Cooperation and conflict over the struggle to reproduce in harsh environments

An experimental field study on ostriches

Melgar, Julian

2021

Cooperation and conflict over the struggle to reproduce in harsh environments

An experimental field study on ostriches

JULIAN MELGAR | FACULTY OF SCIENCE | LUND UNIVERSITY

List of papers

I. Melgar J., Schou M.F., Bonato M., Brand Z., Engelbrecht A., Cloete S.W.P. & Cornwallis C.K. Cooperation and competition over reproduction shape the complexity of cooperative breeding groups. Submitted.

II. Melgar J., Schou M.F., Bonato M., Brand Z., Engelbrecht A., Cloete S.W.P., Muvhali P.T., Hansson B. & Cornwallis C.K. Cheating triggers a tragedy of the commons, group size attenuates it. Manuscript.

III. Schou M.F, Bonato M., Engelbrecht A., Brand Z., Svensson E.I., 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.

IV. Melgar J., Schou M.F., Bonato M., Brand Z., Engelbrecht A., Cloete S.W.P., Muvhali P.T., Hansson B. & Cornwallis C.K. Genetic adaptations to cope with heat predict the emergence of cooperative behaviour. Manuscript.

Printed by Media-Tryck, Lund 2021 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

Lund University,
Faculty of Science
Department of Biology

ISBN 978-91-7895-775-0

Cooperation and conflict over the struggle to reproduce in harsh environments

Cooperation and conflict over the struggle to reproduce in harsh environments

An experimental field study on ostriches

Julian Melgar

LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Science, Lund University, Sweden.
To be defended at in the Blue Hall, Ecology Building, Sölvegatan 37, Lund, Sweden, on Friday 19th of March. 2021 at 09:00 am.

Faculty opponent
Prof. Trine Bilde

Organization	Document name
LUND UNIVERSITY Department of Biology Lund, SWEDEN	DOCTORAL DISSERTATION
Author: Julian Melgar	**Date of issue** 2020-02-08
	Sponsoring organization

Title and subtitle

Cooperation and conflict over the struggle to reproduce in harsh environments: An experimental field study on ostriches

Abstract

Breeding in groups allows the costs of reproduction to be shared amongst multiple individuals, enabling species to occupy environments where independent reproduction is challenging. However, group breeding is also likely to increase levels of competition and other types of conflict. Cooperative groups are, for instance, vulnerable to being exploited by cheats that benefit from the resources provided by cooperators without contributing to the collective effort themselves. How can cooperation be maintained in face of cheating and competition, and how does this influence the complexity of social groups in cooperative breeding animals?

To answer these questions, I experimentally established groups of varying complexity, defined by the number of male and female members, in the ostrich, Struthio camelus. This created variable conditions for cooperation and conflict to arise. The need for cooperation over offspring care was also manipulated by artificially incubating eggs during a part of the breeding season. Finally, I examined if the emergence of cooperation in groups was influenced by variation in tolerance to a key environmental challenge for ostriches, high temperatures. Individual genetic tolerance to heat stress was estimated using long-term egg production data spanning, temperature records and a population pedigree.

The results of this thesis show that cooperation and variation in group complexity are maintained in a number of different ways: a) The benefits of cooperation over offspring care and the costs of competition over mates are determined by the number of males and females in groups. These costs and benefits differ between the sexes. b) In large cooperative groups, the detrimental effects of cheating on cooperative behaviour and reproductive success are buffered by the presence of many cooperators, whereas in small groups cheating leads to the collapse of cooperation. c) Individual tolerance to heat stress promotes cooperative behaviour, and variation in heat tolerance within groups increases the benefits of cooperation for individuals with low heat tolerance.

These results provide experimental evidence that sexual conflict over optimal group size can promote variation in the complexity of social groups. The occurrence of cheats in the population favours greater group complexity by increasing the relative benefits of being in bigger groups, in which cooperation buffers the effects of cheating. This in turn leads to the coexistence of cheats and cooperators in large groups, adding yet another level of social complexity to the system. Finally, this thesis shows that measures of individual tolerance to environmental stress are important for predicting how cooperative behaviour and environmental factors interact. The presence of heat tolerant individuals in cooperative groups allows individuals with low heat tolerance to breed under hot conditions with a lower reproductive investment. This suggests that cooperation may mediate the maintenance of variation in individual environmental sensitivity.

Key words

Social, group, competition, conflict, cheating, heat, stress, tolerance, complexity, variation.

Classification system and/or index terms (if any)

Supplementary bibliographical information		**Language** English
ISSN and key title		**ISBN** (print) 978-91-7895-775-0 (pdf) 978-91-7895-776-7
Recipient's notes	**Number of pages** 214	Price
	Security classification	

Signature  Date 2021-02-08

Cooperation and conflict over the struggle to reproduce in harsh environments

An experimental field study on ostriches

Julian Melgar

LUND
UNIVERSITY

Thesis art by Violeta Caballero López and Philip Downing (back cover)

Faculty of Science
Department of Biology

ISBN (print) 978-91-7895-775-0
ISSN (pdf) 978-91-7895-776-7

Printed in Sweden by Media-Tryck, Lund University
Lund 2021

Y a la ternurosa avestruz
como que la ha querido, como que la ha adorado.
Pero ella se ha calzado todas sus diferencias.
César Vallejo

Table of Contents

List of papers

I. Melgar J., Schou M.F., Bonato M., Brand Z., Engelbrecht A., Cloete S.W.P. & Cornwallis C.K. Cooperation and competition over reproduction shape the complexity of cooperative breeding groups. Submitted.

II. Melgar J., Schou M.F., Bonato M., Brand Z., Engelbrecht A., Cloete S.W.P., Muvhali P.T., Hansson B. & Cornwallis C.K. Cheating triggers a tragedy of the commons, group size attenuates it. Manuscript.

III. Schou M.F, Bonato M., Engelbrecht A., Brand Z., Svensson E.I., 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.

IV. Melgar J., Schou M.F., Bonato M., Brand Z., Engelbrecht A., Cloete S.W.P., Muvhali P.T., Hansson B. & Cornwallis C.K. Genetic adaptations to cope with heat predict the emergence of cooperative behaviour. Manuscript.

Author contributions

I. Conceptualization, JM, SWPC, CKC; Methodology, JM, CKC; Formal analysis, JM, MFS, CKC; Investigation, JM, MFS, MB, SWPC, CKC; Data curation, JM, MFS, MB, ZB, AE, SWPC, CKC; Writing – original draft, JM, CKC; Writing – Reviewing & Editing, JM, MFS, MB, ZB, AE, SWPC, CKC; Visualization, JM, MFS, CKC; Supervision, JM, MFS, CKC; Funding acquisition, JM, SWPC, CKC.

II. Conceptualization, JM, SWPC, CKC; Methodology, JM, CKC; Formal analysis, JM, MFS, CKC; Investigation, JM, MFS, MB, PTM, SWPC, CKC; Data curation, JM, MFS, MB, ZB, AE, SWPC, CKC; Writing – original draft, JM, CKC; Writing – Reviewing & Editing, JM, MFS, MB, ZB, AE, SWPC, BH, CKC; Visualization, JM, MFS, CKC; Supervision, JM, MFS, CKC; Funding acquisition, JM, SWPC, CKC.

III. Conceptualization: MFS, CKC; Data curation: MFS, CKC, MB, AE, ZB, PTM, SWPC; Formal analysis: MFS; Funding acquisition: MFS, SWPC, CKC; Investigation: MFS., MB, AE, ZB, JM, PTM, SWPC, CKC; Methodology: MFS, EIS., CKC; Project administration: MFS, MB, CKC; Writing—original draft: MFS, CKC; Writing—reviewing and editing: MFS, MB, AE, ZB, EIS, JM, PTM, SWPC, CKC

IV. Conceptualization, JM, SWPC, CKC; Methodology, JM, CKC; Formal analysis, JM, MFS, CKC; Investigation, JM, MFS, MB, SWPC, CKC; Data curation, JM, MFS, MB, ZB, AE, SWPC, CKC; Writing – original draft, JM, CKC; Writing – Reviewing & Editing, JM, MFS, MB, ZB, AE, SWPC, BH, CKC; Visualization, JM, MFS, CKC; Supervision, JM, MFS, CKC; Funding acquisition, JM, SWPC, CKC.

Abstract

Breeding in groups allows the costs of reproduction to be shared amongst multiple individuals, enabling species to occupy environments where independent reproduction is challenging. However, group breeding is also likely to increase levels of competition and other types of conflict. Cooperative groups are, for instance, vulnerable to being exploited by cheats that benefit from the resources provided by cooperators without contributing to the collective effort themselves. How can cooperation be maintained in face of cheating and competition, and how does this influence the complexity of social groups in cooperative breeding animals?

To answer these questions, I experimentally established groups of varying complexity, defined by the number of male and female members, in the ostrich, Struthio camelus. This created variable conditions for cooperation and conflict to arise. The need for cooperation over offspring care was also manipulated by artificially incubating eggs during a part of the breeding season. Finally, I examined if the emergence of cooperation in groups was influenced by variation in tolerance to a key environmental challenge for ostriches, high temperatures. Individual genetic tolerance to heat stress was estimated using long-term egg production data spanning, temperature records and a population pedigree.

The results of this thesis show that cooperation and variation in group complexity are maintained in a number of different ways: a) The benefits of cooperation over offspring care and the costs of competition over mates are determined by the number of males and females in groups. These costs and benefits differ between the sexes. b) In large cooperative groups, the detrimental effects of cheating on cooperative behaviour and reproductive success are buffered by the presence of many cooperators, whereas in small groups cheating leads to the collapse of cooperation. c) Individual tolerance to heat stress promotes cooperative behaviour, and variation in heat tolerance within groups increases the benefits of cooperation for individuals with low heat tolerance.

These results provide experimental evidence that sexual conflict over optimal group size can promote variation in the complexity of social groups. The occurrence of cheats in the population favours greater group complexity by increasing the relative benefits of being in bigger groups, in which cooperation buffers the effects of cheating. This in turn leads to the coexistence of cheats and cooperators in large groups, adding yet another level of social complexity to the system. Finally, this thesis shows that measures of individual tolerance to environmental stress are important for predicting how cooperative behaviour and environmental factors interact. The presence of heat tolerant individuals in cooperative groups allows individuals with low heat tolerance to breed under hot conditions with a lower reproductive investment. This suggests that cooperation may mediate the maintenance of variation in individual environmental sensitivity.

Popular science summary

People are used to hearing that evolution is about the survival of the fittest. They imagine a world full of bullies that ruthlessly fight their way to the top in the struggle for life, leaving the weak behind. But this doesn't need be the case. This thesis reveals a different side of evolution, where survival means working together, and where the weak can ride on the shoulders of the fit.

Ostriches are awkward and goofy-looking. They are the biggest bird alive, easily reaching 2.5 meters in height, and can weigh over 150 kg. These massive birds are adapted to dry and hot environments. In the past, their habitat extended from the Arabian Peninsula to the southernmost tip of Africa. But their habitat is now confined to Africa, south of the Sahara Desert, and it keeps shrinking due to human activities.

Ostriches are social animals. They breed in groups (often several males and several females), and their nests are communal, which means that females lay eggs in the same nest. Adult ostriches cooperate over the incubation of eggs and in the protection of young chicks. They are caring parents and go through a lot of trouble to raise their chicks in the harsh and dangerous environment in which they thrive. And no, ostriches do not bury their heads in the sand at the first sign of trouble. I know from first-hand experience that they can chase you down if they feel threatened. In nature, ostriches fight cheetahs. They are definitely not head-burying cowards.

In this thesis, I experimentally manipulated more than one hundred groups of breeding ostriches. This allowed me to study the social behaviour of these fascinating birds. I did this using a pair of binoculars and a birding telescope, sitting hidden on top of a 10-meter tall camouflaged tower in a research farm in Oudtshoorn, South Africa.

Studying ostriches has taught me many things about living in a group. Being in a group can lead to conflict, of course. Ostrich males in particular do not want other males around. In groups with several males, they often compete with each other over females. This competition can get so frantic that, in their crazed attempts to mate before their rivals do, males sometimes break the eggs in their own nest.

Females are generally at ease with having other females in their group; they even benefit from it. Given that incubating eggs is tedious and time consuming, having more females around is welcomed, since it means that there are more bottoms to help with incubation. Males incubate as well, but they mostly take the night shift. During the day, they are busy fighting each other and breaking the eggs!

Under the peaceful surface of female coexistence, however, trouble lurks: some females sneak their eggs into the communal nest, without contributing to incubation. These cheats let other females do all the hard work. This kind of cheating behaviour

is most common in big groups. This is probably because cheats can easily pass unnoticed when there are lots of females in the group, or maybe because the other females don't mind a cheat or two when there are plenty of others that do help with incubation.

Cheat are very uncommon small in small groups, but do occasionally occur. When cheats do occur in small groups, they are often discovered. What happens next is striking. When a hard-working female discovers that the other female in her group is not contributing to incubation, she herself stops incubating. This is bad for both females, because the nest fails completely. Does this reaction to cheating mean that ostriches have a sense of fairness? If you don't do your fair share, I won't either! I don't know if this is about fairness in the human sense. Nonetheless, it is incredibly relatable behaviour.

Enough about conflict. What about the kinder side of evolution I promised? Well, this relates to heat-stress. Some female ostriches can tolerate high temperatures but others are sensitive to the heat and struggle to lay eggs in hot conditions. Even 25°C is too hot for some females, suggesting that heat-sensitive females should avoid hot environments. But wait! Ostriches are supposed to be adapted to hot and dry habitats, like the savannahs of sub-Saharan Africa. How can it be that some female ostriches would suffer on a nice summer day in Sweden? The answer to this question lies in shared incubation. Females that can tolerate the heat incubate more. This lets heat-sensitive females survive and reproduce in environments where they probably couldn't breed on their own.

This shows how nature's fittest help the vulnerable survive. Evolution may not be so ruthless after all and cooperation could be a way for ostriches, and other species, to cope with climate change.

Dedicated parents. In De Hoop National Park.

Populärvetenskaplig sammanfattning

Man brukar höra att evolution handlar om att de starkaste, de bäst lämpade, överlever. Detta för tankarna till en värld full med översittare, som hänsynslöst driver fram och lämnar de svaga bakom sig. Men det behöver inte vara så. Denna avhandling visar oss att gemenskap är styrka, och att evolutionen skapar liv där de svaga ibland sitter på de starkas axlar.

Strutsar är märkliga varelser. De ser ut som befjädrade, spralliga jättar. De är de största nu levande fåglarna, och blir ofta över 2,5 meter höga och väga väl över 150 kilo. De här gigantiska fjäderfäna är anpassade till ett liv i varma, torra områden. Fram tills mitten av förra seklet kunde man hitta strutsar hela vägen från den Arabiska halvön till sydligaste Afrika. Deras utbredningsområde är nu begränsat till Afrika söder om Saharaöknen, och fortsätter minska på grund av mänsklig påverkan.

Strutsar är sociala djur. De häckar i grupper som ofta består av flera hannar och flera honor. Hannarna gräver stora bon in marken där alla honorna i gruppen, och ibland en och annan utomstående, lägger sina ägg. Strutsarna hjälps sedan åt att tillsammans ruva äggen och skydda ungarna. De är flitiga föräldrar som sliter hårt för att föda upp sina ungar i den karga, farofyllda miljön där de trivs. Och nej, de är inga fegisar som sticker huvudet i sanden. De sticker definitivt inte huvudet i sanden när de känner sig hotade; sannolikt skulle de jaga efter dig om du kom för nära (jag vet av egen erfarenhet!). I naturen slåss strutsar mot geparder, så några försvarslösa duvungar är de inte.

I samband med denna avhandling åkte jag till en forskningsfarm i Oudtshoorn, i sydvästra Sydafrika. Där genomförde jag sociala experiment med strutsgrupper. Jag manipulerade antalet hannar och antalet honor i mer än hundra grupper av häckande strutsar. Detta tillät mig att, på behörigt avstånd och med ett par kikare, studera dessa fåglars fascinerande sociala beteende.

Mina studier av strutsarnas sociala beteende har lärt mig mycket om innebörden av att leva i grupp. Samvaron med andra innebär givetvis att konflikter ibland uppstår. Strutshannar i synnerhet visar en viss motvilja mot att ha andra hannar i sin grupp. I grupper med fler än en hanne konkurrerar hannarna ofta om honornas gunst. Den här konkurrensen kan ibland bli så hård att hannar, i sin iver att hinna para sig med honorna före sina konkurrenter, kan trampa sönder sina egna, och andras, ägg. Honorna är generellt mer tillfreds med att ha andra honor i gruppen. För honor kan det nämligen vara fördelaktigt att vara många. Eftersom ruvning av ägg är ett tidskrävande och ledsamt arbete är det ofta välkommet att ha några extra vingar att dela arbetet med. Hannar hjälper också till med ruvningen, men de tar oftast nattskiftet, så de är inte mycket till hjälp under dagen. De är dessutom upptagna med att bråka med varandra och ha sönder ägg!

Under ytan av denna till synes harmoniska samvaro mellan honor döljer sig dock konflikt. Vissa honor kan smyga in sina ägg i ett bo, trots att de sen inte hjälper till med att ta hand om dem. Dessa honor utnyttjar alltså andras hårda arbete utan att själva bidra. Den här typen av fusk är vanligare i stora grupper, förmodligen för att fuskarna lättare kan handla obemärkta när det finns många andra att hålla reda på. Eller kanske är det så att det inte är så noga om några enstaka individer fuskar, så länge det finns tillräckligt många som hjälper till med ruvningen. Fuskare är mycket ovanliga i små grupper, men förekommer även där. I små grupper blir de dock oftast upptäckta, och när det händer, följer något ypperligt fascinerande: förekomsten av en fuskare i en liten grupp, som kanske bara har två honor, leder till att även den honan som till en början gjorde sin del i ruvningen, slutar ruva äggen. Detta är dåligt för båda. Utan honor som ruvar klarar sig inte äggen, och då blir det inte heller några ungar. Betyder denna reaktion på fusk att strutsar har en känsla för rättvisa? Om ingen annan gör sin del, så gör jag inte heller min! Jag vet inte om detta handlar om rättvisa, så som vi människor förstår den, men jag kan i alla fall verkligen relatera till känslan!

Nog sagt om konflikter. Jag har trots allt lovat en vänskapligare bild av evolutionen. Denna avhandling har också avslöjat ett överraskande, och betagande, samarbete mellan strutshonor. För att förstå varför är det viktigt att veta att vissa strutsar är mer tåliga för värmestress än andra. De honor som inte tål värme lägger färre ägg när temperaturen stiger över 25°C. Detta antyder att honor som blir stressade av värme mår bättre av att undvika varma miljöer. Men, vänta! Skulle inte strutsar vara anpassade till ett liv i varma områden? Hur kan det då komma sig att vissa strutsar skulle tycka att det var för varmt på en högsommardag här hemma i Sverige? Svaret på den frågan stavas: samarbete. När strutsar häckar i grupp kan de honor som tål värme bäst ta över en större del av ruvningen. På detta sätt får värmekänsliga honor hjälp på traven, och kan frodas i miljöer där de förmodligen hade haft svårt att klara häckningen själva. Samarbete skulle kunna vara ett sätt för strutsar, och andra djurarter, att klara de utmaningar som följer med den globala uppvärmningen vi människor har skapat. De starkaste, och mest lämpade kan ibland hjälpa de sårbara. Sådana är naturlagarna.

