and one photographed shortly after the sitting individual. Tests were done separately for the surface temperatures of the head and neck. One of the incubating individuals was photographed twice in sequence and we therefore used the mean surface temperatures of the two observations. In three cases, two incubating individuals were paired with the same standing individuals and instead of using the mean surface temperatures of two standing individuals, each incubating individual was paired to the closest standing individual. We also tested if the temperature differences between incubating and standing individuals were more pronounced in either the head or neck than in the other. For this we used paired *t*-tests to compare temperature differences of the head with temperature differences of the neck. Finally, we closely monitored the change in surface temperature for one female during prolonged incubation sessions when she was standing after incubating. Eighteen pictures of this female were taken over 82 minutes, each followed by at least one picture of another standing individual to create a background reference image for comparison. Due to the nature of this dataset, we did not perform any statistical analyses, but present the data as an observation of the dynamics of thermoregulation during incubation.

3.7) Quantifying the evolutionary potential of thermal plasticity

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Using the model of the change in neck-head temperature in each air temperature category (**methods 3.4**), we estimated the repeatability (R) of the neck-head 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 neck-head 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 neck-head 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) [58]:

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

One characteristic of evolvability is that it increases vary 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 variance is included in the reported estimate of evolvability.We also ran identical models with head or neck surface temperature as the response variable. The outcome of these analyses is available in the supplementary materials (**Tables S9-S10**).

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Finally, we modelled neck-head temperature in a random regression model, using the model structure described in **methods 3.1**. This approach was taken to investigate if the neck-head temperature at the optimum temperature (the intercept) influences the rate of change in neck-head temperatures as air temperatures increase or decrease (the slopes). To test if such a relationship is driven by different families, we added a second 3x3 unstructured variance-covariance matrix of individual ID linked to the pedigree and interacted with temperature change and direction. The genetic variance and co-variance was then used to estimate the genetic correlation between the slopes and intercepts (correlation = $\text{covariance}_{\text{trait1,trait2}}$ / $\text{sqrt}(\text{var}_{\text{trait1}}*\text{var}_{\text{trait2}})$) (**Table S11**).

3.8) Current distribution and morphology of ostrich populations

We obtained estimates of current distributions of *S. c. massaicus* (KR) and *S. c. australis* (ZB). This was done by downloading region-based presence/absence data from Avibase (https://avibase.bsc-eoc.org, September, 2020) and plotting these using the R-package "rnaturalearth" v. 0.1.0. To identify climatic differences in their distributions we downloaded 19 bioclimatic variables (10min) from WorldClim v. 2[59]. We performed a Principal Component Analyses (PCA) and inspected the loadings of the first four principal components after varimax transformation (**Table S14**). Based on this inspection we described each principal component by one or two bioclimatic variables to characterize climatic differences.

We estimated population differences in neck morphology between SAB ($n_{\text{individuals}} = 23$), ZB ($n_{\text{individuals}} = 16$) and KR ($n_{\text{individuals}} = 21$) by measuring height and neck length. Neck length was measured from from the cranium to the point where the neck enters the body. Height was measured as the distance from the ground to the cranium. We modelled neck length as a Gaussian response variable in a linear model including population (SAB, KR or ZB), sex (male

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or female) and height (centred and scaled) as fixed effects. We also included the interaction between height and sex and between height and population. The model was run in MCMCglmm v.2.2 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. Model diagnostics were performed as described in **methods 3.1**. To examine population differences in the relative neck length we ran an identical model with neck length/height as Gaussian response variable. In this model height was not included as a fixed effect.

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Competing interests

Authors declare that they have no competing interests.

Data and materials availability

All data extracted from thermal images are available at https://osf.io/fu2wx/. 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.

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Code availability

Code for analyses is available on Github: www.github.com/abumadsen/thermal-images-ostrich/tree/main

