The benefits of long-term international partnerships for ostrich research

by Dr Anel Engelbrecht and Prof. Charlie Cornwallis

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The ostrich research effort of the Western Cape Department of Agriculture (WCDoA) has benefitted tremendously from collaboration with numerous institutions and individuals. One such partnership started more than a decade ago after Prof. Schalk Cloete (Elsenburg) and Prof. Charlie Cornwallis (United Kingdom) were introduced to each other by Prof. Michael Cherry from Stellenbosch University.

Charlie recalls: In 2007 I was working at Oxford University where I shared an office for six months with a visitor from South Africa, Michael Cherry (Zoology Dept, SU). This fortuitous event would tie me to South Africa for the next 17 years, providing insights into one of the strangest farmed species, the ostrich.

At the time, I was working on the reproductive biology of chickens, but I was interested in studying how genetic differences between individuals influence the reproductive success of newly domesticated species. Michael had a PhD student, Maud Bonato, who was collecting data for her study on immunocompetence and mate choice at the Oudtshoorn Research Farm. We got a small grant from the Oppenheimer fund in Oxford that allowed me to visit South Africa and Michael introduced me to Schalk Cloete (a specialist scientist at the WCDoA at the time).

Irek Malecki (Australia), Schalk Cloete (WCDoA) and Charlie Cornwallis (United Kingdom) on their way to the Oudtshoorn Research Farm in 2007.

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Situated in the Klein Karoo, the **Oudtshoorn Research Farm** lies approximately 8 km outside Oudtshoorn in the Eden district. Covering 843 hectares, it is the world's first dedicated ostrich research facility, established in 1964. The farm plays a vital role in supporting the region's primary agricultural enterprise – ostrich farming – and continues to serve as a key resource for both local and international ostrich research.

Schalk was extremely welcoming and broad-minded. I grew up on a farm, but my research up to that point was in Evolutionary Biology, rather than agriculture. Luckily, Schalk saw the value of understanding reproductive behaviour to increase production and we travelled to Oudtshoorn where I got to meet the Oudtshoorn Research Farm team. After some planning and numerous discussions, we first set up an experiment to examine how crossing different breeds (South African Blacks and Zimbabwean Blues) influenced mating, fertilisation and hatching success.

We found that females and males prefer to mate with partners of the same breed, reducing the mating success of crosses. In addition to this result, there were two other interesting findings. First, Blue females reduced their egg-laying rates when paired with Black males, whereas Black females maintained high laying rates irrespective of who they mated with. Secondly, Blue males had higher fertilisation success than Black males, regardless of who they mated with. Together, these results showed that to improve crossbreeding outcomes, it is important to remove possibilities of same-breed matings (e.g., only keep males and females of opposite breeds on farms). Blue males crossed with Black females produced the most chicks because of high fertilisation success and egg laying rates."

This was only the start of a long and fruitful partnership. The complementary expertise of the researchers in South Africa and Europe, and the combined resources, enabled the team to make progress on several fronts.

Over the years they examined the effects of:

1. social breeding group composition on production;
2. the development of the gut microbiome on chick growth and survival;
3. the capacity of breeders to cope with temperature extremes and its effect on reproduction; and
4. different artificial insemination techniques to develop an assisted reproductive technology production system.

Mads Schou and his PhD student, Lea Huber, with an ostrich chick implanted with a data logger to monitor temperature.

Ostrich chick hatching.

What is the optimal breeding group composition for highest production?

In South Africa, ostrich breeders are typically kept in large flocks or in small groups such as trios. However, it is not known what group size maximises chick production and whether the genetic composition of groups is important.

In 2010 Charlie moved to Lund University, and with an injection of funds from the Swedish Research Council, Knut and Alice Wallenberg Foundation along with support from the Western Cape Department of Agriculture, we could test how the composition of social groups influenced the number of eggs and chicks that breeders produced.

An experiment was set up together with PhD student Julian Melgar and Maud Bonato, where groups were established with one to three males and one to six females, respectively, with individuals varying in genetic relatedness to each other. Over seven breeding seasons the rates of mating and aggression, the number of eggs laid, and the genetic parentage of chicks were determined across a total of 110 breeding groups.

It was found that:

- the optimal group size for the production of chicks was one male and four females;
- groups with one male and six females do not suffer fertility problems, as is commonly thought;
- only one male typically reproduces when males are related, especially when competing against unrelated males;
- relatedness amongst females has little

Ostrich chicks, Oudtshoorn.

effect on reproductive success; and
- males and females generally avoid inbreeding where possible.

Why do chicks die?