”Men den ångest, som hon själv bar på, öppnade hennes hjärta. Hon tyckte, att hon inte stod så långt borta från alla levande varelser, som människor annars gör. Hon förstod mycket bättre än någonsin förut hur fåglarna hade det. De hade sina jämna omsorg för hem och barn, de som hon. Det var nog inte så stor skillnad mellan dem och henne, som hon hittills hade trott.”

Ur **Selma Lagerlöfs**
Nils Holgerssons underbara resa genom Sverige

Introduction

Cooperative breeding, where more than two individuals participate in acquiring reproductive partners and/or raising offspring, has evolved numerous times across the animal kingdom (Rubenstein & Abbot, 2017). In some mammals, such as lions, related males cooperate over the acquisition and protection of females that subsequently help each other take care of their offspring (Smith et al., 2017). In cooperative breeding cichlid fish, non-breeding adults care for the progeny of unrelated individuals and in cooperative breeding birds, such as Florida scrub jays, breeding pairs are often joined by mature offspring that help raise subsequent broods (Fitzpatrick & Bowman, 2016; Taborsky & Limberger, 1981). Cooperative breeding has also reached astonishing complexity in insects. Many ant species, and some species of bees, have evolved sterile workers that rear juveniles while queens focus entirely on reproduction (Heinze et al., 2017; Wcislo & Fewell, 2017). The fact that some individuals invest time and energy to help others reproduce has long puzzled evolutionary biologists: Why, given that natural selection is expected to favour individuals to maximize their own fitness, do individuals aid the reproductive success of others in such a wide variety of animals?

Why be a cooperative breeder?

To understand the evolution of cooperative behaviour, we need to consider the different ways in which individuals can transmit their genes to subsequent generations: an individual can pass on its genes by producing its own offspring ("direct fitness"), or by promoting the number of offspring a relative, which shares the same genes, produces ("indirect fitness"). Together, direct and indirect fitness make up inclusive fitness, a term coined by W. D. Hamilton in 1964 (Hamilton, 1964b, 1964a). The importance of this concept for understanding cooperative breeding is captured by Hamilton's rule, which predicts cooperative behaviour will be favoured when $rb > c$; where "c" is the fitness cost an individual pays for helping another individual reproduce, "b" is the fitness benefit that an individual gets from receiving help, and "r" is the degree of relatedness between helpers and recipients, relative to the population (Gardner & West, 2004; Hamilton, 1964a, 1964b).

Inclusive fitness theory tells us that selection can favour individuals to forego their own reproduction and help others when relatedness, r, is high. Inclusive fitness

theory has been successful in explaining patterns of cooperative behaviour amongst relatives in a wide variety of taxa, from bacteria to mole-rats (Bourke, 2014; Stuart A. West et al., 2002, 2021; Stuart A. West & Cooper, 2016). It also illustrates that for selection to favour cooperation amongst unrelated individuals, $r \sim 0$, they must gain net lifetime direct fitness, so c must be negative (Bourke, 2011). Therefore, unrelated helpers are only expected to sacrifice their own reproduction to help in a given breeding season if by doing so they improve their future direct fitness. In this case, cooperation is "mutually beneficial" (Stuart A. West et al., 2008). This can happen in a number of different ways. For example in fish and birds, helpers in social groups have been shown to be more likely than non-helpers to inherit breeding positions, and be protected against predators (Balshine-Earn et al., 1998; Heg et al., 2004; Stacey & Koenig, 1990; Woolfenden & Fitzpatrick, 1978). In mammals, parenting experience gained by helpers during

Table 1: Glossary

Cheat	An individual that benefits from the (cooperative) effort of other individuals without contributing to the effort itself.
Complexity	Property of the behaviour of a system (here a social group) whose components interact in multiple ways. Complexity increases both when the number of components that can interact with each other, and the number of ways in wich they can interact, increase. The complexity of a social group, for example, will increase if the size of the group increases (i.e. the number of components in the system increases), or if the number of strategies that members of the group can adopt increases (i.e. the number of ways in which the components of the system can interact increases). Examples of strategies in a social group are: breeder, helper, cooperator, cheat.
Cooperation	The process of individuals acting together for common benefit.
Cooperative breeding	When more than two individuals participate in the producing or raising of offspring, and/or when two or more individuals cooperate with each other in the acquisition of mates.
Cooperator	An individual that contributes to a collective effort.
Direct fitness	Fitness gained by producing own offspring.
Facultative cooperative breeder	Species capable of, but not restricted to, cooperative breeding .
Family groups	Groups formed by individuals remaining in their natal territories to help relatives reproduce. Members of family groups are often highly related to each other.
Hamilton's rule	Cooperative behaviour should be favoured when rb > c. Where "c" is the fitness cost an individual pays for helping another individual reproduce, "b" is the fitness benefit that an individual gains by receiving help, and "r" is the degree of relatedness between helper and recipient relative to the population.
Inclusive fitness	The sum of direct and indirect fitness.
Independent breeder	An individual that breeds without the assistance of any other individual(s) other than the second parent of its offspring.
Indirect fitness	Fitness gained by increasing the number of offspring produced by relatives.
Non-family groups	Groups formed by individuals aggregating after they disperse from their natal territories. Within group relatedness in non-family groups is often low.
Obligate cooperation breeder	Species restricted to breeding cooperatively.
Reproductive division of labour	An organization principle of some social groups where one or a few individuals specialize in reproduction while others perform strictly non-reproductive tasks.

early life has been shown to have a positive effect on their direct fitness later in life (Salo & French, 1989). By integrating indirect and direct fitness, inclusive fitness theory provides a framework for understanding why cooperation evolves in groups of both relatives and unrelated individuals (Bergmüller et al., 2007; Clutton-Brock, 2002, 2009).

Why do cooperative breeders vary in their social organisation?

Although we now have a good theoretical understanding of why cooperation evolves, it remains a challenge to explain the variation in social organization that occurs within the animal kingdom (Stuart A. West et al., 2021). For example, some species are solitary and rarely meet conspecifics, like the ocean sunfish, whereas others are obligately social, like ants and honey bees, relying on conspecifics for survival and reproduction (Dugatkin, 1997; Koenig & Dickinson, 2016; Rubenstein & Abbot, 2017; Stacey & Koenig, 1990; E. O. Wilson, 1971). Variation in social organization is not only about whether an animal is social or not, but also about how social groups are structured. For instance, groups can vary in their size, sex ratio, relatedness among members, the number of reproductive individuals in relation to (non-reproductive) helpers, and the degree to which members are specialized in specific tasks (Dugatkin, 1997; Koenig & Dickinson, 2016; Rubenstein & Abbot, 2017; Stacey & Koenig, 1990). Moreover, variation in social organization is evident not just across species, but also within species (J. J. Boomsma & Grafen, 1990; Lott, 1984; Schradin et al., 2018; Yamagiwa & Hill, 1998). Variation in social organization within and between species may be explained by two main factors: variation in relatedness, and variation in the benefit to cost ratio of cooperative behaviour. Explaining how and why social organization varies, both between and within species, is a major outstanding problem in social evolution (Stuart A. West et al., 2021).

Relatedness and its influence on variation in social organisation

Relatedness within groups is expected to have an important influence on social group organization by governing the opportunities for direct and indirect fitness (Figure 1) (Bergmüller et al., 2007; Clutton-Brock, 2002, 2009; Downing et al., 2020). The relative magnitudes of direct and indirect fitness will in turn influence the distribution of reproduction among individuals within groups (Keller & Reeve, 1994; Vehrencamp, 1983). In groups with low relatedness where the potential for indirect fitness is limited, selection will act to maximise direct fitness, and reproduction will be more or less evenly distributed among all adult members of the

group (Clutton-Brock, 2002). This is illustrated by the fact that the majority of cooperative breeding groups where relatedness is low do not have non-breeding helpers (Cockburn, 1998; Downing et al., 2020; Koenig & Dickinson, 2016; Riehl, 2017). Instead, individuals in groups with low relatedness generally assist each other in reproducing simultaneously (Koenig & Dickinson, 2016).

In groups with high relatedness, where there is a higher potential for indirect fitness, selection can favour individuals to invest in activities other than reproduction, resulting in what is known as a "reproductive division of labour" (Edward O. Wilson, 2000). Many groups with high relatedness have a strict reproductive division of labour, with a pair, or a few dedicated breeding individuals and a non-reproductive work force that forgoes reproduction (Rubenstein & Abbot, 2017). For example, in grey-crowned babblers, *Pomatostomus temporalis*, offspring stay behind as non-breeding helpers to assist their parents in raising their subsequent broods (Blackmore et al., 2011; McGowan & Woolfenden, 1990; Woolfenden, 1975).

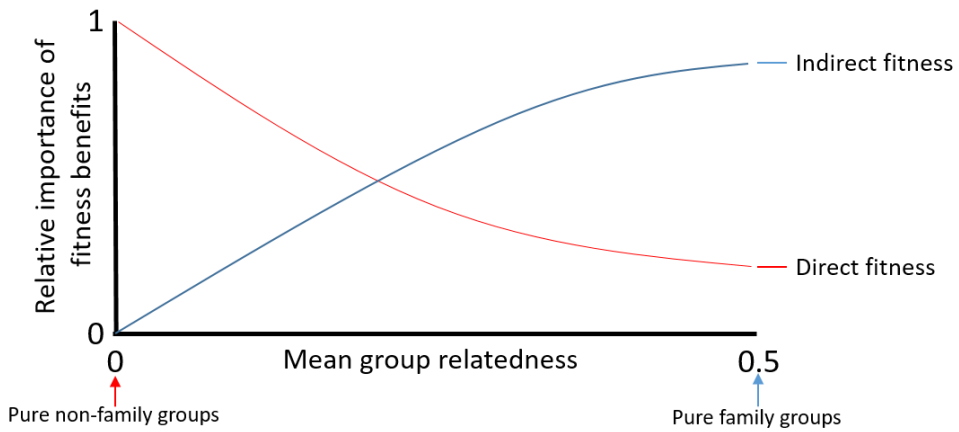


Figure 1. The relative importance of direct and indirect fitness is affected by the degree of relatedness in a cooperative group, which in turn is affected by the way in which the group forms. Mean group relatedness refers to mean relatedness between helpers and breeders.

Reproductive division of labour is predicted to set the scene for further task specialisation (Cooper & West, 2018). The relaxation of the requirement to reproduce to pass on genes may enable selection to favour helpers to specialise in tasks that further increase group productivity. For example, in army ants, where reproductive division of labour is strict, sterile helpers are morphologically and behaviourally differentiated into different soldier and worker castes (Schneirla & Piel, 1948). Soldier castes specialize in defending their group against threats from predators and competitors, while worker castes specialize in brood care and foraging

(Schneirla & Piel, 1948). The specialisation that underlies the complex social organisation in this species would not be possible if helpers in the group had not been freed from the resource demanding task of reproduction.

Why do groups vary in relatedness?

One key factor influencing relatedness is the way groups form, which can happen in two main ways (Downing et al., 2020; Stuart A. West et al., 2015). First, groups can form by offspring remaining with their parents to help raise younger siblings in "family groups", such as in ants, meerkats, *Suricata suricatta*, and Florida scrub jays, *Aphelocoma coerulescens* (Figure 2A-C) (Clutton-Brock & Manser, 2016; Fitzpatrick & Bowman, 2016; Heinze et al., 2017) In family groups relatedness of helpers to offspring is typically high and so is the potential for individuals to gain indirect fitness through helping (Figure 1). Second, groups can form when individuals join together after they disperse from their natal groups, as in greater anis, *Crotophaga major*, daffodil cichlids, *Neolamprologus pulcher*, and Galápagos Hawks, *Buteo galapagoensis* (Figure 2D-F) (Clutton-Brock, 2009; Faaborg et al., 1995; Riehl, 2011, 2013; Taborsky & Limberger, 1981). Groups formed in this way have been referred to as "non-family groups" (Downing et al., 2020). In non-family groups relatedness is typically low and the potential for individuals to gain indirect fitness is limited (Figure 1). Individuals in non-family groups are therefore expected to cooperate as a result of gains in direct fitness, for instance through gaining access to mates, reduced adult mortality and/or increased offspring survival (Baglione et al., 2002; Cameron et al., 2009; Kokko et al., 2001).

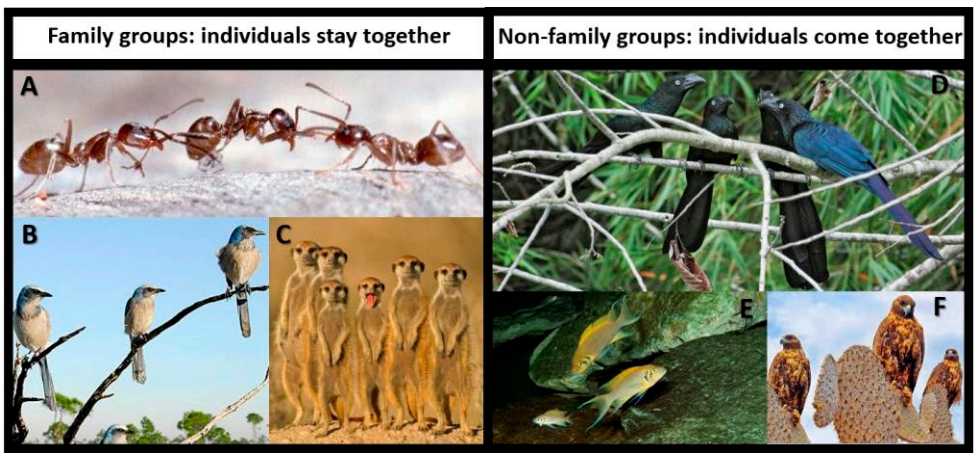


Figure 2. Some cooperative breeding species reproduce in family groups (e.g. Argentine ants, *Linepithema humile* (A), Florida scrub jays, *Aphelocoma coerulescens* (B) and Meerkats, *Suricata suricatta* (C), whereas other cooperative breeding species form non-family groups (e.g. greater anis, *Crotophaga major* (D), daffodil cichlids, *Neolamprologus pulcher* (E) and Galápagos hawks, *Buteo galapagoensis* (F)).

There are also cases where cooperative groups are a mix of related and unrelated individuals (Dierkes et al., 2005; Horn et al., 2004; Magrath & Whittingham, 1997; Reyer, 1984; Wright et al., 2010). These groups typically arise when unrelated immigrants join family groups or family members disperse together to join other, unrelated, individuals (Rubenstein & Abbot, 2017). Furthermore, even when groups form through the same mechanisms, relatedness can vary due to mating behaviour, breeder turnover and levels of reproductive competition (Hartley & Davies, 1994; Riehl, 2011; Russell, 2016). For example, in family groups, relatedness between retained offspring ("helpers") and offspring raised in subsequent broods ("recipients") is reduced when breeding females are polyandrous and/or breeding females are replaced between reproductive events (Hartley & Davies, 1994; Russell, 2016). Under such circumstances, cooperative groups may still be maintained when relatedness is low, but are expected to involve helpers gaining direct fitness from helping, for example by inheriting breeding positions or by getting a greater benefit to cost ratio from helping (Downing et al., 2018).

In non-family groups, where all adult members reproduce, relatedness to offspring can also vary due to mating competition, mate choice and policing behaviour to avoid brood parasitism (Bertram, 1979; Hartley & Davies, 1994; Riehl, 2010). However, research on the effects of variation in relatedness has focused mainly on family groups, where relatedness is easier to measure. Consequently, we know much less about how relatedness affects the cost to benefit ratio of cooperation in non-family groups.

To summarize: relatedness in cooperative groups can vary in a number of different ways, and when relatedness varies, cooperation is generally maintained by a change in the relative importance of direct and indirect fitness gains (Figure 1). However, an increase in the cost to benefit ratio of cooperation, resulting from for instance low relatedness, can also drive individuals to change strategy, from cooperation to independent breeding.

The benefit to cost ratio of cooperative behaviour and its influence on variation in social organisation

The benefits and the costs of helping are predicted to depend on environmental factors (Emlen, 1982a; Hatchwell & Komdeur, 2000). Imagine an individual that has just reached sexual maturity and is ready to enter its first breeding season. It is faced with the decision of whether to disperse and attempt to breed on its own, to join a group where relatedness is low or uncertain, or stay in its natal group as a non-breeding helper and forego its own reproduction. The outcome of this decision will depend on the cost of helping, which is defined by the probability that the individual will be able to successfully breed on its own. This probability is affected by: a) how likely it is that an individual will establish a breeding position, and b)

how likely an individual is to successfully breed once it has gained a breeding position. Both "a" and "b" are dependent on environmental factors, such as food availability and weather conditions during dispersal, and demographic factors, such as mate availability and population density. If the likelihood of successfully breeding independently is low, then the costs of foregoing reproduction (measured as fitness loss through not reproducing independently) will be low as well. Under such circumstances, selection for cooperative breeding will be stronger than when opportunities for independent breeding are high.

The idea that environmental constraints drive the evolution of cooperative breeding has found support from both observational and experimental studies, as well as from comparative studies (Hatchwell & Komdeur, 2000). However, it is not entirely clear what type of environments favour cooperative breeding. Some studies suggest that cooperative breeding is favoured in benign environments while others show that cooperative breeding is associated with harsh environments (Arnold & Owens, 1999; J. L. Brown, 1974; Gonzalez et al., 2013; Jetz & Rubenstein, 2011; Ricklefs, 1975; Rubenstein & Lovette, 2007). Whether an environment is harsh or benign is typically defined by the degree of stability and predictability, with more unstable and unpredictable environments being considered harsher (Emlen, 1982a).

Cooperative breeding in harsh and in benign environments

Both harsh and benign environments can be challenging for independent breeding (Emlen, 1982a). Harsh environments often have low carrying capacity and impose constraints on survival and breeding due to extreme ecological conditions, such as high temperatures (Chesson & Huntly, 1997), or drought (Lukas & Clutton-Brock, 2017). Cooperative breeding has been proposed to buffer individuals from these kinds of challenges (Emlen, 1982a). For instance, in birds inhabiting hot environments, heat stress during incubation could potentially be alleviated if several individuals take it in turns to incubate eggs (AlRashidi et al., 2010; Deeming, 2001). Benign environments, on the other hand, typically have a higher carrying capacity due to lower rates of mortality (Gonzalez et al., 2013). Favourable conditions can, however, lead to habitats becoming saturated, which can also restrict opportunities for independent breeding (Komdeur, 1992). These constraints come mainly from intraspecific competition for mates and breeding sites (Pen & Weissing, 2000). Selection is thus likely to favour non-breeding individuals to remain on their natal territories and help raise siblings, or to join other already established breeders in the hope of getting a breeding position in the future. Consequently, in benign environments selection for cooperation is likely to be driven by interactions with the biotic environment and with conspecifics (e.g. mate availability, competition) (Komdeur, 1992; Pen & Weissing, 2000). In harsh environments on the other hand, interactions with abiotic factors, such as temperature and rainfall, may play a more prominent role (Emlen, 1982a, 1982b).