Supporting Information

Tables S1-S16

Figures S1-S5

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Figures and captions

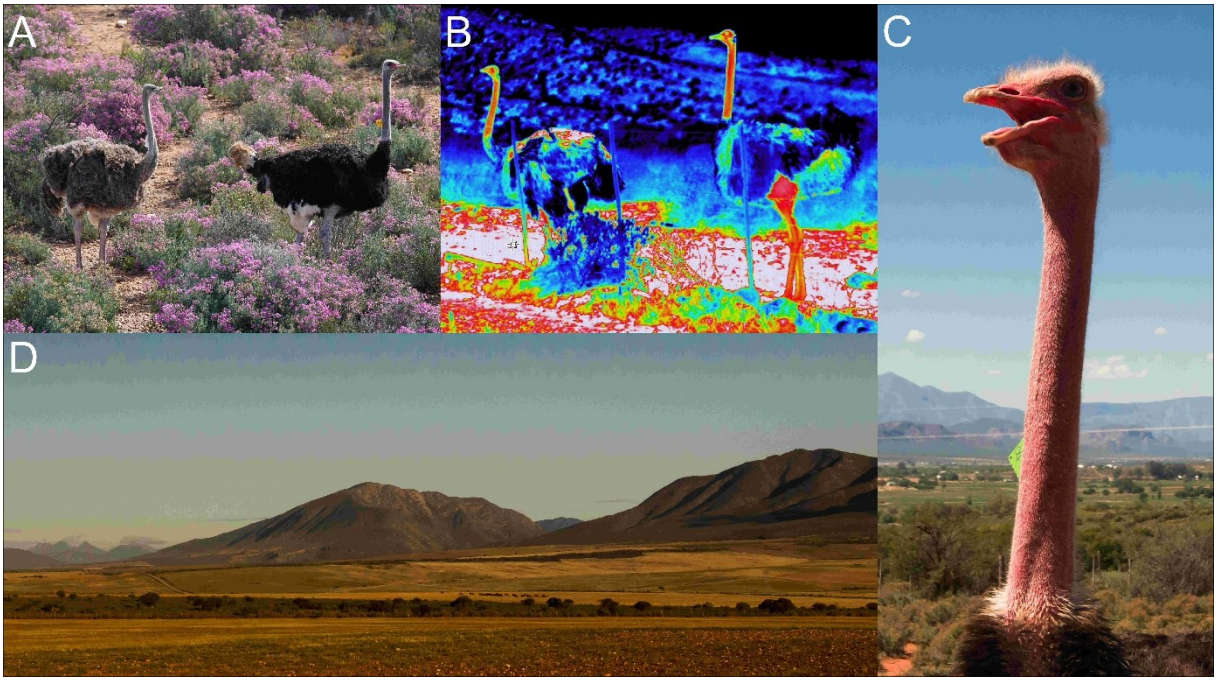

Fig. 1. Thermoregulation in the world's largest living bird in the Karoo desert environment in South Africa. **A.** The ostrich (*Struthio camelus*) is the world's largest living bird with a feathered body and featherless neck and head. *Left*: female. *Right:* male (photograph by C. K. Cornwallis in 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**. Close-up photo of a male ostrich of East African origin ("Kenyan Red"; KR) from our individually-marked study population in Oudtshoorn during the heat of the day (photograph by E. I. Svensson). The open bill is due to panting behaviour that gets rid of excess heat when ambient temperatures are high. **D.** 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 [4] (photograph by E. I. Svensson).