In 2014, a project was started to investigate why ostrich chicks have such variable growth and often suffer high mortality. Evidence from other animals was accumulating that the gut microbiome is very important for juvenile health, and autopsies on ostrich chicks often showed that bacterial enteritis was a problem. However, it was unknown what bacteria were involved and if this was just symptomatic of another underlying issue.

The development of the gut microbiome was first characterised, showing that both harmful (e.g. *Enterobacteriaceae*, *Peptostreptococcaceae*, *Porphyromonadaceae*, *Clostridia*) and beneficial bacteria (*Lachnospiraceae*, *Ruminococcaceae*, *Erysipelotrichaceae*, *Turicibacter*, *Roseburia*) are present soon after hatching. The effects of these bacteria on chick health,

however, are typically not observed until four to six weeks of age, when dysbiosis was typically associated with chick mortalities. It was also found (by experimentally manipulating access to breeders' faeces) that chicks can acquire beneficial bacteria and develop a mature gut sooner by frequently ingesting adult faeces. Often chicks are raised separately from breeders, however, without access to adult faeces, which may contribute to the malformation of the gut microbiome, adversely affecting chick

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How will climate change influence ostrich production?

One of the striking things about ostriches is their ability to cope with extreme temperatures. This has led to many conversations over the years about how they do this, and whether they will be the only ones left as climate change continues! A colleague in Lund, Erik Svensson, was particularly interested in examining how much genetic variation there was in temperature tolerance using a thermal camera he had just bought. This is important as genetic variation in thermal tolerance gives an indication of how populations might cope with more extreme temperatures, and with it, how ostrich farming will be affected by climate change.

After about 10 years of taking thousands of thermal images, Mads Schou joined the group as a post-doc. Mads had been working on thermal tolerance in fruit flies, which is all well and good but obviously can't compete with the charismatic ostrich. He started by analysing how temperature fluctuations influence the reproductive success of males (sperm traits) and females (egg-laying rates) and whether this was linked to their ability to keep cool, as measured by thermal imaging. The findings were extremely surprising.

Charlie Cornwallis with one of his PhD students, Elin Videvall, who investigated the ostrich gut microbiome.

Even though ostriches are famous for their ability to survive in deserts, the fertilisation capacity of males and egg-laying rates of females were significantly reduced by temperatures above 30°C and below 15°C, even when birds had *ad libitum* food and water.

Common ostrich (*Struthio camelus*), Kgalagadi Transfrontier Park.
Photo © Barry Lovegrove as published in *The Living Deserts of Southern Africa*.

Oudtshoorn Research Farm.
Photo © Quintus Strauss

The thermal images indicated that individuals that are better at keeping head temperatures within a certain range (which they potentially do by dissipating/conserving heat through their long necks) have higher reproduction. Further follow-ups showed that individuals with genes for tolerance to high temperatures were more susceptible to cold temperatures, and vice versa. Consequently, it will be difficult to breed ostriches that are able to cope with both high and low temperatures, which may be a problem in the future as temperatures become more volatile.

Mads was able to secure funding from the European Union to continue this work, which landed him a lectureship at Aarhus University in Denmark. He now coordinates the research on thermal tolerance, which is examining the effects of temperature fluctuations across the entire ostrich lifecycle from egg to adult.

These projects have all run in parallel with the development of assisted reproductive technology, initiated by Schalk, Irek Malecki (from Australia) and Maud Bonato (from France), and continued by Dr Pfunzo Tonny Muvhali and Nelleke Lotz. This has provided key insights into the processes underlying variation in fertility of males and females and has demonstrated that artificial insemination of ostriches is possible.

The whole is greater than the sum of the parts, and the partnership has enabled research that would otherwise not have been possible. The work has been greatly helped through the support of the WCDoA management, everyone at the Oudtshoorn Research Farm, as well as Dr Adriaan Olivier (industry veterinarian in Oudtshoorn).

Technician Bernard Snyman (left) with some of the support personnel on the farm.

Numerous students that worked on these projects have since graduated, while investigations into acclimation, optimal breeding group composition and genotyping continue. The research directly benefits the ostrich industry by providing insights into optimal breeding flock ratios, reproduction and health. Some of the publications stemming from the collaborations are listed in the references.

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References

Bonato, M., Rybnik, P.K., Malecki, I.A., Cornwallis, C.K. and Cloete, S.W.P., 2010. Between male variation in semen characteristics and preliminary results on the dilution of semen in the ostrich. *South African Journal of Animal Science*, 40(5), pp.438-441.