The effects of the social environment

Above, I have provided an overview of how within-group relatedness affects the benefit to cost ratio of cooperation, and how this, in combination with environmental factors, is predicted to shape the evolution of cooperation. There are, however, characteristics of social groups other than relatedness, that are likely to affect the evolution of cooperation. The social environment, which results from interactions between members of a group, has also been shown to affect the costs and benefits of cooperation (Smiseth & Moore, 2004; Wong et al., 2013).

The complexity of cooperative groups is likely to influence the way in which individuals interact with each other. For instance, in groups that do not have a strict reproductive division of labour, group size, and relative numbers of males and females (sex ratio), are likely to influence the levels of competition over mates within the group (Alexander, 1974; Davies & Houston, 1986; Hauber & Lacey, 2005). If levels of competition over mates are high, individuals are likely to be driven to pursue reproductive opportunities at the expense of others, which can potentially lead to the collapse of cooperation in the group, or prevent cooperation from evolving in the first place (Galliard et al., 2005; Rankin et al., 2011). Moreover, the level of competition over mates in a given set of social conditions might differ between males and females, leading to sexual conflict, which arises when the fitness interest of males and females diverge from each other (Arnqvist, 2004; Chapman et al., 2003). Such conflict is likely to lead to female and male fitness being optimized in different groups sizes, or different sex ratios, providing a potential explanation to why social groups vary in their social organization.

Cooperative groups are also vulnerable to a different kind of conflict between group members: cheating (C. R. Brown, 1984; Emlen & Wrege, 1986; Ghoul et al., 2014; Michod & Herron, 2006; Wade & Breden, 1980). Cheating arises when individuals increase their fitness by exploiting the benefits of cooperation without contributing to cooperation themselves. Cheating can, in a similar way to competition over mates, lead to the collapse of cooperation (Haig & Grafen, 1991; Hardin, 1968; Rankin et al., 2007; Riehl & Strong, 2019; Sachs et al., 2004; Sachs & Rubenstein, 2007; Strassmann & Queller, 2011; Van Dyken et al., 2011; Stuart A. West, Gardner, et al., 2006; Stuart A. West, Griffin, et al., 2006). The occurence of cheating in a group has been shown to be frequency dependent, with cheats being more successful when they are rare (Gore et al., 2009; Ross-Gillespie et al., 2007; Stuart A. West et al., 2021), cheting is thus likely to be yet another factor contributing to variation in the complexity of social groups.

What is the causal relationship between the environment and cooperative breeding?

The environment as a promotor of cooperative breeding is an intuitive idea that has dominated research on cooperative breeders for several decades (J. L. Brown, 1974; Emlen, 1982a, 1982b). However, the causal direction of that relationship is not entirely clear. The fact that there is an overabundance of cooperative breeding species in harsh environments has nurtured the idea that the evolution of cooperative breeding is driven by a high benefit to cost ratio of cooperation imposed by ecological constraints (J. L. Brown, 1974; Emlen, 1982a; Jetz & Rubenstein, 2011; Koenig et al., 1992; Lukas & Clutton-Brock, 2017). The environment is, in other words, viewed as a cause of cooperative breeding.

However, there is an alternative explanation for the relationship between cooperative breeding and the environment that invokes the reverse causal relationship: cooperative breeding evolves in the absence of strong ecological constraints but enables inhospitable environments to be invaded. Cooperative breeding is instead favoured by high indirect fitness ensured by high within group relatedness, mediated by high levels of monogamy, with only a small benefit to cost ratio required for the evolution of cooperation (Jacobus J. Boomsma, 2007, 2009). Cooperative groups with a work force of helpers may then be able to expand into ecological niches unsuitable for independent breeders (Cornwallis et al., 2017; Duffy & Macdonald, 2010). The environment is hence not a cause, but rather a consequence, of cooperative breeding.

The idea that cooperative breeding is a cause and not a consequence of the environment has gained support in the last decade. Phylogenetic studies have shown that cooperative breeding has facilitated niche expansion in birds (Cornwallis et al., 2017) and social shrimps (Duffy & Macdonald, 2010). Studies like these are very valuable in providing general inferences about the order in which traits evolve (e.g. cooperative breeding first, and then environment, or vice versa). However, conclusions from phylogenetic studies can be complicated by, among other things, traits evolving at different rates, uneven distributions of species exhibiting different traits (e.g. cooperative versus independent breeding) and difficulties in reconstructing past evolutionary events. As a result, experimental studies are needed to complement and verify patterns seen in phylogenetic studies.

Only one experimental study to date has tested the idea that cooperative breeding determines the environments species can inhabit. This study was carried out in a facultative cooperative breeding species of burying beetle and showed that cooperative groups thrived in a wider range of thermal environments than non-cooperative groups (Sun et al., 2014). Experimental studies are invaluable for our understanding of the causal relationship between environmental conditions and cooperation and more are needed.

Hamilton's b's and c's are not always equal

Understanding how environmental conditions affect the costs and benefits of cooperation can help us understand why species vary in their social organization. However, a given environmental factor, temperature for instance, is likely to affect the cooperative traits of different species in idiosyncratic ways. Consequently, if we want to explain why a given environmental factor affects cooperative traits in some species, but not in others, we need to take into account non-social traits that have the potential of effecting interactions with the environment (Figure 3).

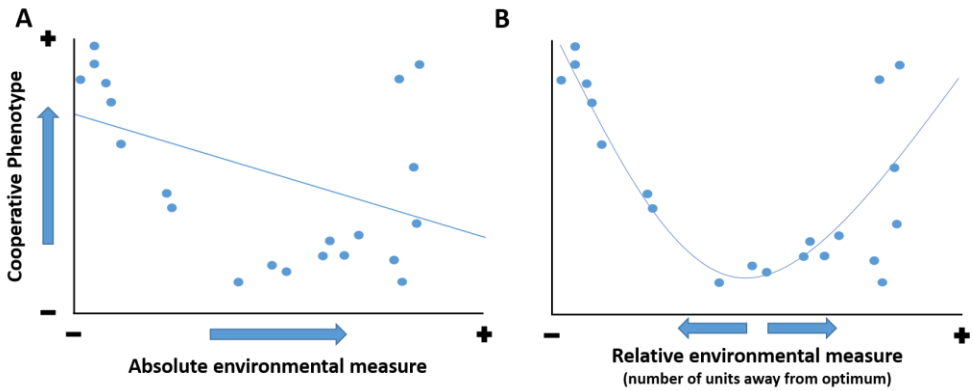


Figure 3. A relative measure of environmental factors: Conceptual figures 3 **A** and **B** show the same dummy data but ask different questions that might lead to different conclusions. Figure **A** asks the question: How does an increase in environmental factor "*x*" affect cooperative phenotype "*y*"? The answer to this question is that the cooperative phenotype *y* generally decreases when environmental factor *x* increases. However, the cooperative phenotype "*y*" has high values both at the low and the high end of the environmental range and the curve does not fit the data very well. A more sensible question to ask might instead be: Does distance away from the optimum in environmental factor "*x*" affect cooperative phenotype "*y*"? (**B**). The curve fits the data much better in this case, and shows that cooperative phenotype "*y*" increases with increasing distance from optimum at both sides of the environmental range. If we are interested in understanding the relationship between cooperative behaviour and environmental constraints, then taking into account species, or individual, optima, as in B, is likely to give us more relevant answers.

In a similar way, understanding how the costs and benefits of cooperation vary in relation to the environment across individuals within a population can help us understand variation in social organization within species. The costs and benefits of cooperative behaviour are likely to vary across individuals, due to, for instance, variation in their genetic predisposition to withstand environmental stress (Mackinnon et al., 1991; Ravagnolo & Misztal, 2000). For example, in a hot environment, an individual with a high heat tolerance would not be constrained by heat stress in the same way as an individual more sensitive to heat. If we view cooperative breeding as an adaptation that enables individuals to overcome environmental constraints, then it follows that cooperative breeding will be expected to increase when environmental conditions shift away from the individual, or species, optimum (Figure 3B). Since individuals, and species, are likely to vary in

their optimal environmental conditions, and in how sensitive they are to environmental change; this variation may explain the coexistence of cooperative and non-cooperative strategies in the same environments (Lott, 1984; Schradin, 2013; Schradin et al., 2018).

Thesis Aims

The main aim of this thesis is to further our understanding of the relationship between variation in the social organization of cooperative groups and their environment (both the social and abiotic environment). To do this, I: a) manipulated the complexity of groups of cooperative breeding ostriches (*Struthio camelus*) by establishing groups with different numbers of males and females (**papers I, II and IV**), and b) quantified individual tolerance to temperature stress to examine its influence on fitness and cooperation under challenging temperature conditions (**papers III and IV**).

In **paper I,** I investigated why groups vary in complexity by experimentally engineering entire social groups of cooperatively breeding ostriches, mirroring the complexity of groups observed in natural populations. To disentangle the effects of competition and cooperation across different phases of breeding, I compared the reproductive success of individuals when offspring care was experimentally removed to when individuals were allowed to provide offspring care.

In a similar way, in **paper II,** I experimentally manipulated the opportunities for cheating and cooperation in groups of breeding ostriches. This allowed me to examine the role of cheating in shaping the complexity of social groups, and also what social conditions allow cheats to proliferate.

In **paper III** the effect of fluctuating temperatures on male and female reproductive success, without any opportunities for cooperative breeding, was quantified. In **paper IV** the knowledge gained from **paper III** on the temperature effects on reproduction was used to predict the heat tolerance of males and females used in experiments where opportunities for cooperation were manipulated. This paper addresses the question of why individuals of the same species show so much variation in how cooperative they are, adding a piece to the great puzzle of what causes variation in social organization.

Methods

Study system: The ostrich (*Struthio camelus*), a facultative cooperative breeder

Ostriches have a communal nesting system in which males dig and protect a nest where multiple females lay their eggs (Bertram, 1992). As several females lay eggs in each nest, and because females can be polyandrous, numerous males and females potentially contribute offspring to a single nest (Kimwele & Graves, 2003). Both males and females participate cooperatively in the incubation of the eggs and protection of offspring (Bertram, 1992; Kimwele & Graves, 2003; Magige et al., 2009). Ostrich groups are thought to largely consist of unrelated individuals (Kimwele & Graves, 2003), but the details of how ostrich groups form in the wild (i.e. whether they are family, of non-family groups) is very limited. We know, however, that the size and sex ratio of breeding ostrich groups varies greatly in nature (Figure 5), ranging from pairs to groups of well over a dozen individuals of various sex ratios (Figure 5B) (Bertram, 1992; Kimwele & Graves, 2003; Magige et al., 2009). The natural variation in complexity, and the flexibility of the social organisation of ostrich groups make these birds an ideal species for the study of social evolution.

Natural variation social organization

Natural variation in the complexity of breeding groups (group size, number of males and number of females) was examined using published literature (Bertram, 1992; Magige et al., 2009), and directly estimated by conducting transects along the roads of the south eastern part of Karoo National Park. Each transect was carried out 2 to 3 times in two separate years (2014 and 2018). Ostriches were typically observed in clearly defined groups, judged by their coordinated movement and close proximity to each other (< ~100m). In a few instances, individuals were separated by more than 100m and in these situations they were observed until it was clear whether they were part of the group or moving separately. The location of groups and single individuals was recorded using GPS on an iPhone 4 in 2018 and using maps in 2014.

Experimental population

Study site

The research presented in this thesis on captive ostriches was done at Oudtshoorn Research Farm, South Africa (33° 38’ 21.5“S, 22° 15’ 17.4”E) in natural Karoo habitat, where wild ostriches thrive (Figure 4).

Figure 4. Field work in South Africa. A) a group of female ostriches guarding their nest in one of the experimental groups. B) Charlie Cornwallis surveying the wild ostrich population. C) A daily routine; Jakob Baartman (right) and Niklaas Appel counting and marking eggs in one of the experimental groups. The observation tower can be seen in the background. D) Mads Schou (standing) and Charlie Cornwallis examining the remains of a wild ostrich nest.

Study population

The captive ostriches used in this thesis are derived from 139 founding individuals. From 1998 to 2018 the reproduction of captive breeding pairs ($n_{females}$ = 756, n_{males} = 701) was monitored in 197 enclosures of ~0.25 ha. A male and a female ostrich were assigned to each enclosure in May/June each year and kept together until the end of the breeding season in December/January. During this period, pairs were checked twice daily and any eggs were collected. Male–female combinations were

established to prevent inbreeding and, when possible, generate new combinations each year. From 2008 to 2018 the fertility of males (n = 22) kept in solitary enclosures (20m × 17 m) and trained to ejaculate into an artificial cloaca using a dummy female (Rybnik et al., 2007) was monitored.

During the breeding season ostriches received a balanced ostrich breeder diet (90 to 120 g protein, 7.5 to 10.5 MJ metabolizable energy, 26 g calcium and 6 g phosphorus per kg feed) and ad-libitum water.

Estimating individual tolerance to heat stress

Daily records of egg production between 1998 and 2018 of 678 pair-breeding female ostriches, as well as daily temperature records, and an available pedigree were used to estimate the tolerance to heat stress of each individual included in the experimental part of this thesis. This was done by constructing a random regression animal model of the individual change in egg-laying rate with increasing or decreasing temperatures from the optimum (20ºC). The animal model was run in R v.3.6.0 (R Core Team, 2019) using the Bayesian framework implemented in the R-package MCMCglmm v.2.29 (Hadfield, 2010).

Experimental manipulations of groups

The complexity of 118 groups of breeding ostriches, involving 309 adult ostriches (145 males and 164 females), was experimentally manipulated over a seven-year period (between 2012 and 2018, 16-18 groups per year). Groups were kept in fenced areas (range: 2400 and 70600 m^2, median = 4700 m^2). The number of males in groups ranged from 1 to 3 and the number of females ranged from 1 to 6. Due to limitations in the number of birds accessible for the experiments, and other experiments being conducted on the same population, not all combinations of male and female group sizes were possible. All individuals in the population were individually identifiable by coloured and numbered neck tags.

The breeding season was typically from May to December every year. During the first ~5 months of the season, eggs were collected and put in artificial incubators to measure reproductive success independently of the effects of incubation behaviour. During the last ~2 months, eggs were left in nests and incubation behaviour was monitored to examine patterns of reproductive success when individuals had to care for offspring. The number of eggs and number of chicks produced in every group was recorded.

Behavioural observations

During the last ~2 months of every breeding season, the incubation and copulation behaviours of individuals were monitored by conducting ~3 hour observations at least three times a week using binoculars (10 x 40) and a telescope (12-36 x 50). The observer sat camouflaged in a 10-meter-tall observation tower in the middle of

the field site. Groups were observed for between 47 and 91 hours. The identity of each individual involved in the focal behaviours, the time of every copulation, and the time for the start and end of incubation were recorded.

Parentage analysis

Blood samples were collected from all adults included in the experimental groups, from all chicks hatched from these groups, and, when possible, blood or tissue samples were collected from eggs that failed to hatch. From these samples, seven highly polymorphic tracts of repetitive DNA (microsatellites) were amplified using Phusion Blood Direct PCR Kit (Thermo Scientific™) and fluorescently labelled primers. These seven microsatellites have previously been used to assign parentage in ostriches with high confidence (Bonato, 2009). After DNA-amplification, the amplicons were separated by size using capillary electrophoresis. Microsatellite scoring was then performed visually using the software Geneious 10.2.3 (*Geneious | Bioinformatics Software for Sequence Data Analysis*, 2017). Finally, a parentage analysis was run in the software Cervus 3.0.7 (Marshall et al., 1998).

Statistical analyses

Data were analysed in R (R Core Team, 2020) with Bayesian Linear Mixed Models (BLMM) with Markov chain Monte Carlo (MCMC) estimation in the package MCMCglmm (Hadfield, 2010). Convergence was checked by running models three times and examining the overlap of traces, levels of autocorrelation, and testing with Gelman and Rubin's convergence diagnostic (Brooks & Gelman, 1998). Fixed effects were considered significant when 95% credible intervals (CIs) did not overlap with 0 and pMCMC were less than 0.05. Random effects were used, when applicable, to model the non-independence of data arising from multiple data points per individual, per group, per enclosure and per year. To estimate the magnitude of random effects, the percentage of the total random effect variance explained by each random term on the expected data scale (I2%: (Vi/Vtotal)*100) was calculated (de Villemereuil et al., 2016). To obtain estimates of I2 on the expected scale from binomial models, the distribution variance for the logit link function was included in the denominator (Vi/Vtotal + π^3/2)*100).

Results and discussion

Here I present, in an abbreviated form, some of the most important results found in the four papers of this thesis, together with a brief discussion. I have, across this section, indicated the paper in the thesis to which each result (or set of results) belongs. In this way, readers interested in gaining a more in-depth knowledge of a given result can find it in the corresponding paper.

Natural variation in group complexity (paper I)

Groups of wild ostriches co-occurring in the same habitat at the same time have previously been shown to be highly variable in their complexity (Bertram, 1992; Kimwele & Graves, 2003; Magige et al., 2009). I confirmed this phenomenon when I conducted transects in Karoo National Park, South Africa, and found that ostrich groups inside the national park varied greatly in size and sex ratio, with sizes of between 2 and 18 individuals, containing 1 to 12 same sexed individuals. However, groups usually consisted of 1 to 6 females and 1 to 3 males (Figure 5). This is similar to the complexity of groups reported in East African populations (Bertram, 1992; Kimwele & Graves, 2003; Magige et al., 2009), showing that local variation in ostrich groups is widespread across their geographical range.

Experimental ostrich population (papers I-IV)

Using estimates from the wild as a guide, I experimentally established 118 groups involving a total of 309 individuals in enclosures of natural Karoo habitat. These experimental groups covered the typical range of group complexities observed in the wild (i.e. groups composed of 1 to 3 males with 1 to 6 females).