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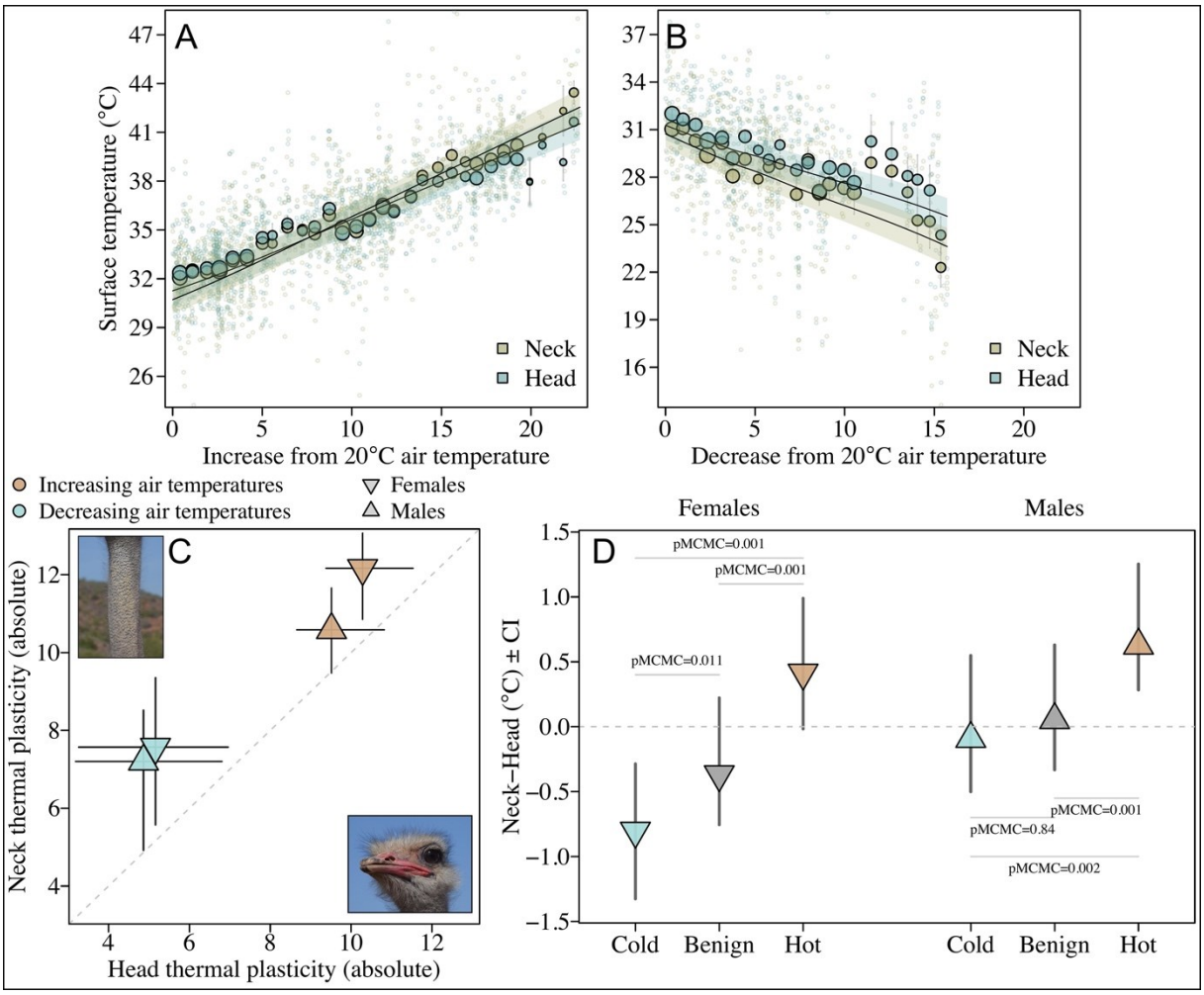

Fig. 2. The ostrich neck acts as a thermal radiator regulating head temperature. (A-B) The surface temperatures of the head and neck were sensitive to increases (n_{images} = 3848) and decreases (n_{images} = 1683) in air temperatures (Tables S1-S2). Only females are shown with five extreme datapoints in A removed for graphical purposes (see Fig. S3 which shows very similar patterns for males). **(C)** The rate of surface temperature change was steeper for the neck compared to the head (Table S3). This difference in thermal plasticity between the two body parts was consistent across both deceasing and increasing temperatures, and in both males ($n_{individuals}$ = 371) and females ($n_{individuals}$ = 423). **(D)** The difference in thermal plasticity between the head and neck led to higher discrepancy between neck to head surface temperatures during hot ambient conditions (air temperatures >= 30°C, n_{images} = 1696) and cold (air temperatures <= 20°C, n_{images} = 1683) but not under benign (air temperatures > 20°C & < 30°C, n_{images} = 2152) ambient conditions (Table S6).