Bonato, M., Rybnik, P.K., Malecki, I.A., Cornwallis, C.K. and Cloete, S.W., 2011. Twice daily collection yields greater semen output and does not affect male libido in the ostrich. *Animal Reproduction Science*, 123(3-4), pp.258-264.

Bonato, M., Cornwallis, C.K., Malecki, I.A., Rybnik-Trzaskowska, P.K. and Cloete, S.W., 2012. The effect of temperature and pH on the motility and viability of ostrich sperm. *Animal Reproduction Science*, 133(1-2), pp.123-128.

Bonato, M., Malecki, I.A., Rybnik-Trzaskowska, P.K., Cornwallis, C.K. and Cloete, S.W., 2014. Predicting ejaculate quality and libido in male ostriches: effect of season and age. *Animal Reproduction Science*, 151(1-2), pp.49-55.

Videvall, E., Strandh, M., Engelbrecht, A., Cloete, S. & Cornwallis, C.K., 2017. Direct PCR Offers a Fast and Reliable Alternative to Conventional DNA Isolation Methods for Gut Microbiomes. *mSystems*, November/December 2017, Volume 2, Issue 6, e00132-17, 13pp, http://msystems.asm.org/content/2/6/e00132-17

Videvall, E., Strandh, M., Engelbrecht, A., Cloete, S. & Cornwallis, C.K., 2018. Measuring the gut microbiome in birds: comparison of faecal and cloacal sampling. *Molecular Ecology Resources* 18(3), 424-434.

Cloete, S.W.P., Engelbrecht, A., Gilmour, A.R., Schou, M.F., Brand, Z. & Cornwallis, C.K., 2019. The genetic analysis of adult bird performance together with slaughter traits in ostriches. Proc. Assoc. Advmt. *Journal of Animal Breeding and Genetics*. 23: 394-397.

Videvall, E., Song, S.J., Bensch, H.M., Strandh, M., Engelbrecht, A., Serfontein, N., Hellgren, O., Olivier, A., Cloete, S., Knight, R. & Cornwallis, C.K., 2019. Major shifts in gut microbiota during development and its relationship to growth in ostriches. *Molecular Ecology* 28 (10): 2653-2667. https://doi.org/10.1111/mec.15087

Videvall, E., Song, S.J., Bensch, H.M., Strandh, M., Engelbrecht, A., Serfontein, N., Hellgren, O., Olivier, A., Cloete, S., Knight, R. & Cornwallis, C.K., 2020. Early-life gut dysbiosis linked to juvenile mortality in ostriches. *Microbiome 8*, 147 (2020). https://doi.org/10.1186/s40168-020-00925-7

Schou, M., Bonato, M., Engelbrecht, A., Brand, Z., Svensson, E., Melgar, J., Muvhali, P.T., Cloete, S.W.P., Cornwallis, C.K., 2021. Extreme temperatures compromise male and female fertility in a large desert bird. *Nature Communications 12: 666*. https://doi.org/10.1038/s41467-021-20937-7

Schou, M., Engelbrecht, A., Brand, Z., Svensson, E.I., Cloete, S.W.P., Cornwallis, C.K., 2022. Evolutionary trade-offs between heat and cold tolerance limit responses to fluctuating climates. *Science Advances 8*, eabn9580.

Melgar, J., Schou, M.F., Bonato, M., Brand, Z., Engelbrecht, A., Cloete, S.W.P. & Cornwallis, C.K., 2022. Experimental evidence that group size generates divergent benefits of cooperative breeding for male and female ostriches. *Elife*, 11: e77170. DOI: https://doi.org/10.7554/eLife.77170.

Videvall, E., Bensch, H.M., Engelbrecht, A., Cloete, S. & Cornwallis, C.K., 2023. Coprophagy rapidly matures juvenile gut microbiota in a precocial bird. *Evolution Letters*, 7(4), pp. 240-251, https://doi.org/10.1093/evlett/qrad021

Yazdi, H.P., Olito, C., Kawakami, T., Unneberg, P., Schou, M.F., Cloete, S.W., Hansson, B. and Cornwallis, C.K., 2023. The evolutionary maintenance of ancient recombining sex chromosomes in the ostrich. *PLoS Genetics*, 19(6), p.e1010801.

Svensson, E.I., Schou, M.F., Melgar, J., Waller, J., Engelbrecht, A., Brand, Z., Cloete, S. & Cornwallis, C.K., 2024. Heritable variation in thermal profiles is associated with reproductive success in the world's largest bird, *Evolution Letters*, Volume 8, Issue 2, April 2024, Pages 200-211, https://doi.org/10.1093/evlett/qrad049

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