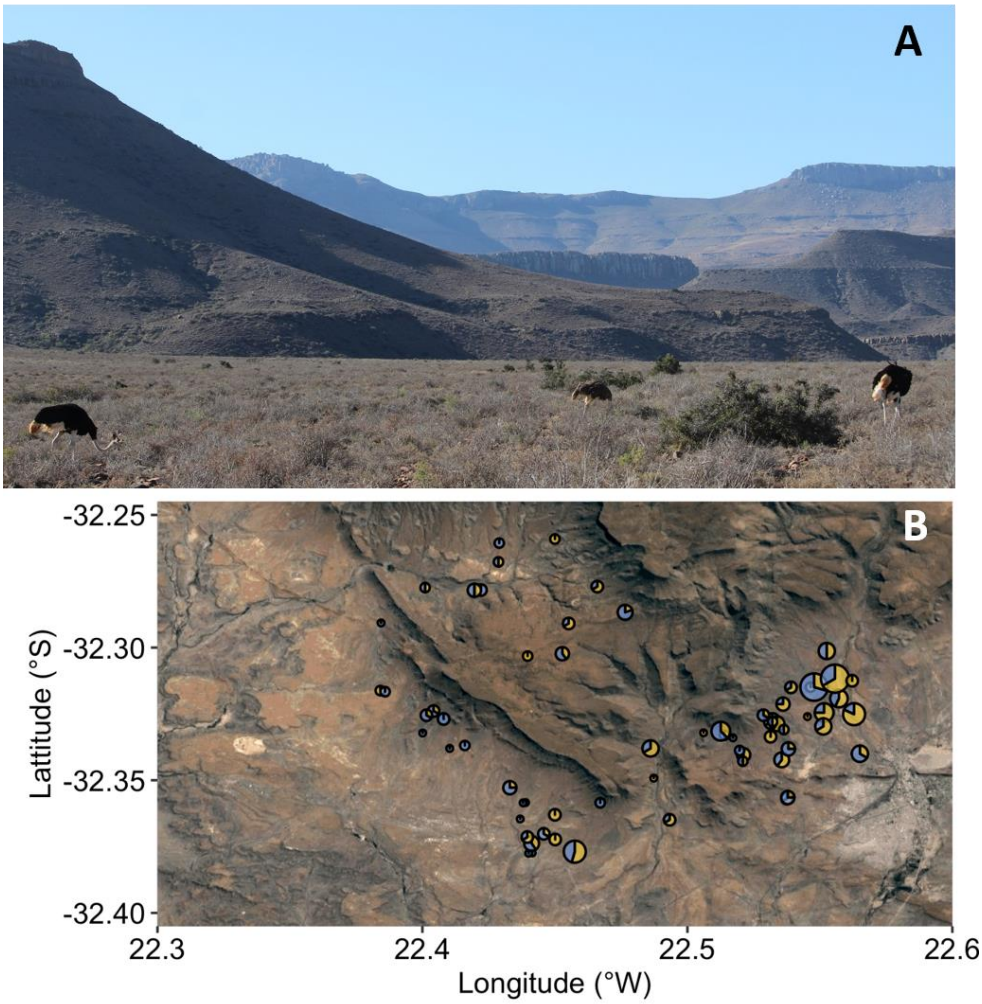


Figure 5: Variation in the complexity of cooperative breeding groups of ostriches. (A) Group of two males and one female breeding ostriches in Karoo National Park. (B) A map of Karoo National Park with the complexity of each group plotted. The size of the circles indicate the number of individuals (maximum = 18, minimum = 1), the blue and yellow segments indicate the proportion of males and females respectively.

The costs and benefits of cooperation differ between males and females in relation to group complexity (paper I)

Individual reproductive success was measured using data on eggs and chicks produced in the experimental groups over a period of six to seven months each breeding season. I used mean reproductive success per individual (total number of eggs and chicks / number of same sex individuals in group) as I was interested in the average reproductive returns for individuals in groups with different

complexities, irrespective of within-group individual variation in reproductive success. These data were combined with behavioural observations (59.25±1.17 (mean±se) hours per group spread over the season) that allowed me to quantify the incubation and mating behaviour of all individuals.

To disentangle the effects of competition and cooperation in different stages of breeding, the need for cooperation over incubation was experimentally removed. This was done by collecting eggs and incubating them artificially during the initial ~5 months of the breeding season. In the final ~2 months of the season, eggs were left in groups to allow natural incubation. When the need for cooperation over incubation was removed, average male reproductive success declined as the number of competitors in groups increased (Figure 6A. Number of $males_{chicks}$ posterior mode (PM) and credible interval (CI) = -0.38 (-0.57 , -0.23), pMCMC = 0.001). For example, single males on average sired three times the number of chicks compared to when males had competitors (Figure 6A). Male reproductive success was also influenced by the number of sexual partners in groups (Figure 6A). As the number of females in groups increased to four, male reproductive output went up markedly (Figure 6A. Number of $females_{chicks}$ PM (CI) = 0.48 (0.33 , 0.69), pMCMC = 0.001), after which it plateaued (Number of $females^2_{eggs}$ PM (CI) = -0.2 (-0.29 , -0.1), pMCMC = 0.001). In contrast, female reproductive success, expressed as both the number of eggs and number of chicks, was largely independent of the number of males and females in groups (Figure 6B).

When eggs were left with their parents, and individuals cooperated over incubation, the way female reproductive success was maximised changed (Figure 6D). The number of chicks that females produced during this period was dependent on the number of females in their group, and also on whether there were multiple or single males (Number of males:Number of $females^2_{chicks}$ PM (CI) = 0.38 (0.07 , 0.77), pMCMC = 0.018). In groups with multiple males, the average number of chicks hatched per female was highest when there were low and high numbers of females (Figure 6D). Conversely, in groups with single males, the number of chicks hatched per female was highest in groups with four females and lowest when females were on their own (Figure 6D). Female reproductive success was therefore lowest in groups with intermediate numbers of each sex and in pairs, which was not the case when individuals did not cooperate over incubation (Figure 6). In contrast, patterns of male reproductive success were not influenced by the need to incubate offspring (Figure 6A & C).

The results above suggest that in ostriches, sexual competition and gaining access to females dominates male reproductive success. In contrast, female reproductive success is strongly dependent on cooperative care and there was little evidence of sexual competition amongst females. The differences in the relative effect of cooperation and competition on male and female reproductive success may explain why groups with a certain level of complexity are more common than others in the wild. The reproductive interests of males and females were best balanced in groups

with single males and four females. Interestingly this is the most common group composition observed in the wild (Bertram, 1992; Magige et al., 2009). Female reproductive success was, nevertheless, equally high when on their own in groups with multiple males and in multi-male multi-female groups, which may provide an answer to why group complexity is so variable in nature.

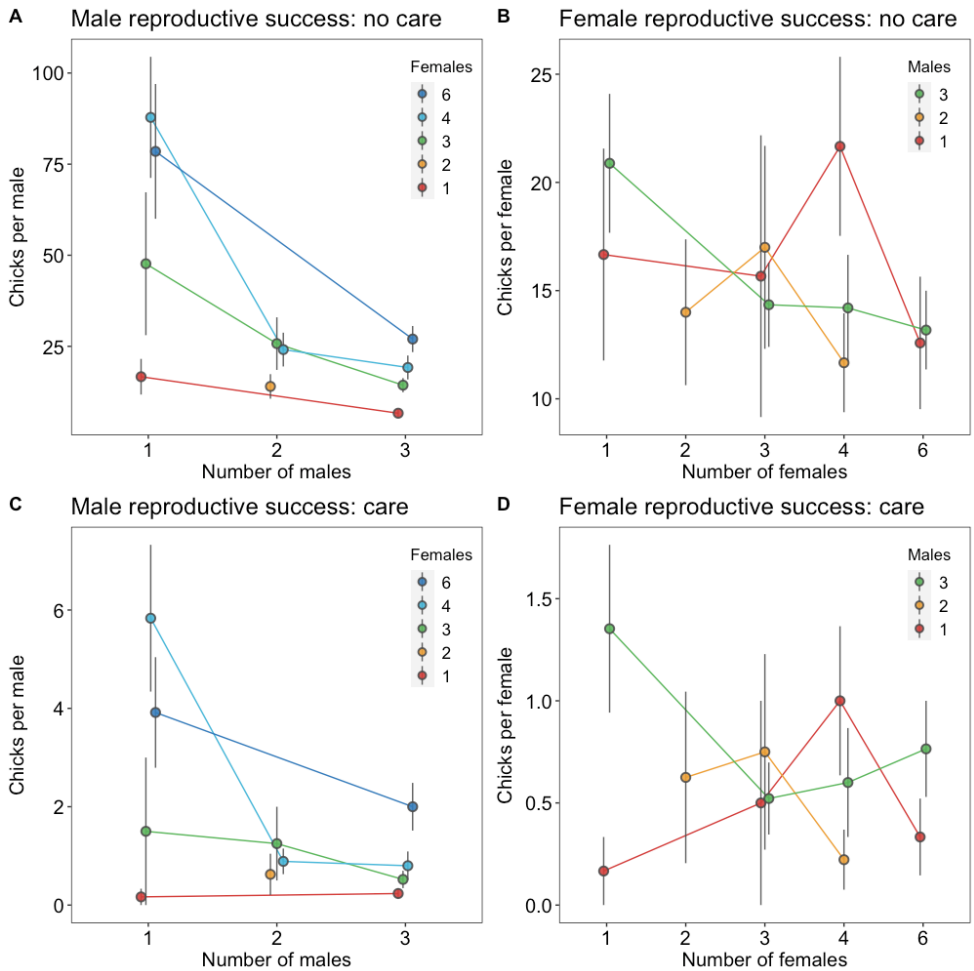


Figure 6: Group complexity and the need for offspring care influence male and female reproductive success. (A) The average number of chicks males sired decreased with the number of males in the group and increased with the number of females, irrespective of whether there was offspring care (C). The number of chicks females produced depended on the number of males in groups and offspring care. In groups with single males, the number of chicks females produced was highest in groups with four females both with (D) and without offspring care (B). In groups with more males, the number of chicks females produced declined with increasing numbers of females when offspring care was removed (B), but was highest in groups with few and many females when there was offspring care (D). See figure S1 for plots of egg production. Means ± SE are plotted.

Group size buffers the costs of cheating in cooperative groups (paper II)

Using the behavioural data obtained on experimental groups, I identified two different strategies among breeding females: "cooperators" and "cheats". Cooperators were individuals that contributed to the collective incubation effort, whereas cheats were individuals that were active breeders, but did not contribute to incubation.

My results show that, although the relative frequency of cheats is higher in big groups (PM (CI) = 0.25 (0.01 , 0.43), pMCMC = 0.04), cheats appeared to have higher reproductive success in small groups (Cheats : number of cooperators^2: PM (CI) = 0.34 (-0.04 , 0.78), pMCMC = 0.074). Why are not cheats then more frequent in small groups, where they seem to maximize their reproductive success? The results of this paper suggest that the answer to this question is that cooperators in small groups respond to cheats by decreasing their incubation effort (Figure 7A. PM (CI) = 0.29 (0.04 , 0.7), pMCMC = 0.03), leading to the collapse of cooperative incubation.

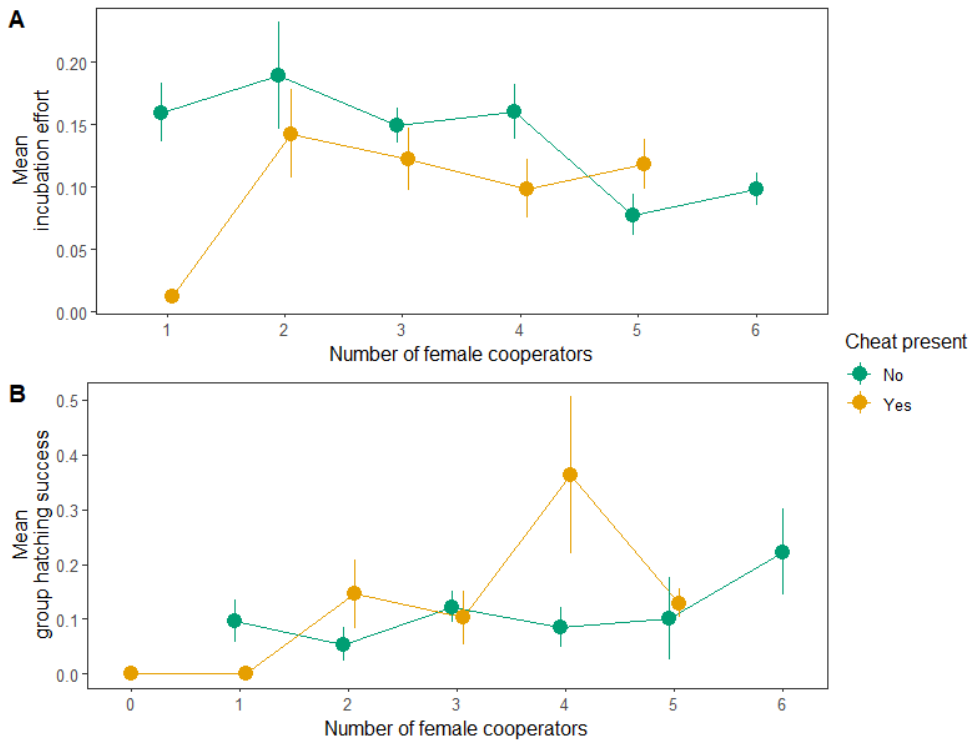


Figure 7: The effect of cheats. A) Mean individual incubation effort across diferent breeding group size in groups with (orange) and without (green) cheats. B) Mean hatching success of eggs in groups with (orange) and without (green) cheats. In groups without female cooperators (x = 0), incubation was taken care of by males only. Groups where no incubation was recorded were excluded from the data. Points and error bars show mean +/- 1 standard error.

This in turn triggers a "tragedy of the commons" (Hardin, 1968; Rankin et al., 2007), by severely compromising reproductive success in small groups (Figure 7B. PM (CI) = 1.09 (0.21 , 1.84), pMCMC = 0.006). Together, these results suggest that group size buffers the negative effects of cheating in ostrich groups, contributing to the stability of cooperation.

Variation in individual temperature tolerance and its effects on fitness (paper III)

In **paper III**, daily records from 20 years of egg production between 1998 and 2018 from 678 pair-breeding female ostriches, as well as ten years of data (2008-2018) of ejaculates of 22 solitary males, were combined with daily temperature records (that ranged from ranged from −5 to 45 °C), to quantify individual variation in temperature tolerance and its effects on gametic traits. Moreover, hatching success data of artificially incubated eggs on the same population was used to assess the fitness effects of thermal tolerance.

The results of **paper III** show that the number of eggs females laid and the number of sperm males ejaculated were significantly reduced by both increasing and decreasing ambient temperature (Figure 8a, b). The effects of temperature were not immediate, but resulted from a critical thermal window 2–4 days before laying and ejaculation. During this critical thermal window, egg laying rate peaked at 20 °C, dropping by 18% and 15% when temperatures decreased and increased by 5 °C, respectively (Figure 8a). Similar reductions were seen in the number of sperm males ejaculated (19% with 5 °C increases and decreases from the optimum; Figure 8b), but the thermal optimum appeared to be slightly higher than for egg laying, peaking at ~26 °C.

A significant part of the variation in the reduction of gamete production was explained by individual differences, suggesting that there is individual variation in thermal tolerance in the study population. Differences between individual females explained 24% of variation in egg laying rate declines when temperatures increased, and 18% of variation when temperatures decreased. Similarly, some males were much more resilient to temperature change than others, as indicated by the number of sperm they ejaculated. When temperatures increased, 47% of variation in the decline in sperm numbers was explained by differences between males, and 57% when temperature decreased.

Hatching success was significantly affected by the temperature birds experienced prior to laying: hatching success was reduced by 4–7% with 5 °C increases and decreases from 20 °C (Figure 8c; $T_{heat\ stress}$ (credible interval, CI) = −0.26 (−0.43, −0.09), pMCMC = 0.002; $T_{cold\ stress}$ (CI) = −0.57 (−0.98, −0.01), pMCMC = 0.028). Combined with changes in laying rates, this resulted in the total number of offspring decreasing by 28% with an increase in temperature of 5 °C, and 44% with a

temperature decrease of 5 °C from 20 °C (Figure 8d; $T_{cold\ stress}$ (CI) = −2.10 (−2.57, −1.60), pMCMC = 0.001; $T_{heat\ stress}$ (CI) = −1.42 (−1.61, −1.21), pMCMC = 0.001).

The results of **paper III** show that ostriches experience thermal stress when temperatures are below 15 °C, or above 25 °C, but that individuals varied substantially in their thermal tolerance. This leads to the question of how this variation is maintained in an environment with highly fluctuating temperatures,

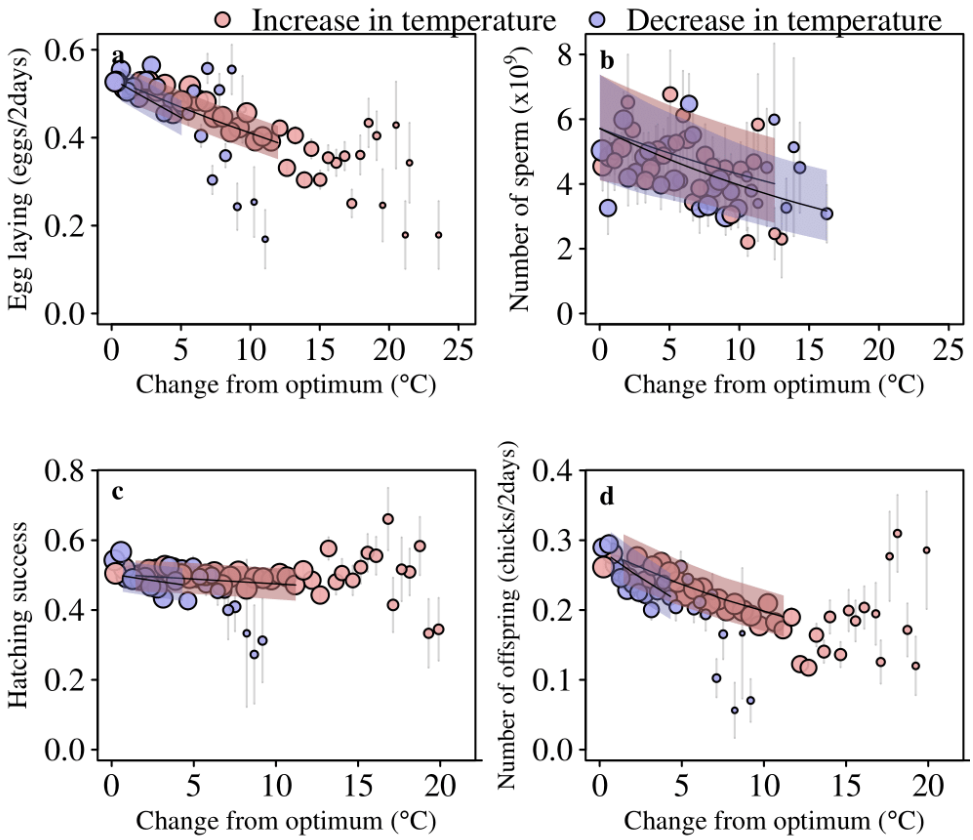


Figure 8 Temperature extremes compromise male (n =22) and female (n = 652) fertility. Female egg laying rate (a) and number of sperm ejaculated by males (b) were both highly sensitive to increases and decreases in temperature. Hatching success (c), which is influenced by the egg mass, sperm numbers and sperm viability, was also less affected by temperature change. The number of offspring (d) is a product of hatching success as well as egg laying and showed sensitivity to changes in temperature during egg laying. Ostrich females can only lay an egg every other day and we therefore used number of eggs or chicks per number of two-day intervals (eggs/2 days or chicks/2 days). The range of temperatures that sperm traits were measured at differed from the other traits, because it was not possible to collect sperm across all years. Fitted lines and 95% credible intervals (shaded area) from the primary set of models are shown for traits significantly affected by temperature. For binomial models the fitted lines span the modelled binned temperature classes making them robust to outliers. Points are averages with standard errors binned according to the temperature variable. Point size illustrates relative number of observations.

where fitness is likely to be maximized with high thermal tolerance. One possible explanation is that variation in thermal tolerance is maintained by the cooperative breeding nature of ostriches. The results in **paper III** were obtained from individuals breeding in pairs. In **paper IV** I therefore test whether the reproductive consequences of low heat tolerance can be buffered by benefits of breeding in cooperative groups.