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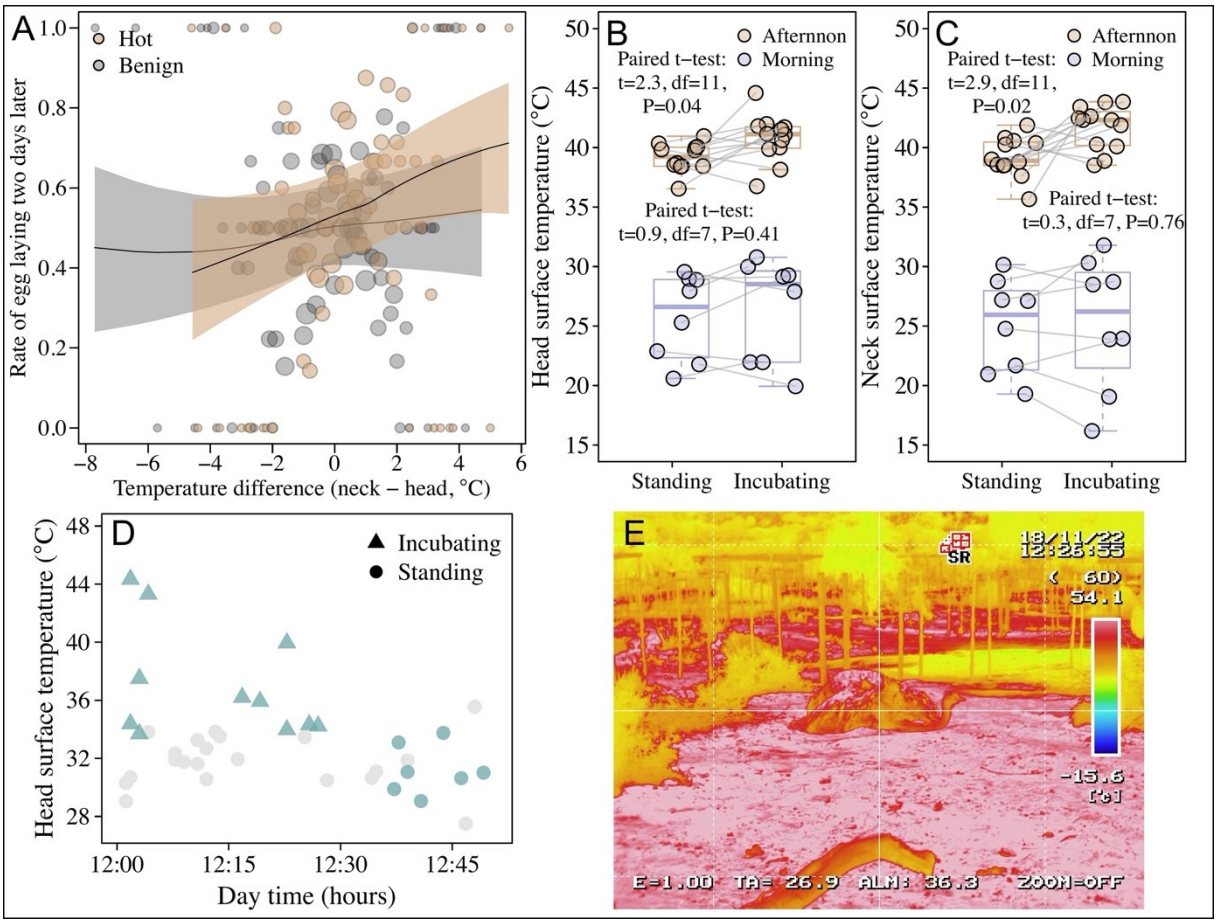

Fig 3: The reproductive benefits of the neck as a thermal radiator. (A) A high temperature difference between neck and head is associated with a higher egg-laying rate two days later during hot temperatures (air temperatures >= 30°C, n_{images} = 471, $n_{females}$ = 228), but not at benign temperatures (air temperatures = 20-30°C, n_{images} = 615, $n_{females}$ = 220) (Tables S7). **(B-C)** During hot afternoons incubating females have significantly higher head and neck temperatures than standing females, revealing high thermal costs of incubation that were not evident during colder mornings. **(D)** A female ostrich was monitored during incubation under natural conditions. Her head temperatures were elevated compared to other standing individuals ($n_{individuals}$ = 21). When she halted incubation and moved to the shade of a tree, her head temperature quickly dropped, becoming similar to those of other standing individuals. **(E)** Thermal image of the incubating female in the sun (warmer colours indicate higher temperatures). These findings are consistent with the dissipation of heat through the neck having reproductive benefits for females.

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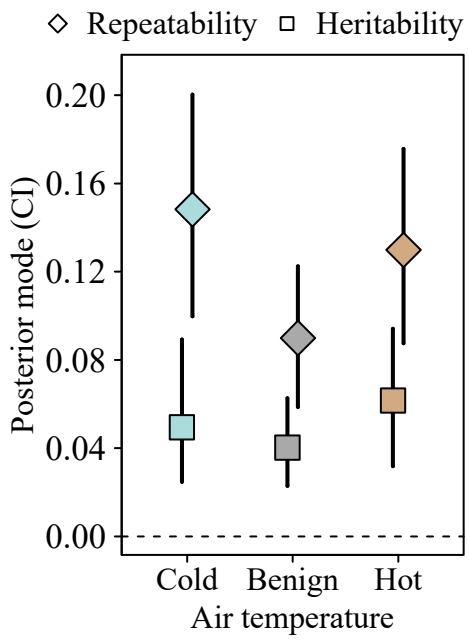

Fig. 4. Evolutionary potential of thermal radiator efficiency. The efficiency of the neck in regulating head temperatures was measured as the difference between neck and head temperatures for each individual. Repeatability and heritability were estimated at different air temperatures (cold < 20°C, benign = 20-30°C and hot >= 30°C) and were generally low, but significantly different from zero (Table S8). Note that the low repeatabilities may result in an underestimation of our heritability measures.