Individual heat tolerance explains variation in cooperative behaviour (paper IV)

In **paper IV**, an additional element was added to the complexity of the experimental groups of cooperatively breeding ostriches: within-group variation in heat tolerance. The methods and data in **paper III** were used together with an available pedigree of the population to quantify genetic heat tolerance in all adult birds. Experimental birds were then selected so that relative frequency of heat tolerant individuals varied across groups. In this way, I could study the effect of individual heat tolerance, and its interaction with other elements of group complexity, such as numbers of males and females, on cooperative incubation behaviour. Note that, since incubation behaviour is likely to interfere with the behavioural response to heat (Maloney, 2008), the focus of this paper is on heat tolerance, the cold-tolerance element of thermal tolerance has consequently been omitted. Thus, all temperatures below 25 °C are referred to as "benign".

The results of **paper IV** show that individual contributions to cooperative incubation were highly variable, and that this variation was predicted by individual heat tolerance. Heat tolerant females were much more likely to engage in incubation, both at benign and hot temperatures (Figure 9A. Benign: PM (CI) = 0.38 (0.1 , 0.55), pMCMC = 0.004. Hot: PM (CI) = 0.2 (-0.03 , 0.44), pMCMC = 0.07). Heat tolerant females also invested more time in incubation (Figure 9C. Benign: PM (CI) = 0.67 (0.29 , 1.46), pMCMC = 0.006. Hot: PM (CI) = 0.67 (-0.06 , 1.29), pMCMC = 0.054). Females with low heat tolerance reduced their incubation effort in groups with more females, whereas females with high heat tolerance largely maintained their incubation effort across different group sizes, even at high temperatures (Figure 9C). In contrast to females, male genetic heat tolerance did not influence the probability that they incubated or the amount of time they invested in incubation at either high or benign temperatures (Figures 8B & 8D).

In **paper IV**, I also tested whether differences in the incubation behaviour of individuals with low and high heat tolerance influenced the emergence of cooperation at the group level. This was done by examining whether the frequency of heat tolerant individuals in groups predicted the number of individuals that contributed to cooperation over incubation. I found that as the opportunity for cooperation increased (big groups), groups with a higher frequency of heat tolerant

females had more cooperators under hot conditions than groups with lower frequencies of heat tolerant females (Figure 10A). This effect disappeared under benign temperatures, suggesting that higher frequencies of heat tolerant females results in greater cooperation over incubation during periods of heat stress (Hot: PM (CI) = 0.45 (0.04 , 0.94), pMCMC = 0.032; Benign: PM (CI) = 0.35 (-0.19 , 0.68), pMCMC = 0.246).

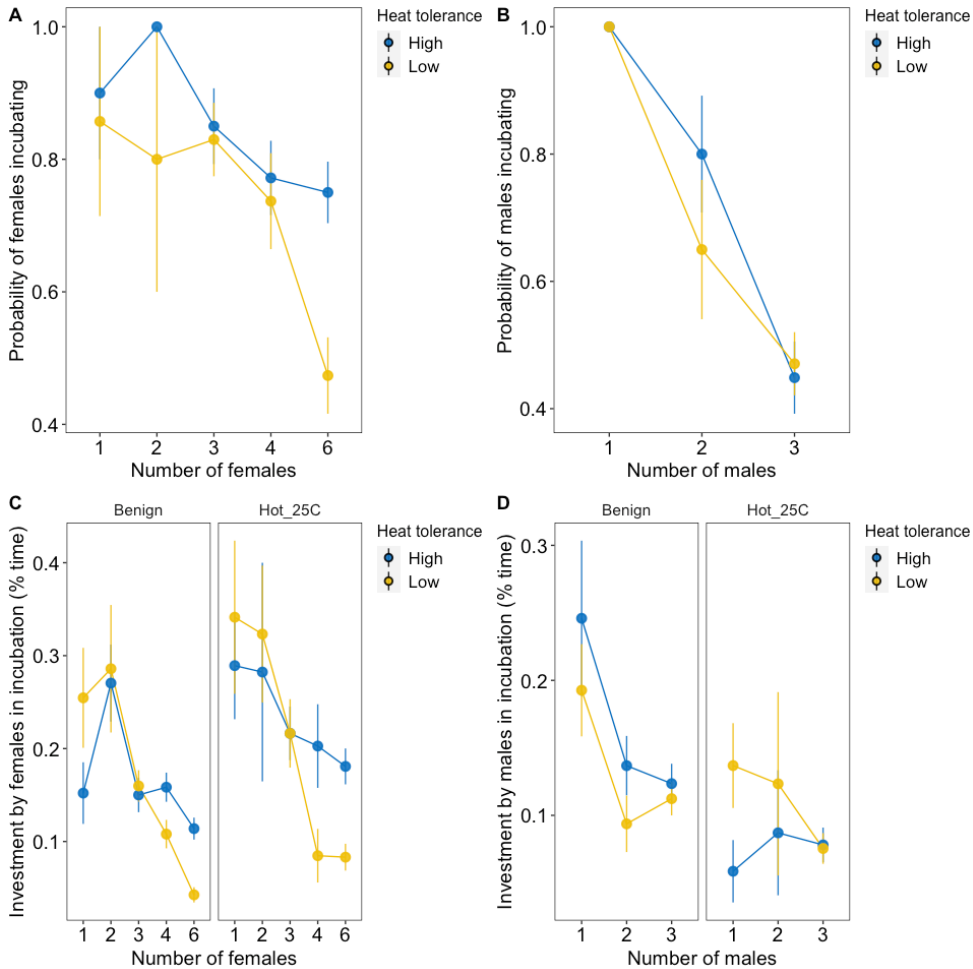


Figure 9: Genetic heat tolerance influences incubation behaviour. The probability that females (A) and males (B) with high (>median) and low (<=median) heat tolerance contributed to incubation. The proportion of time females (C) and males (D) with high and low heat tolerance invested in incubation under benign (<25°C) and hot temperatures (>25°C). Heat tolerance was categorised for illustration purposes only, models analyse continuous values. Means ± SE are plotted.

Heat tolerance in males had the opposite effect to that in females under hot conditions. As the frequency of heat tolerant males increased, the number of males that cooperated over incubation decreased (Figure 10B. Hot: PM (CI) = -0.35 (-0.58 , -0.05), pMCMC = 0.018. Benign: PM (CI) = -0.07 (-0.3 , 0.1), pMCMC = 0.262).

The results in **paper IV** suggest that individual sensitivity to environmental stress affects the cost to benefit ratio of cooperation. Moreover, the effects of tolerance to environmental stress differ between the sexes, adding yet another level of complexity that can help us better account for the variation in social organization.

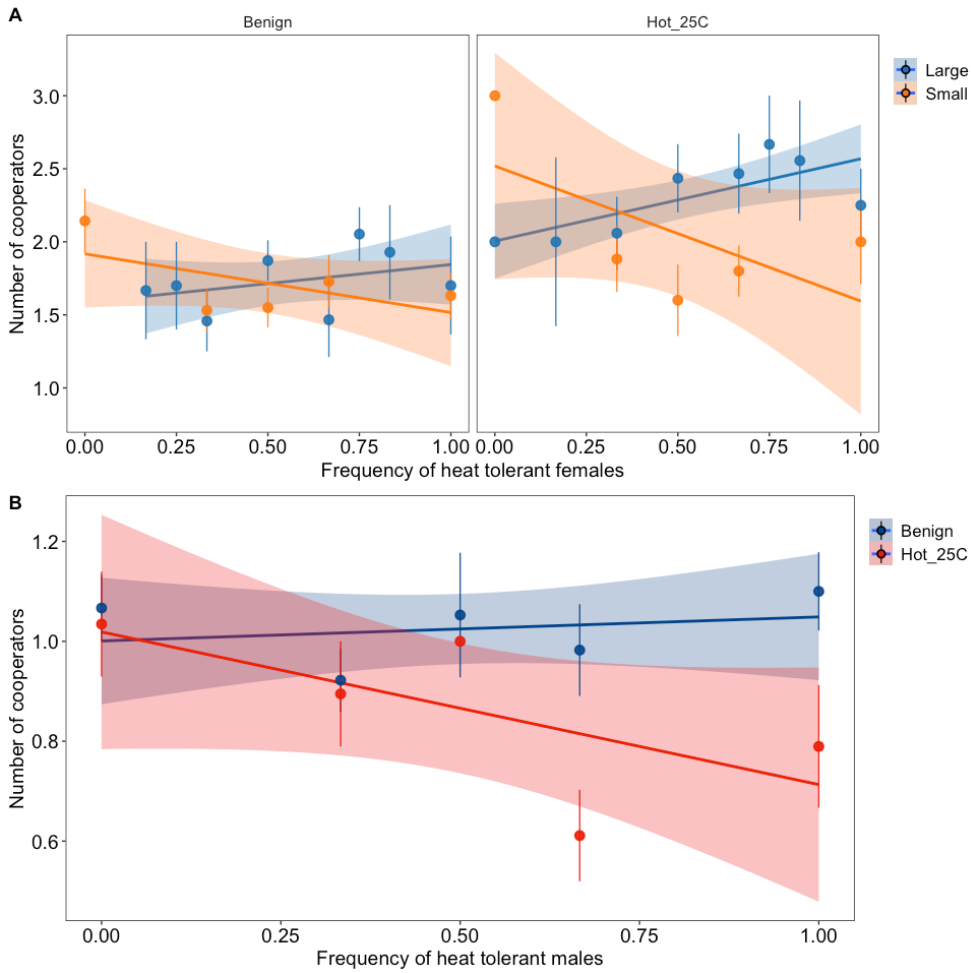


Figure 10: The emergence of cooperation in groups with different frequencies of heat tolerant individuals. (A) The number of females that cooperated over incubation in relation to the frequency of heat tolerant females in small (<=3 females) and large groups (>3 females) under benign (<25°C) and hot temperature (>25°C) conditions. Female group size was categorised for graphical purposes only. (B) The number of males that cooperated over incubation in relation to the frequency of heat tolerant males under benign (<25°C) and hot temperature (>25°C).

The effect of heat tolerance on the cost to benefit ratio of cooperation is expected to lead to a reduction in the need for cooperation under hot conditions. However, the fact that I found a higher frequency of heat tolerant females resulted in greater levels of cooperation in big groups at hot temperatures (Figure 10A, see panel to the right), seems contrary to this idea. Instead, my results suggest that higher heat tolerance in females may in fact drive cooperation.

It is important to note that these increased levels of cooperation were not because heat tolerant females had a greater propensity to cooperate in hot environments: among cooperators, heat tolerance did not affect the likelihood of cooperation over incubation in a given temperature condition (Benign: PM (CI) = 0.02 (-0.05 , 0.06), pMCMC = 0.782. Hot: PM (CI) = -0.02 (-0.07 , 0.05), pMCMC = 0.654). Instead, higher levels of cooperation appear to emerge in groups with more heat tolerant females due to their inherent higher probability of engaging in incubation. Additionally, the incubation behaviour of heat tolerant females was largely insensitive to variations in group size (Figure 9A & C), suggesting that heat tolerant females are relatively unaffected by the benefits of cooperation. This might be because they pay a lower cost of incubation (although we do not test this), which in turn results in lower benefits of cooperation. For females with low heat tolerance, on the other hand, incubation is likely to be costlier, and thus they get relatively large benefits by being able to reduce their incubation effort when being part of a big cooperative group (Figure 9A & C).

Figure 11. A schematic representation of the author.
By Sina Melgar Hilz.

Conclusions

Disentangling the effects of the many factors that affect the cost to benefit ratio of cooperation is difficult (Stuart A. West et al., 2021). This is true, in part due to the difficulties of finding systems amenable to experimental manipulation of entire cooperative groups. Much of the novelty and value of this thesis, thus lies in its experimental approach.

This thesis shows that the cost to benefit ratio of cooperative versus competitive behaviour can change with the complexity of social groups. In particular, the interests of individuals within groups can differ in a number of ways, helping explain why cooperative groups can be so variable in their complexity, even under the same ecological conditions. The relationship between cooperation and group complexity has long been discussed (Alexander, 1974; Williams, 1996). However, how and why they are linked has been challenging to explain due to lack of experimental tests. The results presented here, therefore provide important pieces of evidence that help us understand why social groups vary in complexity in nature.

Individuals vary in their response to the conditions that affect cooperative behaviour. Environmental factors, mediated by individual variation in environmental sensitivity, are key drivers of this variation. The fact that some individuals are more prone to cooperating than others also implies that when a cooperative group has formed, it is vulnerable to exploitation. Some individuals do not cooperate at all but reap the rewards of cooperation, compromising the stability of the group. Others are constrained by the environment and do less than their fair share. This individual variability, and the fact that the social and physical environments interact with each other, have implications for predictions about how cooperative species are able to expand their ranges to habitats that pose environmental challenges. Stress tolerant individuals might pave the way for conspecifics less tolerant to environmental stress, so that the species' range can be expanded. This has also implications for our understanding of how species will be able to maintain their ranges in face of the global climate crisis.

My results on the effects of variation in heat tolerance on cooperative ostrich behavior suggest that there are benefits for groups to having individuals with varying genotypes in fluctuating environments. Ostriches typically form groups of unrelated individuals (Kimwele & Graves, 2003). Genetic variation within groups is therefore likely to be higher than in species where groups are formed of relatives.

For example, in family groups, where relatedness is high, variation in environmental sensitivity within groups is likely to be lower due to all individuals having similar genotypes.

Although cooperation still has a buffering effect against environmental stress in family groups (Cornwallis et al., 2017; Covas et al., 2008; Duffy & Macdonald, 2010; Emlen, 1982a; McLeod & Wild, 2014; Pen & Weissing, 2000; Rubenstein, 2011). Fluctuating environmental conditions might select for higher genetic variation (Husby et al., 2011; Rowiński & Rogell, 2017). This can be achieved by for instance increased levels of polyandry (El-Niweiri & Moritz, 2011; E. O. Wilson, 1971), resulting in decreased group relatedness. Fluctuating environmental conditions can also lead to the dissolution of family groups by favouring direct over indirect fitness (Bourke, 2014). This illustrates how environmental conditions can shift the relative importance of direct and indirect fitness in cooperative groups (see figure 1). Fluctuating environments are likely to promote higher genetic variation, and thus lower relatedness, while more stable environments allow lower levels of genetic variation, paving the way for indirect fitness benefits.

Another important consideration when examining the results of this thesis is that they are a snap shot in time, not accounting for longer term effects, such as life time reproductive success, longevity or cumulative effects of heat stress. Therefore, a potential future avenue of research would be to examine how the effects of successive breeding attempts affect the cost to benefit ratio of cooperation. Using a novel and versatile study system, this thesis provides a starting point for such research, along with new insights into the question of why cooperative groups vary in social organization.

Figure 12. An attentive observer. Julian Melgar demostrates how field work can be on the observation tower at Oudtshoorn's research farm.

Acknowledgments

Charlie, it's hard to sufficiently express how thankful I'm for having had the opportunity of studying under your supervision. I could dedicate this whole section entirely to you, but that would be weird. I'll therefore limit myself to thanking you for providing such a sound scientific example, and such an admirable example of humanity. Thanks!

Mads, you have been one of the main contributors of this thesis and a source of inspiration. Thanks for always being so supportive, and so willing to answer my questions and help me fix the countless R-scripts that I broke.

Bengt, I know you feel like you have been free-riding this supervisor role a little, me having two other supervisors and all. But remember the early days, when I spent most of my time in the lab, or in front of a computer screen trying to make sense of microsatellite data. You were there then, and you were a big help, Thanks for that, and thanks for always having your door open for me!

Maud, you are not formally my supervisor, but in practice you have supervised all my field seasons in South Africa, and collected a big part of the data that are included in this thesis. You have been truly indispensable. You have also become a good friend and I really hope I can come and pet your new little pets soon!

Philip, you have been my office mate and my close friend during practically my whole PhD. You have contributed enormously, both to my scientific development, and to my mental sanity. Thanks for being such a good friend and such a kind human being!

Hanna S, imagine that we have studied together since we were master students! In a way, it feels a little like we are siblings. Such a nice feeling!

Hanna B, I feel like I have made a friend for life in you. I hope you feel the same way. Maybe when we are old we can meet and exaggerate all our stories about South Africa. Like the one about the time we saw a shark eat a whale and Charlie missed it because he was taking pictures of some undistinguishable little grey bird. It was epic!

Tonny, you have truly made my time in South Africa so much richer and enjoyable. All our pizza nights and our evening chats in the veranda of the big house will always be happy memories. Thank you for sharing those moments with me! Thank

you as well for helping me with so many things in the lab. Your help and your friendship have meant a lot to me.

I also want to thank the members of the Cornwallis group that I haven't already mentioned. **Emily**, **Maria**, **Qinyang**. I feel very lucky to be part of such a stimulating and friendly scientific environment. Thanks for always being so supportive!

To the people in the Biology Show. **Therese**, **Lana**, **Pierre**, **Jacob**, **Lina**, **Marie**, **Emily**. Thanks for all the fun and all the laughter. Thank you as well for letting me dress up as Spiderman, and for only waiting like three years before you told me that the suit was way too tight for me, and that I was not giving the kids a show about zoology, but one about anatomy.

I have taught in a number of courses during my time as a PhD candidate, and as it often is with teaching, I have learned a lot in the process. Much of what I learned came from other PhD students that I taught together with. **Linus**, **Atticus**, **David**, **Ann-Kathrin**, **Sofia**, **Samantha**. Thanks for being a part of my learning process!

Inga, thank you for all the fun adventures and for your good sense of humour. It's always fun to hang out with you!

Emma, thanks for all the lovely excursions. Now that I'll be unemployed, we maybe can pick up on the bird watching and butterfly hunting again.

Suvi, thanks for all the lunches and for your wonderful friendship. Even though I'll never forgive you for all the acts of vandalism against the MEEL Vegan Society. I know it was you! They have stopped since you left.

Beatriz, your friendship has been very valuable, and your scientific example a real inspiration. Thank you for that, and thanks for the dance!

Dovilė, thanks for your friendship and your love. It feels beautiful that distance and time don't seem to matter. I will always keep collecting seashells, small sticks and rocks for you to have.

Violeta, thanks for embellishing my science!

Rachel, you were my first scientific mentor and are still a big source of inspiration. Thank you for being so kind and encouraging, and thank you for your friendship!

Kristaps, **David**, **Hans**, and the rest of the Humming Bored Gamers. Thanks for all the game nights!

Jessica, thank you so much for reading and giving feedback on my half-time seminar, that was the foundation for the introduction in this thesis. And thank you for being such a refreshing element of change and renewal in the department!

Anders, thank you for making sure that I complied with all the formalities involved with PhD-education. I know I wasn't the best at that, but you succeeded with keeping me on track. Thanks!

Staffan, you have such a nice and inspiring way of leadership. It has always felt very reassuring to know that you are in charge.

I want to thank all the people at Oudtshoorn Research Farm in South Africa for always being so welcoming and helpful. **Anel**, **Zanel**, **Bernard**, **Molatelo**, **Stefan,** thank you so much for your hospitality.

A special thanks goes to **Madeli**, for your friendship, and for teaching me how to dance sokkie, Oudtshoorn-style; although I might have taught Oudtshoorn how to dance sokkie Latino-style.

I also want to thank you, **Naomi**, for all the help in the lab, and for your fantastic kindness.

I am also very grateful towards the staff in the hatchery and breeding facilities of the farm. **Jakob**, **Lilly**, **Ursula**, **Phillip**, **Isak**, **Jan**, **Damon**, **Fortuin**, **Samuel**, **Niklaas**. Without you this thesis wouldn't have been possible.

To my family. **Pa**, **Nahiel**, you two have always been there to encourage and challenge me, and have taught me, by example, to follow my dreams. I admire and love you both enormously. **Sina**, **Jakob**, gracias por alegrarme la vida! **Sari, Pepe, André, Nadja,** thank you for all the love and support that you have given me. It warms my heart knowing that I have you close.

Stone, you have always been a source of admiration and inspiration for me. You helped me widen my horizons already when I was a little kid. It is much thanks to you that I am who I am today. Thank you tiger-mom!

Ma, you have been the most important person in my life. Your example and your love have taught me most things that really matter. It is awfully painful not being able to share this moment, any moment, with you. Your absence is a dark, frightening emptiness.

And to my friends that have become family. **Ulf**, **Nadia**, your friendship and love mean the world to me.

Tack, **Henrik Åhl**!

Ode to Thanks

By: **Pablo Neruda**—translated by Ken Krabbenhoft

Thanks to the word that says thanks!
Thanks to thanks,
word
that melts
iron and snow!
The world is a threatening place
until
thanks
makes the rounds
from one pair of lips to another,
soft as a bright
feather
and sweet as a petal of sugar,
filling the mouth with its sound
or else a mumbled
whisper.
Life becomes human again:
it's no longer an open window.
A bit of brightness
strikes into the forest,
and we can sing again beneath the leaves.
Thanks, you're the medicine we take
to save us from
the bite of scorn.
Your light brightens the altar of harshness.
Or maybe
a tapestry
known
to far distant peoples.

Travelers
fan out
into the wilds,
and in the jungle
of strangers,
merci
rings out
while the hustling train
changes countries,
sweeping away borders,
then spasibo
clinging to pointy
volcanoes, to fire and freezing cold,
or danke, yes! and gracias, and
the world turns into a table:
a single word has wiped it clean,
plates and glasses gleam,
silverware tinkles,
and the tablecloth is as broad as a plain.
Thank you, thanks,
for going out and returning,
for rising up
and settling down.
We know, thanks,
that you don't fill every space-
you're only a word-
but
where your little petal
appears
the daggers of pride take cover,
and there's a penny's worth of smiles.

Literature

Alexander, R. D. (1974). The Evolution of Social Behavior. *Annual Review of Ecology and Systematics*, *5*(1), 325–383. https://doi.org/10.1146/annurev.es.05.110174.001545

AlRashidi, M., Kosztolányi, A., Küpper, C., Cuthill, I. C., Javed, S., & Székely, T. (2010). The influence of a hot environment on parental cooperation of a ground-nesting shorebird, the Kentish plover Charadrius alexandrinus. *Frontiers in Zoology*, *7*(1), 1. https://doi.org/10.1186/1742-9994-7-1

Arnold, K. E., & Owens, I. P. F. (1999). Cooperative breeding in birds: The role of ecology. *Behavioral Ecology*, *10*(5), 465–471. https://doi.org/10.1093/beheco/10.5.465

Arnqvist, G. (2004). Sexual Conflict and Sexual Selection: Lost in the Chase. *Evolution*, *58*(6), 1383–1388.

Baglione, V., Marcos, J. M., Canestrari, D., & Ekman, J. (2002). Direct fitness benefits of group living in a complex cooperative society of carrion crows, Corvus corone corone. *Animal Behaviour*, *64*(6), 887–893. https://doi.org/10.1006/anbe.2002.2007

Balshine-Earn, S., Neat, F. C., Reid, H., & Taborsky, M. (1998). Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. *Behavioral Ecology*, *9*(5), 432–438. https://doi.org/10.1093/beheco/9.5.432

Bergmüller, R., Johnstone, R. A., Russell, A. F., & Bshary, R. (2007). Integrating cooperative breeding into theoretical concepts of cooperation. *Behavioural Processes*, *76*(2), 61–72. https://doi.org/10.1016/j.beproc.2007.07.001

Bertram, B. C. R. (1979). Ostriches recognise their own eggs and discard others. *Nature*, *279*(5710), 233–234. https://doi.org/10.1038/279233a0

Bertram, B. C. R. (1992). *The Ostrich Communal Nesting System:* Princeton University Press; JSTOR. https://doi.org/10.2307/j.ctt7ztm99

Blackmore, C. J., Peakall, R., & Heinsohn, R. (2011). The absence of sex-biased dispersal in the cooperatively breeding grey-crowned babbler. *The Journal of Animal Ecology*, *80*(1), 69–78. https://doi.org/10.1111/j.1365-2656.2010.01761.x

Bonato, M. (2009). *Mate choice and immunocompetence in ostriches (Struthio camelus)* [Thesis, Stellenbosch : University of Stellenbosch]. https://scholar.sun.ac.za:443/handle/10019.1/1257

Boomsma, J. J., & Grafen, A. (1990). Intraspecific Variation in Ant Sex Ratios and the Trivers-Hare Hypothesis. *Evolution*, *44*(4), 1026–1034. https://doi.org/10.1111/j.1558-5646.1990.tb03823.x

Boomsma, Jacobus J. (2007). Kin Selection versus Sexual Selection: Why the Ends Do Not Meet. *Current Biology*, *17*(16), R673–R683. https://doi.org/10.1016/j.cub.2007.06.033

Boomsma, Jacobus J. (2009). Lifetime monogamy and the evolution of eusociality. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1533), 3191–3207. https://doi.org/10.1098/rstb.2009.0101

Bourke, A. F. G. (2011). Principles of Social Evolution. In *Principles of Social Evolution*. Oxford University Press. https://oxford.universitypressscholarship.com/view/10.1093/acprof:oso/9780199231157.001.0001/acprof-9780199231157

Bourke, A. F. G. (2014). Hamilton's rule and the causes of social evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1642), 20130362. https://doi.org/10.1098/rstb.2013.0362

Brooks, S. P., & Gelman, A. (1998). General Methods for Monitoring Convergence of Iterative Simulations. *Journal of Computational and Graphical Statistics*, *7*(4), 434–455. https://doi.org/10.1080/10618600.1998.10474787

Brown, C. R. (1984). Laying Eggs in a Neighbor's Nest: Benefit and Cost of Colonial Nesting in Swallows. *Science*, *224*(4648), 518–519. https://doi.org/10.1126/science.224.4648.518

Brown, J. L. (1974). Alternate Routes to Sociality in Jays—With a Theory for the Evolution of Altruism and Communal Breeding. *American Zoologist*, *14*(1), 63–80. https://doi.org/10.1093/icb/14.1.63

Cameron, E. Z., Setsaas, T. H., & Linklater, W. L. (2009). Social bonds between unrelated females increase reproductive success in feral horses. *Proceedings of the National Academy of Sciences*, *106*(33), 13850–13853. https://doi.org/10.1073/pnas.0900639106

Chapman, T., Arnqvist, G., Bangham, J., & Rowe, L. (2003). Sexual conflict. *Trends in Ecology & Evolution*, *18*(1), 41–47. https://doi.org/10.1016/S0169-5347(02)00004-6

Chesson, P., & Huntly, N. (1997). The Roles of Harsh and Fluctuating Conditions in the Dynamics of Ecological Communities. *The American Naturalist*, *150*(5), 519–553. https://doi.org/10.1086/286080

Clutton-Brock, T. (2002). Breeding Together: Kin Selection and Mutualism in Cooperative Vertebrates. *Science*, *296*(5565), 69–72. https://doi.org/10.1126/science.296.5565.69

Clutton-Brock, T. (2009). Cooperation between non-kin in animal societies. *Nature*, *462*(7269), 51–57. https://doi.org/10.1038/nature08366

Clutton-Brock, T., & Manser, M. (2016). Meerkats: Cooperative breeding in the Kalahari. In J. L. Dickinson & W. D. Koenig (Eds.), *Cooperative Breeding in Vertebrates: Studies of Ecology, Evolution, and Behavior* (pp. 294–317). Cambridge University Press. https://doi.org/10.1017/CBO9781107338357.018

Cockburn, A. (1998). Evolution of Helping Behavior in Cooperatively Breeding Birds. *Annual Review of Ecology and Systematics*, *29*(1), 141–177. https://doi.org/10.1146/annurev.ecolsys.29.1.141

Cooper, G. A., & West, S. A. (2018). Division of labour and the evolution of extreme specialization. *Nature Ecology & Evolution*, *2*(7), 1161–1167. https://doi.org/10.1038/s41559-018-0564-9

Cornwallis, C. K., Botero, C. A., Rubenstein, D. R., Downing, P. A., West, S. A., & Griffin, A. S. (2017). Cooperation facilitates the colonization of harsh environments. *Nature Ecology & Evolution*, *1*(3), 0057. https://doi.org/10.1038/s41559-016-0057

Covas, R., du Plessis, M. A., & Doutrelant, C. (2008). Helpers in colonial cooperatively breeding sociable weavers Philetairus socius contribute to buffer the effects of adverse breeding conditions. *Behavioral Ecology and Sociobiology*, *63*(1), 103–112.

Davies, N. B., & Houston, A. I. (1986). Reproductive Success of Dunnocks, Prunella modularis, in a Variable Mating System. II. Conflicts of Interest Among Breeding Adults. *Journal of Animal Ecology*, *55*(1), 139–154. https://doi.org/10.2307/4698

de Villemereuil, P., Schielzeth, H., Nakagawa, S., & Morrissey, M. (2016). General Methods for Evolutionary Quantitative Genetic Inference from Generalized Mixed Models. *Genetics*, *204*(3), 1281–1294. https://doi.org/10.1534/genetics.115.186536

Deeming, D. C. (Ed.). (2001). *Avian Incubation: Behaviour, Environment and Evolution*. Oxford University Press.

Dierkes, P., Heg, D., Taborsky, M., Skubic, E., & Achmann, R. (2005). Genetic relatedness in groups is sex-specific and declines with age of helpers in a cooperatively breeding cichlid. *Ecology Letters*, *8*(9), 968–975. https://doi.org/10.1111/j.1461-0248.2005.00801.x

Downing, P. A., Griffin, A. S., & Cornwallis, C. K. (2018). Sex differences in helping effort reveal the effect of future reproduction on cooperative behaviour in birds. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1885), 20181164. https://doi.org/10.1098/rspb.2018.1164

Downing, P. A., Griffin, A. S., & Cornwallis, C. K. (2020). Group formation and the evolutionary pathway to complex sociality in birds. *Nature Ecology & Evolution*, *4*(3), 479–486. https://doi.org/10.1038/s41559-020-1113-x

Duffy, J. E., & Macdonald, K. S. (2010). Kin structure, ecology and the evolution of social organization in shrimp: A comparative analysis. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1681), 575–584. https://doi.org/10.1098/rspb.2009.1483

Dugatkin, L. A. (1997). *Cooperation Among Animals: An Evolutionary Perspective*. Oxford University Press.

El-Niweiri, M. A. A., & Moritz, R. F. A. (2011). Mating in the rain? Climatic variance for polyandry in the honeybee (Apis mellifera jemenitica). *Population Ecology*, *53*(3), 421. https://doi.org/10.1007/s10144-011-0271-8

Emlen, S. T. (1982a). The Evolution of Helping. I. An Ecological Constraints Model. *The American Naturalist*, *119*(1), 29–39. https://doi.org/10.1086/283888

Emlen, S. T. (1982b). The Evolution of Helping. II. The Role of Behavioral Conflict. *The American Naturalist*, *119*(1), 40–53. https://doi.org/10.1086/283889

Emlen, S. T., & Wrege, P. H. (1986). Forced Copulations and Intra-specific Parasitism: Two Costs of Social Living in the White-fronted Bee-eater. *Ethology*, *71*(1), 2–29. https://doi.org/10.1111/j.1439-0310.1986.tb00566.x

Faaborg, J., Parker, P. G., DeLay, L., de Vries, Tj., Bednarz, J. C., Maria Paz, S., Naranjo, J., & Waite, T. A. (1995). Confirmation of cooperative polyandry in the Galapagos hawk (Buteo galapagoensis). *Behavioral Ecology and Sociobiology*, *36*(2), 83–90. https://doi.org/10.1007/BF00170712

Fitzpatrick, J. W., & Bowman, R. (2016). Florida scrub-jays: Oversized territories and group defense in a fire-maintained habitat. In J. L. Dickinson & W. D. Koenig (Eds.), *Cooperative Breeding in Vertebrates: Studies of Ecology, Evolution, and Behavior* (pp. 77–96). Cambridge University Press. https://doi.org/10.1017/CBO9781107338357.006

Galliard, J.-F. L., Fitze, P. S., Ferrière, R., & Clobert, J. (2005). Sex ratio bias, male aggression, and population collapse in lizards. *Proceedings of the National Academy of Sciences*, *102*(50), 18231–18236. https://doi.org/10.1073/pnas.0505172102

Gardner, A., & West, S. A. (2004). Spite and the scale of competition. *Journal of Evolutionary Biology*, *17*(6), 1195–1203. https://doi.org/10.1111/j.1420-9101.2004.00775.x

Geneious | Bioinformatics Software for Sequence Data Analysis. (2017). Geneious. https://www.geneious.com/

Ghoul, M., Griffin, A. S., & West, S. A. (2014). Toward an Evolutionary Definition of Cheating. *Evolution*, *68*(2), 318–331. https://doi.org/10.1111/evo.12266

Gonzalez, J.-C. T., Sheldon, B. C., & Tobias, J. A. (2013). Environmental stability and the evolution of cooperative breeding in hornbills. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1768), 20131297. https://doi.org/10.1098/rspb.2013.1297

Gore, J., Youk, H., & van Oudenaarden, A. (2009). Snowdrift game dynamics and facultative cheating in yeast. *Nature*, *459*(7244), 253–256. https://doi.org/10.1038/nature07921

Hadfield, J. D. (2010). MCMC Methods for Multi-Response Generalized Linear Mixed Models: The **MCMCglmm** *R* Package. *Journal of Statistical Software*, *33*(2). https://doi.org/10.18637/jss.v033.i02

Haig, D., & Grafen, A. (1991). Genetic scrambling as a defence against meiotic drive. *Journal of Theoretical Biology*, *153*(4), 531–558. https://doi.org/10.1016/S0022-5193(05)80155-9

Hamilton, W. D. (1964a). The genetical evolution of social behaviour. I. *Journal of Theoretical Biology*, *7*(1), 1–16. https://doi.org/10.1016/0022-5193(64)90038-4

Hamilton, W. D. (1964b). The genetical evolution of social behaviour. II. *Journal of Theoretical Biology*, *7*(1), 17–52. https://doi.org/10.1016/0022-5193(64)90039-6

Hardin, G. (1968). The Tragedy of the Commons. *Science*, *162*(3859), 1243–1248. https://doi.org/10.1126/science.162.3859.1243

Hartley, I. R., & Davies, N. B. (1994). Limits to cooperative polyandry in birds. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *257*(1348), 67–73. https://doi.org/10.1098/rspb.1994.0095

Hatchwell, B. J., & Komdeur, J. (2000). Ecological constraints, life history traits and the evolution of cooperative breeding. *Animal Behaviour*, *59*(6), 1079–1086. https://doi.org/10.1006/anbe.2000.1394

Hauber, M. E., & Lacey, E. A. (2005). Bateman's Principle in Cooperatively Breeding Vertebrates: The Effects of Non-breeding Alloparents on Variability in Female and Male Reproductive Success1. *Integrative and Comparative Biology*, *45*(5), 903–914. https://doi.org/10.1093/icb/45.5.903

Heg, D., Bachar, Z., Brouwer, L., & Taborsky, M. (2004). Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1555), 2367–2374. https://doi.org/10.1098/rspb.2004.2855

Heinze, J., Kellner, K., & Seal, J. (2017). Sociality in Ants. In D. R. Rubenstein & P. Abbot (Eds.), *Comparative Social Evolution* (pp. 21–49). Cambridge University Press. https://doi.org/10.1017/9781107338319.003

Horn, R. C. V., Engh, A. L., Scribner, K. T., Funk, S. M., & Holekamp, K. E. (2004). Behavioural structuring of relatedness in the spotted hyena (Crocuta crocuta) suggests direct fitness benefits of clan-level cooperation. *Molecular Ecology*, *13*(2), 449–458. https://doi.org/10.1046/j.1365-294X.2003.02071.x

Husby, A., Visser, M. E., & Kruuk, L. E. B. (2011). Speeding Up Microevolution: The Effects of Increasing Temperature on Selection and Genetic Variance in a Wild Bird Population. *PLOS Biology*, *9*(2), e1000585. https://doi.org/10.1371/journal.pbio.1000585

Jetz, W., & Rubenstein, D. R. (2011). Environmental Uncertainty and the Global Biogeography of Cooperative Breeding in Birds. *Current Biology*, *21*(1), 72–78. https://doi.org/10.1016/j.cub.2010.11.075

Keller, L., & Reeve, H. K. (1994). Partitioning of reproduction in animal societies. *Trends in Ecology & Evolution*, *9*(3), 98–102. https://doi.org/10.1016/0169-5347(94)90204-6

Kimwele, C. N., & Graves, J. A. (2003). A molecular genetic analysis of the communal nesting of the ostrich (Struthio camelus). *Molecular Ecology*, *12*(1), 229–236. https://doi.org/10.1046/j.1365-294X.2003.01727.x

Koenig, W. D., & Dickinson, J. L. (Eds.). (2016). *Cooperative Breeding in Vertebrates: Studies of Ecology, Evolution, and Behavior*. Cambridge University Press. https://doi.org/10.1017/CBO9781107338357

Koenig, W. D., Pitelka, F. A., Carmen, W. J., Mumme, R. L., & Stanback, M. T. (1992). The Evolution of Delayed Dispersal in Cooperative Breeders. *The Quarterly Review of Biology*, *67*(2), 111–150. https://doi.org/10.1086/417552