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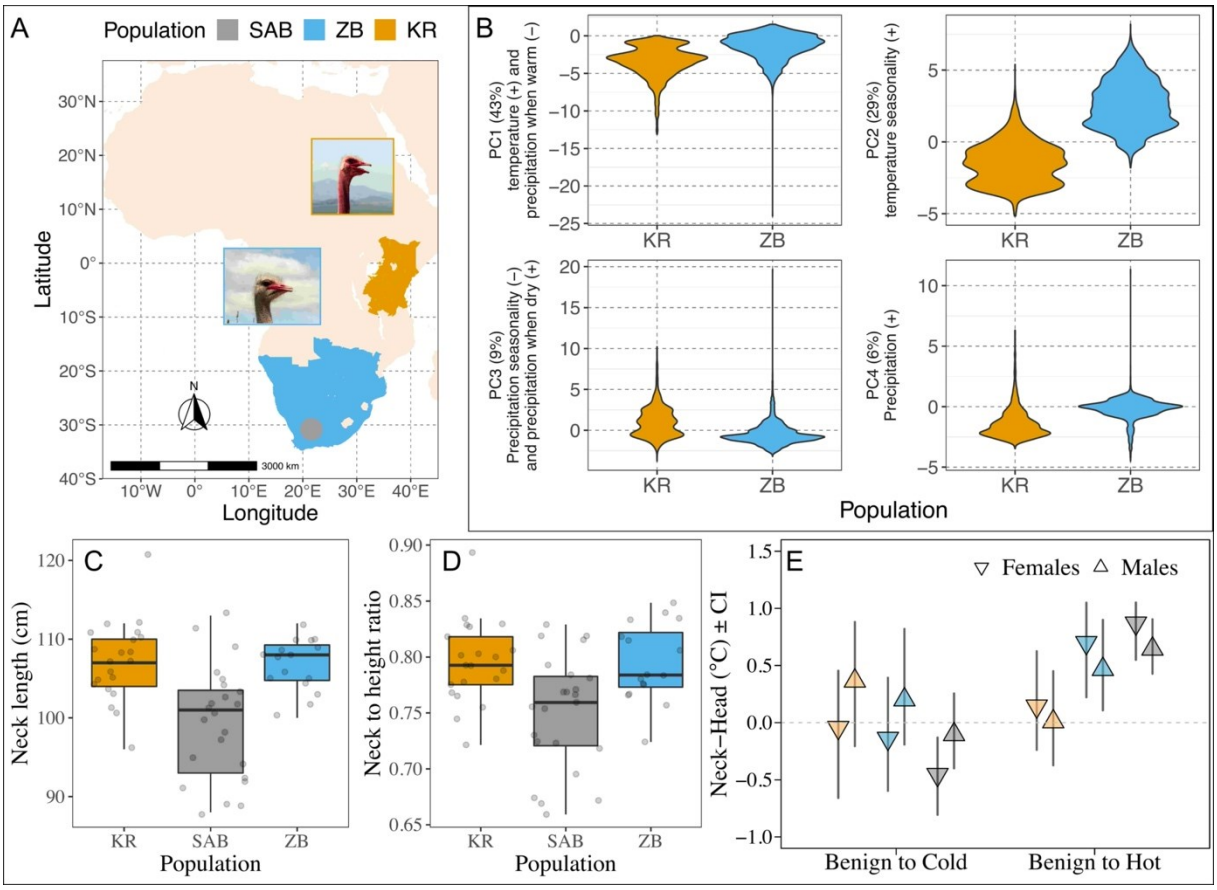

Fig. 5. Ostrich populations originating from more variable climatic regions exhibit greater thermal plasticity. **(A-B)** Kenyan Reds (KR) inhabit eastern Africa that is less seasonal and has lower temperature fluctuations compared to Southern Africa, where Zimbabwean Blues (ZB) and South African Blacks (SAB) occur. Distribution ranges were estimated from regional presence/absence data from Avibase (https://avibase.bsc-eoc.org) and climatic data was obtained from WorldClim[59] (Table S14). **(C-D)** These three populations also differ significantly in morphology: SAB ($n_{individuals}$ = 23) have shorter necks and lower neck to height ratio than KR ($n_{individuals}$ = 21) and ZB ($n_{individuals}$ = 16) (Tables S12-S13). **(E)** For both the SAB ($n_{individuals}$ = 556) and ZB ($n_{individuals}$ = 71) populations the neck-head temperature difference increased from benign to hot temperatures, but this was not the case for KR ($n_{individuals}$ = 55) (Tables S6).