Kokko, H., Johnstone, R. A., & T. H., C.-B. (2001). The evolution of cooperative breeding through group augmentation. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *268*(1463), 187–196. https://doi.org/10.1098/rspb.2000.1349

Komdeur, J. (1992). Importance of habitat saturation and territory quality for evolution of cooperative breeding in the Seychelles warbler. *Nature*, *358*(6386), 493–495. https://doi.org/10.1038/358493a0

Lott, D. F. (1984). Intraspecific Variation in the Social Systems of Wild Vertebrates. *Behaviour*, *88*(3–4), 266–325. https://doi.org/10.1163/156853984X00353

Lukas, D., & Clutton-Brock, T. (2017). Climate and the distribution of cooperative breeding in mammals. *Royal Society Open Science*, *4*(1), 160897. https://doi.org/10.1098/rsos.160897

Mackinnon, M. J., Meyer, K., & Hetzel, D. J. S. (1991). Genetic variation and covariation for growth, parasite resistance and heat tolerance in tropical cattle. *Livestock Production Science*, *27*(2), 105–122. https://doi.org/10.1016/0301-6226(91)90090-D

Magige, F. J., Stokke, B. G., Sortland, R., & Røskaft, E. (2009). Breeding biology of ostriches (Struthio camelus) in the Serengeti ecosystem, Tanzania. *African Journal of Ecology*, *47*(3), 400–408. https://doi.org/10.1111/j.1365-2028.2008.01002.x

Magrath, R. D., & Whittingham, L. A. (1997). Subordinate males are more likely to help if unrelated to the breeding female in cooperatively breeding white-browed scrubwrens. *Behavioral Ecology and Sociobiology*, *41*(3), 185–192. https://doi.org/10.1007/s002650050378

Maloney, S. K. (2008). Thermoregulation in ratites: A review. *Aust. J. Exp. Agric.*, *48*(10), 1293. https://doi.org/10.1071/EA08142

Marshall, T. C., Slate, J., Kruuk, L. E. B., & Pemberton, J. M. (1998). Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, *7*(5), 639–655. https://doi.org/10.1046/j.1365-294x.1998.00374.x

McGowan, K. J., & Woolfenden, G. E. (1990). Contributions to Fledgling Feeding in the Florida Scrub Jay. *Journal of Animal Ecology*, *59*(2), 691–707. https://doi.org/10.2307/4889

McLeod, D. V., & Wild, G. (2014). The relationship between ecology and the optimal helping strategy in cooperative breeders. *Journal of Theoretical Biology*, *354*, 25–34. https://doi.org/10.1016/j.jtbi.2014.03.003

Michod, R. E., & Herron, M. D. (2006). Cooperation and conflict during evolutionary transitions in individuality. *Journal of Evolutionary Biology*, *19*(5), 1406–1409. https://doi.org/10.1111/j.1420-9101.2006.01142.x

Pen, I., & Weissing, F. J. (2000). Towards a unified theory of cooperative breeding: The role of ecology and life history re-examined. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1460), 2411–2418. https://doi.org/10.1098/rspb.2000.1299

R Core Team. (2019). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/

R Core Team. (2020). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rankin, D. J., Bargum, K., & Kokko, H. (2007). The tragedy of the commons in evolutionary biology. *Trends in Ecology & Evolution*, *22*(12), 643–651. https://doi.org/10.1016/j.tree.2007.07.009

Rankin, D. J., Dieckmann, U., & Kokko, H. (2011). Sexual Conflict and the Tragedy of the Commons. *The American Naturalist*, *177*(6), 780–791. https://doi.org/10.1086/659947

Ravagnolo, O., & Misztal, I. (2000). Genetic Component of Heat Stress in Dairy Cattle, Parameter Estimation. *Journal of Dairy Science*, *83*(9), 2126–2130. https://doi.org/10.3168/jds.S0022-0302(00)75095-8

Reyer, H.-U. (1984). Investment and relatedness: A cost/benefit analysis of breeding and helping in the pied kingfisher (Ceryle rudis). *Animal Behaviour*, *32*(4), 1163–1178. https://doi.org/10.1016/S0003-3472(84)80233-X

Ricklefs, R. E. (1975). The Evolution of Co-Operative Breeding in Birds. *Ibis*, *117*(4), 531–534. https://doi.org/10.1111/j.1474-919X.1975.tb04252.x

Riehl, C. (2010). A Simple Rule Reduces Costs of Extragroup Parasitism in a Communally Breeding Bird. *Current Biology*, *20*(20), 1830–1833. https://doi.org/10.1016/j.cub.2010.09.005

Riehl, C. (2011). Living with strangers: Direct benefits favour non-kin cooperation in a communally nesting bird. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1712), 1728–1735. https://doi.org/10.1098/rspb.2010.1752

Riehl, C. (2013). Evolutionary routes to non-kin cooperative breeding in birds. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1772), 20132245. https://doi.org/10.1098/rspb.2013.2245

Riehl, C. (2017). Kinship and Incest Avoidance Drive Patterns of Reproductive Skew in Cooperatively Breeding Birds. *The American Naturalist*, *190*(6), 774–785. https://doi.org/10.1086/694411

Riehl, C., & Strong, M. J. (2019). Social parasitism as an alternative reproductive tactic in a cooperatively breeding cuckoo. *Nature*, *567*(7746), 96–99. https://doi.org/10.1038/s41586-019-0981-1

Ross-Gillespie, A., Gardner, A., West, S. A., & Griffin, A. S. (2007). Frequency Dependence and Cooperation: Theory and a Test with Bacteria. *The American Naturalist*, *170*(3), 331–342. https://doi.org/10.1086/519860

Rowiński, P. K., & Rogell, B. (2017). Environmental stress correlates with increases in both genetic and residual variances: A meta-analysis of animal studies. *Evolution*, *71*(5), 1339–1351. https://doi.org/10.1111/evo.13201

Rubenstein, D. R. (2011). Spatiotemporal environmental variation, risk aversion, and the evolution of cooperative breeding as a bet-hedging strategy. *Proceedings of the National Academy of Sciences*, *108*(Supplement 2), 10816–10822. https://doi.org/10.1073/pnas.1100303108

Rubenstein, D. R., & Abbot, P. (Eds.). (2017). *Comparative Social Evolution*. Cambridge University Press. https://doi.org/10.1017/9781107338319

Rubenstein, D. R., & Lovette, I. J. (2007). Temporal Environmental Variability Drives the Evolution of Cooperative Breeding in Birds. *Current Biology*, *17*(16), 1414–1419. https://doi.org/10.1016/j.cub.2007.07.032

Russell, A. F. (2016). Chestnut-crowned babblers: Dealing with climatic adversity and uncertainty in the Australian arid zone. In J. L. Dickinson & W. D. Koenig (Eds.), *Cooperative Breeding in Vertebrates: Studies of Ecology, Evolution, and Behavior* (pp. 150–164). Cambridge University Press. https://doi.org/10.1017/CBO9781107338357.010

Rybnik, P. K., Horbanczuk, J. O., Naranowicz, H., Lukaszewicz, E., & Malecki, D. I. A. (2007). Semen collection in the ostrich (Struthio camelus) using a dummy or a teaser female. *British Poultry Science*, *48*(5), 635–643. https://doi.org/10.1080/00071660701573078

Sachs, J. L., Mueller, U. G., Wilcox, T. P., & Bull, J. J. (2004). The Evolution of Cooperation. *The Quarterly Review of Biology*, *79*(2), 135–160. https://doi.org/10.1086/383541

Sachs, J. L., & Rubenstein, D. R. (2007). The evolution of cooperative breeding; is there cheating? *Behavioural Processes*, *76*(2), 131–137. https://doi.org/10.1016/j.beproc.2006.12.018

Salo, A. L., & French, J. A. (1989). Early experience, reproductive success, and development of parental behaviour in Mongolian gerbils. *Animal Behaviour*, *38*(4), 693–702. https://doi.org/10.1016/S0003-3472(89)80015-6

Schneirla, T. C., & Piel, G. (1948). The army ant. *Scientific American*, *178*(6), 16–23.

Schradin, C. (2013). Intraspecific variation in social organization by genetic variation, developmental plasticity, social flexibility or entirely extrinsic factors. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1618), 20120346. https://doi.org/10.1098/rstb.2012.0346

Schradin, C., Hayes, L. D., Pillay, N., & Bertelsmeier, C. (2018). The evolution of intraspecific variation in social organization. *Ethology*, *124*(8), 527–536. https://doi.org/10.1111/eth.12752

Smiseth, P. T., & Moore, A. J. (2004). Behavioral dynamics between caring males and females in a beetle with facultative biparental care. *Behavioral Ecology*, *15*(4), 621–628. https://doi.org/10.1093/beheco/arh053

Smith, J. E., Lacey, E. A., & Hayes, L. D. (2017). Sociality in Non-Primate Mammals. In D. R. Rubenstein & P. Abbot (Eds.), *Comparative Social Evolution* (pp. 284–319). Cambridge University Press. https://doi.org/10.1017/9781107338319.011

Stacey, P. B., & Koenig, W. D. (Eds.). (1990). *Cooperative Breeding in Birds: Long Term Studies of Ecology and Behaviour*. Cambridge University Press.

Strassmann, J. E., & Queller, D. C. (2011). Evolution of cooperation and control of cheating in a social microbe. *Proceedings of the National Academy of Sciences*, *108*(Supplement_2), 10855–10862. https://doi.org/10.1073/pnas.1102451108

Sun, S.-J., Rubenstein, D. R., Chen, B.-F., Chan, S.-F., Liu, J.-N., Liu, M., Hwang, W., Yang, P.-S., & Shen, S.-F. (2014). Climate-mediated cooperation promotes niche expansion in burying beetles. *ELife*, *3*, e02440. https://doi.org/10.7554/eLife.02440

Taborsky, M., & Limberger, D. (1981). Helpers in fish. *Behavioral Ecology and Sociobiology*, *8*(2), 143–145. https://doi.org/10.1007/BF00300826

Van Dyken, J. D., Linksvayer, T. A., & Wade, M. J. (2011). Kin Selection–Mutation Balance: A Model for the Origin, Maintenance, and Consequences of Social Cheating. *The American Naturalist*, *177*(3), 288–300. https://doi.org/10.1086/658365

Vehrencamp, S. L. (1983). A model for the evolution of despotic versus egalitarian societies. *Animal Behaviour*, *31*(3), 667–682. https://doi.org/10.1016/S0003-3472(83)80222-X

Wade, M. J., & Breden, F. (1980). The evolution of cheating and selfish behavior. *Behavioral Ecology and Sociobiology*, *7*(3), 167–172. https://doi.org/10.1007/BF00299360

Wcislo, W., & Fewell, J. H. (2017). Sociality in Bees. In D. R. Rubenstein & P. Abbot (Eds.), *Comparative Social Evolution* (pp. 50–83). Cambridge University Press. https://doi.org/10.1017/9781107338319.004

West, Stuart A., & Cooper, G. A. (2016). Division of labour in microorganisms: An evolutionary perspective. *Nature Reviews Microbiology*, *14*(11), 716–723. https://doi.org/10.1038/nrmicro.2016.111

West, Stuart A., Cooper, G. A., Ghoul, M. B., & Griffin, A. S. (2021). Ten recent insights for our understanding of cooperation. *Nature Ecology & Evolution*, 1–12. https://doi.org/10.1038/s41559-020-01384-x

West, Stuart A., Fisher, R. M., Gardner, A., & Kiers, E. T. (2015). Major evolutionary transitions in individuality. *Proceedings of the National Academy of Sciences*, *112*(33), 10112–10119. https://doi.org/10.1073/pnas.1421402112

West, Stuart A., Gardner, A., & Griffin, A. S. (2006). Altruism. *Current Biology: CB*, *16*(13), R482-483. https://doi.org/10.1016/j.cub.2006.06.014

West, Stuart A., Griffin, A. S., & Gardner, A. (2008). Social semantics: How useful has group selection been? *Journal of Evolutionary Biology*, *21*(1), 374–385. https://doi.org/10.1111/j.1420-9101.2007.01458.x

West, Stuart A., Griffin, A. S., Gardner, A., & Diggle, S. P. (2006). Social evolution theory for microorganisms. *Nature Reviews Microbiology*, *4*(8), 597–607. https://doi.org/10.1038/nrmicro1461

West, Stuart A., Pen, I., & Griffin, A. S. (2002). Cooperation and Competition Between Relatives. *Science*, *296*(5565), 72–75. https://doi.org/10.1126/science.1065507

Williams, G. C. (1996). *Adaptation and natural selection: A critique of some current evolutionary thought*. Princeton Univ. Press.

Wilson, E. O. (1971). *The insect societies.* https://www.cabdirect.org/cabdirect/abstract/19720503745

Wilson, Edward O. (2000). *Sociobiology: The New Synthesis, Twenty-Fifth Anniversary Edition*. Harvard University Press. https://www.jstor.org/stable/j.ctvjnrttd

Wong, J. W. Y., Meunier, J., & Kölliker, M. (2013). The evolution of parental care in insects: The roles of ecology, life history and the social environment. *Ecological Entomology*, *38*(2), 123–137. https://doi.org/10.1111/een.12000

Woolfenden, G. E. (1975). Florida Scrub Jay Helpers at the Nest. *The Auk*, *92*(1), 1–15. https://doi.org/10.2307/4084414

Woolfenden, G. E., & Fitzpatrick, J. W. (1978). The Inheritance of Territory in Group-Breeding Birds. *BioScience*, *28*(2), 104–108. https://doi.org/10.2307/1307423

Wright, J., McDonald, P. G., te Marvelde, L., Kazem, A. J. N., & Bishop, C. M. (2010). Helping effort increases with relatedness in bell miners, but 'unrelated' helpers of both sexes still provide substantial care. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1680), 437–445. https://doi.org/10.1098/rspb.2009.1360

Yamagiwa, J., & Hill, D. A. (1998). Intraspecific variation in the social organization of Japanese macaques: Past and present scope of field studies in natural habitats. *Primates*, *39*(3), 257–273. https://doi.org/10.1007/BF02573076

Paper I

Cooperation and competition over reproduction shape the complexity of cooperative breeding groups

Article type: Report

Julian Melgar[1], Mads F. Schou[1], Maud Bonato[2], Zanell Brand[3], Anel Engelbrecht[3], Schalk W. P. Cloete[4],[3] & Charlie K. Cornwallis[1]

[1] Department of Biology, Lund University, Lund, Sweden [2] Kynsna Elephant Park, Kynsna, South Africa [3] Directorate Animal Sciences, Western Cape Department of Agriculture, Elsenburg, South Africa [4] Department of Animal Sciences, University of Stellenbosch, Matieland, South Africa

*Corresponding authors: Email: julian.melgar@biol.lu.se, charlie.cornwallis@biol.lu.se

In Brief

Cooperative animal societies vary in complexity from simple pairs to complex multi-male, multi-female groups, but explaining why is challenging. Experiments on ostriches show that variation in group complexity emerges because the benefits of cooperative offspring care and the costs of sexual competition differ between males and females at different phases of reproduction.

Highlights (limit of 85 characters per point including spaces)

- The complexity of cooperative breeding groups of ostriches is highly variable
- Group complexity increases competition over reproduction in males
- Cooperative care in complex groups promotes female reproductive success
- Intermediate group sizes harbour conflict over the timing of mating and incubation

Keywords

Cooperative breeding, sexual selection, group complexity, group size, reproductive conflict, birds, ostrich

Summary

Breeding in large cooperative groups allows the costs of reproduction to be shared amongst individuals, enabling environments where independent reproduction is challenging to be inhabited [1–4]. However, in the majority of cooperative breeding animals, groups vary markedly in the number of males and females they contain [3–7]. Why, given the benefits of cooperation, do large breeding groups only emerge sometimes? We addressed this question by experimentally establishing groups with variable numbers of males and females ('group complexity') in the ostrich, *Struthio camelus*, and manipulated the need for cooperation over offspring care by artificially incubating eggs. When the need for offspring care was removed, group complexity had little effect on female reproductive success. However, when eggs were left in nests, cooperative incubation was frequently observed and female reproductive success was dependent on group complexity, being highest in large multi-female groups and groups with single females and multiple males. In contrast, the reproductive success of males was always highest in groups with multiple females without male competitors, regardless of the need for offspring care. The benefits of cooperative care and the costs of sexual competition meant that male and female reproductive success was lowest in pairs and in groups with intermediate numbers of males and females, where there was conflict over the timing of mating and incubation. Our results provide experimental evidence that variation in the complexity of social groups arises because males and females differ in how they increase their reproductive success during different phases of breeding.

Results and Discussion

In cooperatively breeding animals, groups differ markedly in the number of males and females that contribute to reproduction and offspring care [3,4,7,8]. A pervasive idea to explain such variation is that environmental fluctuations change constraints on independent breeding, and in turn the benefits of group living [1,2,9–12]. However, this fails to explain why groups are often highly variable under similar ecological conditions, such as in the same location during the same time [3–5,5–7,13–16]. An alternative explanation is that there are advantages and disadvantages to being in large and small groups that broaden the range of group sizes where reproductive success is maximised [8,17–19]. For example, individuals in small groups may face less sexual competition, but have fewer opportunities for cooperating over offspring care compared to individuals in larger groups [6,20,21].

Investigating why groups vary in complexity is challenging as it requires experiments to disentangle the effects of competition and cooperation, and remove the effects of breeder and territory quality on reproductive success [22–25]. A commonly used experimental approach is the removal of individuals from groups, but this can lead to social upheaval resulting in variable and inaccurate estimates of reproductive success [22,25]. Here we address these issues by experimentally engineering entire social groups of cooperatively breeding ostriches, *Struthio camelus*, that mirror the complexity of groups seen in natural populations (Figure 1). To separate the effects of competition and cooperation across different phases of breeding (mating versus offspring care measured here as time invested in incubation), we compared the reproductive success of individuals when offspring care was experimentally removed (eggs were collected and artificially incubated) to when eggs were incubated naturally (Figure 1).

Ostriches breed in groups where multiple males and multiple females dig and protect a nest (Figure 1) [26,27]. Each nest can contain the offspring of several individuals, as females lay communally and mate with multiple males [28,29]. Males and females participate in the cooperative incubation of eggs that last 42 days, representing a major part of offspring care [27,30]. During this period eggs must be constantly protected, which exposes adults to risks of heat exhaustion and predation [26,27,31]. After hatching, the breeding group often dissolves and the precocial young are typically protected by a single male and female [27].

Groups of wild ostriches co-occurring in the same habitat can be highly variable in their complexity. We conducted transects in Karoo National Park, South Africa, and found that groups consisted of 1 to 12 same sex individuals, although most often groups consisted of 1 to 6 females and 1 to 3 males (Figure 1; Table S1). This is similar to the complexity of groups reported in East

African populations, showing that local variation in groups is widespread across their geographical distribution and across subspecies [27–29]. Using estimates from the wild as a guide, we established 118 groups involving a total of 309 individuals in enclosures of natural Karoo habitat that covered the typical natural range of group complexies (groups composed of 1 to 3 males with 1 to 6 females: Table S1). The reproductive success of individuals was measured by collecting data on the number of eggs and chicks produced over a period of six to seven months each year by each group. The mean reproductive success per individual was examined (total number of eggs and chicks / number of same sex individuals in group) as we were interested in the average reproductive returns for individuals of being in groups with different complexities, irrespective of between individual variation in reproductive success within groups. This data was combined with behavioural observations to quantify the incubation and mating behaviour of all individuals (59.25±1.17 (mean±se) hours per group spread over the season).

Male and female reproductive success is maximised in groups with different complexities

When the need for offspring care was experimentally removed, average male reproductive success declined as the number of competitors in groups increased (Figure 2A. Number of $males_{chicks}$ posterior mode (PM) and credible interval (CI) = -0.38 (-0.57 , -0.23), pMCMC = 0.001. Table S3. For similar statistical support for eggs see Figure S1 and Table S2). For example, single males on average sired three times the number of chicks compared to when males had competitors (Figure 2A). Male reproductive success was also influenced by the number of sexual partners in groups (Figure 2A). As the number of females in groups increased to four, male reproductive output went up markedly (Figure 2A. Number of $females_{chicks}$ PM (CI) = 0.48 (0.33 , 0.69), pMCMC = 0.001. Table S3. For similar statistical support from eggs see Figure S1 and Table S2), after which it plateaued (Number of $females^2_{eggs}$ PM (CI) = -0.2 (-0.29 , -0.1), pMCMC = 0.001. Table S2). In contrast, female reproductive success, expressed as both the number of eggs and number of chicks, was largely independent of the number of males and females in groups (Figure 2B & S1. Tables S2 & S3).

Offspring care changes how group complexity influences female, but not male, reproductive success

Caring for offspring changed the way female reproductive success was maximised (Figure 2D. Table S2 & S3). The number of chicks females produced when they naturally incubated eggs was dependent on the number of females in groups, and whether there were multiple or single males (Number of males:Number of $females^2_{chicks}$ PM (CI) = 0.38 (0.07 , 0.77), pMCMC = 0.018). In groups with multiple males, the average number of chicks hatched per female was highest when there were low and high numbers of females (Figure 2D). Conversely, in groups with single males, the number of chicks hatched per female was highest in groups with four females and

lowest when females were on their own (Figure 2D). Female reproductive success was therefore lowest in groups with intermediate numbers of each sex and in pairs, which was not the case when individuals did not incubate offspring (Figure 2. Tables S2 & S3). In contrast, patterns of male reproductive success were not influenced by the need to incubate offspring (Figure 2A & 2C), with single males in groups with four or more females hatching the most chicks (Number of $males_{chicks}$ PM (CI) = -0.32 (-0.54 , -0.1), pMCMC = 0.002; Number of $females_{chicks}$ PM (CI) = 0.84 (0.64 , 1.19), pMCMC = 0.001; Number of males:Number of $females^2_{chicks}$ PM (CI) = 0.43 (0.11 , 0.74), pMCMC = . Table S3. For similar statistical support from eggs see Figure S1 and Table S2).

Cooperative care in larger groups offsets the costs of competition

Next, we investigated why female reproductive success increased in more complex groups and decreased in pairs when there was offspring care. When observing groups, multiple males and multiple females were frequently seen sharing incubation. We therefore examined cooperation over incubation in relation to group complexity and quantified the influence this had on hatching success. The amount of time eggs were incubated increased with the number of females and males in groups (Figure 3A. Number of females PM (CI) = 0.87 (0.51 , 1.32), pMCMC = 0.001. Number of males PM (CI) = 0.63 (0.24 , 1.03), pMCMC = 0.001. Table S4). In turn, hatching success was positively related to the amount of time eggs were incubated (Figure 3B. PM (CI) = 0.28 (0.05 , 0.61), pMCMC = 0.014. Table S5).

Individuals did not, however, spend more time incubating in larger groups. On the contrary, the amount of time males and females invested in incubation decreased with the number of same sex individuals, although not significantly for females (Figure 3C & 3D. Females: Number of females PM (CI) = -0.38 (-0.89 , 0.09), pMCMC = 0.164. Males: Number of males PM (CI) = -0.9 (-1.74 , -0.52), pMCMC = 0.002. Table S6). The incubation eggs received and their hatching success were therefore greatest in groups with multiple males and multiple females, and yet individuals in these groups did not work any harder. This suggests that cooperation over incubation in larger groups increases hatching success while spreading the load of parental care across more individuals.

Conflict over the timing of mating and incubation disfavours intermediate group sizes

Low female reproductive success in pairs when there was offspring care may be explained by cooperation over incubation being limited (Figure 2 & 3). However, the question remains as to why female reproductive success was lower in groups with intermediate numbers of each sex. In some groups, males were frequently seen trying to copulate with incubating females that superficially resemble soliciting females. This not only disturbed incubation, resulting in nests

being protected for less time, but can also causes eggs to be displaced and broken. We investigated whether such lack of coordination over mating and incubation could explain reductions in the reproductive success of individuals in intermediate group sizes.

The number of interruptions to incubation increased with the number of males in groups (Figure 4A. Number of males PM (CI) = 0.78 (0.28 , 1.17), pMCMC = 0.001. Table S7). However, the effect of males was dependent on the number of females (Figure 4A. Number of males^2: Number of females PM (CI) = 0.97 (-0.13 , 3.38), pMCMC = 0.072. Table S7). In groups with the lowest and highest numbers of females, interruptions to incubation were rare irrespective of the number of males in groups (Figure 4. Number of females^2 PM (CI) = -0.48 (-0.97 , -0.08), pMCMC = 0.028. Table S7). In contrast, when there were intermediate numbers of females, interruptions to incubation increased markedly with the number of males (Figure 4A). Interruptions to incubation were associated with a mismatch in the amount of time males and females spent incubating (Figure 4B. Difference in incubation PM (CI) = -0.49 (-0.71 , -0.25), pMCMC = 0.002. Table S8). When females invested more time than males in incubation, interruptions were frequent, which was not the case when males invested more than females (Figure 4B).

The number of interruptions increased the proportion of broken eggs that were found in nests, which contributed significantly to lower hatching success (Figure 4C & 4D. Broken eggs PM (CI) = 0.22 (0.05 , 0.45), pMCMC = 0.028. Hatching success PM (CI) = -0.98 (-1.17 , -0.59), pMCMC = 0.001. Table S9 & S10). Consequently, the greater the disparity between males and females in the amount they invested in incubation, the greater the proportion of broken eggs, which reduced the reproductive success of groups (PM (CI) = -0.13 (-0.32 , 0), pMCMC = 0.046. Table S11). These results are consistent with the idea that in groups with intermediate numbers of males and females, reproductive success is jeopardised by males pursuing copulations after females initiated incubation. Conflicts over the timing of reproduction between the sexes have been found to influence reproductive success in other species [32,33], but these results show that it may be an important factor shaping the complexity of cooperative breeding groups.

Conclusions

In cooperative breeding species, levels of sexual selection and opportunities for cooperation can vary with the number of males and females in groups [8,17,34]. Our results suggest that in ostriches, sexual competition and gaining access to females dominates male reproductive success, even though cooperation over care with other males reduces the burden of offspring care. In contrast, female reproductive success was strongly dependent on cooperative care and there was little evidence of sexual competition amongst females. Although the importance of competition and cooperation for group living species has long been recognised [8,35], experimental evidence

of their relative importance for males and females, and how this shapes the complexity of social groups during different stages of reproduction, has been challenging to obtain.

The differences in the relative effect of cooperation and competition on male and female reproductive success may explain why groups with a certain level of complexity are more common than others in the wild. For example, the reproductive success of individuals in groups with intermediate numbers of males and females was compromised by a lack of reproductive coordination, that bared a signature of male competition cancelling the benefits of cooperative care. The pursuit of reproductive opportunities by individuals at the expense of others may therefore lead to a 'sexual tragedy of the commons' and the disappearance of such groups from natural populations [36–38]. This does not appear to happen in groups with single females, potentially where access to females is more easily controlled. Because of the benefits of cooperative care, the adverse effects of sexual competition do not simply lead to smaller groups, as illustrated by the reduction in the reproductive success of individuals in pairs when there was offspring care. The reproductive interests of males and females were best balanced in groups with single males and four females. Interestingly this is the most common group composition observed in the wild [27,29]. Female reproductive success was nevertheless equally high when on their own in groups with multiple males and in multi-male multi-female groups, which may provide an answer to why group complexity is so variable in nature.

Data and Code Availability

All data and code are available on request.

Acknowledgments

We are thankful to all the staff at Oudtshoorn Research Farm for assistance with data collection and maintenance of the birds and to the Western Cape Government for use of their resources. This research was funded by the Swedish Research Council (grant number 2017-03880), the Knut and Alice Wallenberg Foundation (Wallenberg Academy fellowship number 2013.0129 & 2018.0138), Carl Tryggers (grant numbers 12: 92 & 19: 71) to CKC and a stipend from the Carlsberg Foundation to MFS and by the Jörgen Lindström stipendium to JM. The maintenance and development of the ostrich populations used in this study was funded by the Western Cape Department of Agriculture and supported by grants from the Western Cape Agricultural Research Trust (Grant number 0070/000VOLSTRUISE: Cloete) as well as the Technology and Human Resources for Industry program (THRIP – Grant number TP14081390585) of the South African National Research Foundation to SWPC.

Author contributions

Conceptualization JM, SWPC, CKC; Methodology JM, CKC; Formal analysis JM, MFS, CKC; Investigation JM, MFS, MB, SWPC, CKC; Data curation JM, MFS, MB, ZB, AE, SWPC, CKC; Writing original draft JM, CKC; Writing, Reviewing & Editing JM, MFS, MB, ZB, AE, SWPC, CKC; Visualization JM, MFS, CKC; Supervision JM, MFS, CKC; Funding acquisition JM, SWPC, CKC.

Declaration of interests

The authors declare no competing interests

Figures

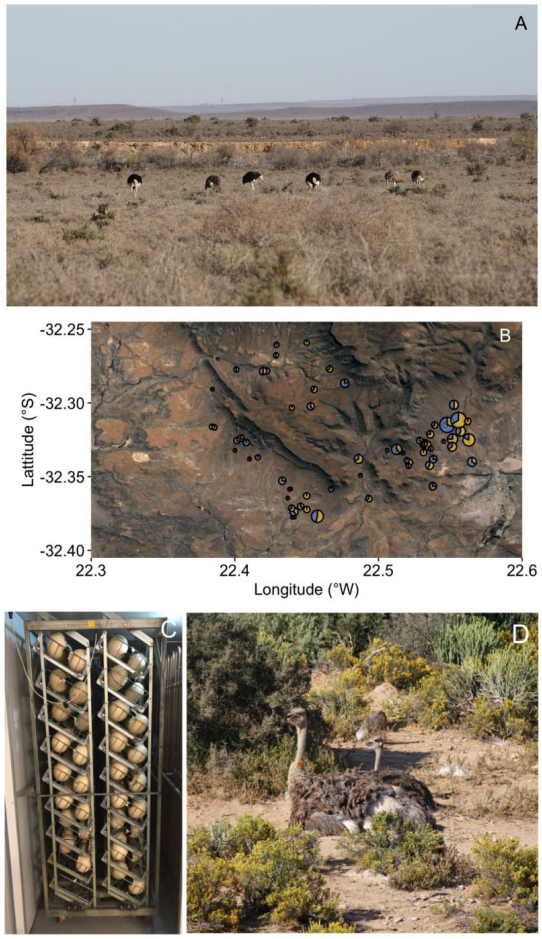


Figure 1: Variation in the complexity of cooperative breeding groups of ostriches. (A) Group of three male and three female breeding ostriches in Karoo National Park. (B) A map of Karoo National Park with different group compositions plotted. The size of the circles indicate the number of individuals (maximum = 18, minimum = 1), the blue and yellow segments indicate the proportion of males and females respectively. (C) The need for offspring care was manipulated at the experimental study site by collecting eggs and using artificial incubators. (D) The reproductive success of individuals when incubating naturally, such as these females, was compared to periods where eggs were removed.

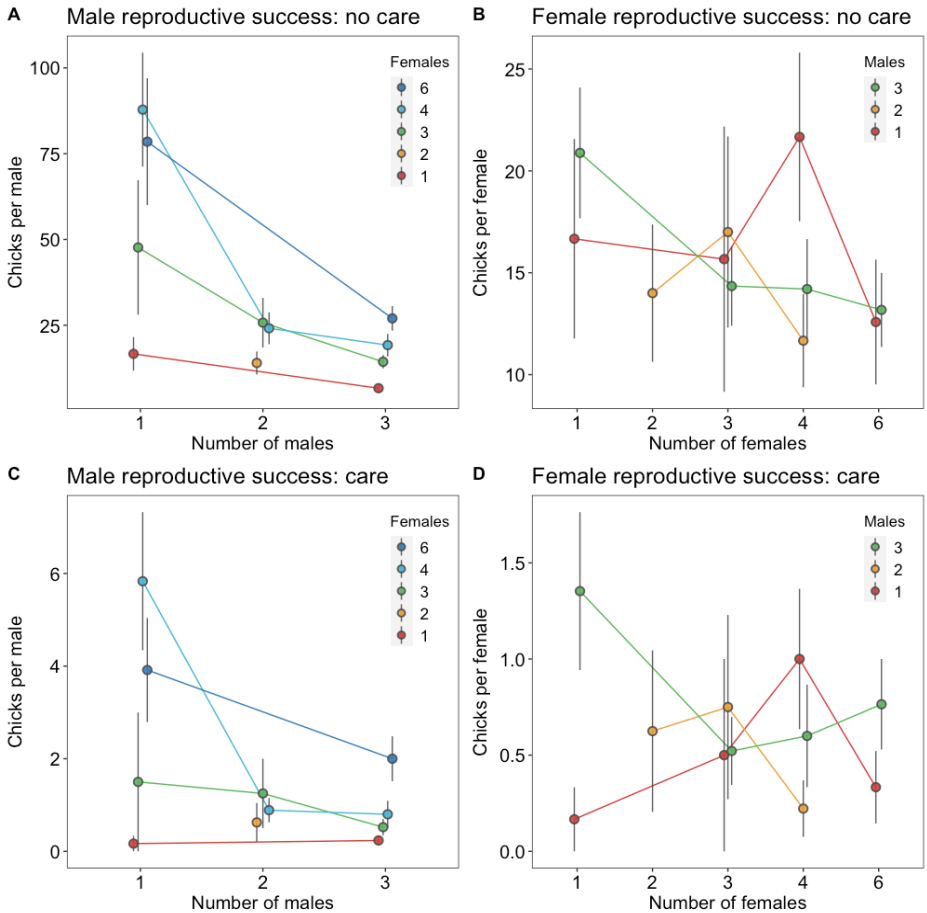


Figure 2: Group complexity and the need for offspring care influence male and female reproductive success. (A) The average number of chicks males sired decreased with the number of males in the group and increased with the number of females, irrespective of whether there was offspring care (C). The number of chicks females produced depended on the number of males in groups and offspring care. In groups with single males, the number of chicks females produced was highest in groups with four females both with (D) and without offspring care (B). In groups with more males, the number of chicks females produced declined with increasing numbers of females when offspring care was removed (B), but was highest in groups with few and many females when there was offspring care (D). See figure S1 for plots of egg production. Means ± SE are plotted.

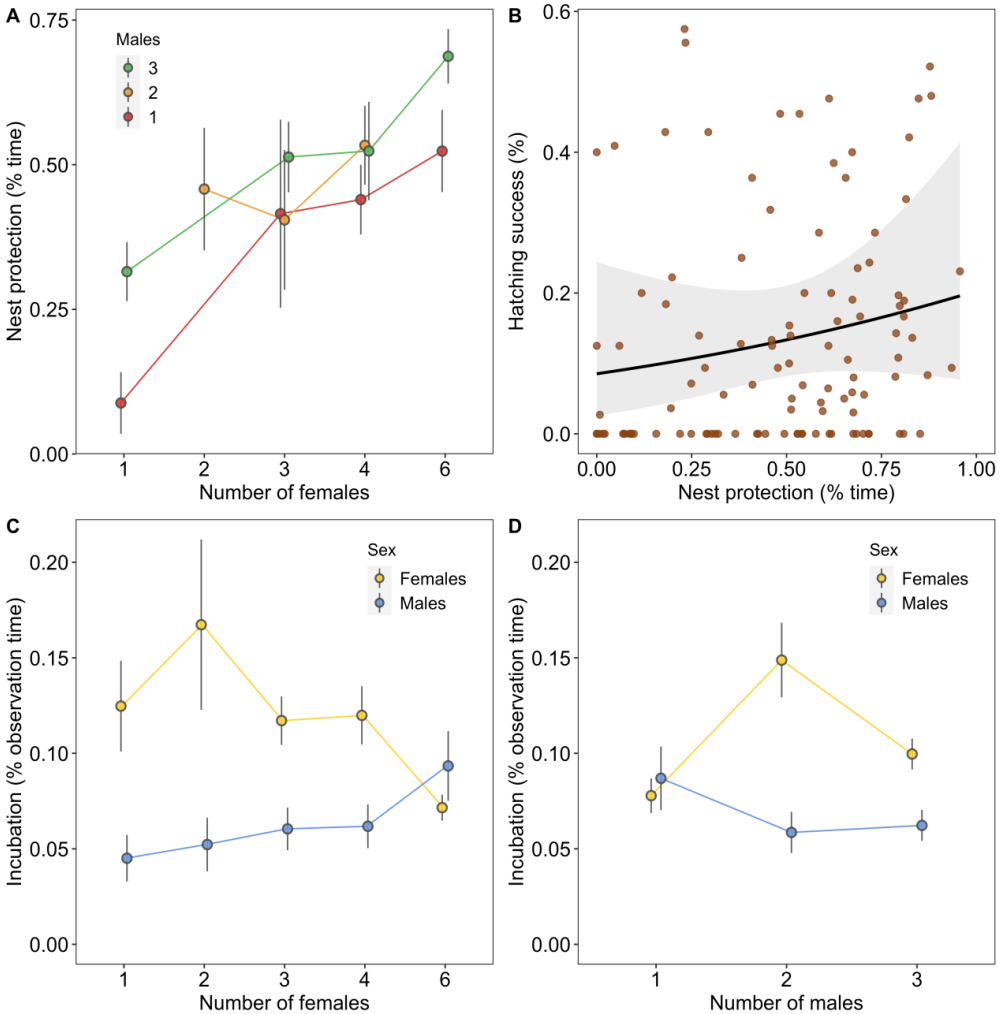


Figure 3: The benefits of cooperative offspring care in relation to group complexity. (A) The amount of time nests were protected was higher in groups with more males and females. (B) Hatching success increased with the amount of time nests were protected. Regression line from a binomial glm with 95% confidence intervals is shown. (C) When the number of females in groups went up, females decreased and males increased the amount of time they invested in incubation. (D) As the number of males in groups went up, females increased and males decreased the amount of time they invested in incubation. Means ± SE are plotted in A, B and C.

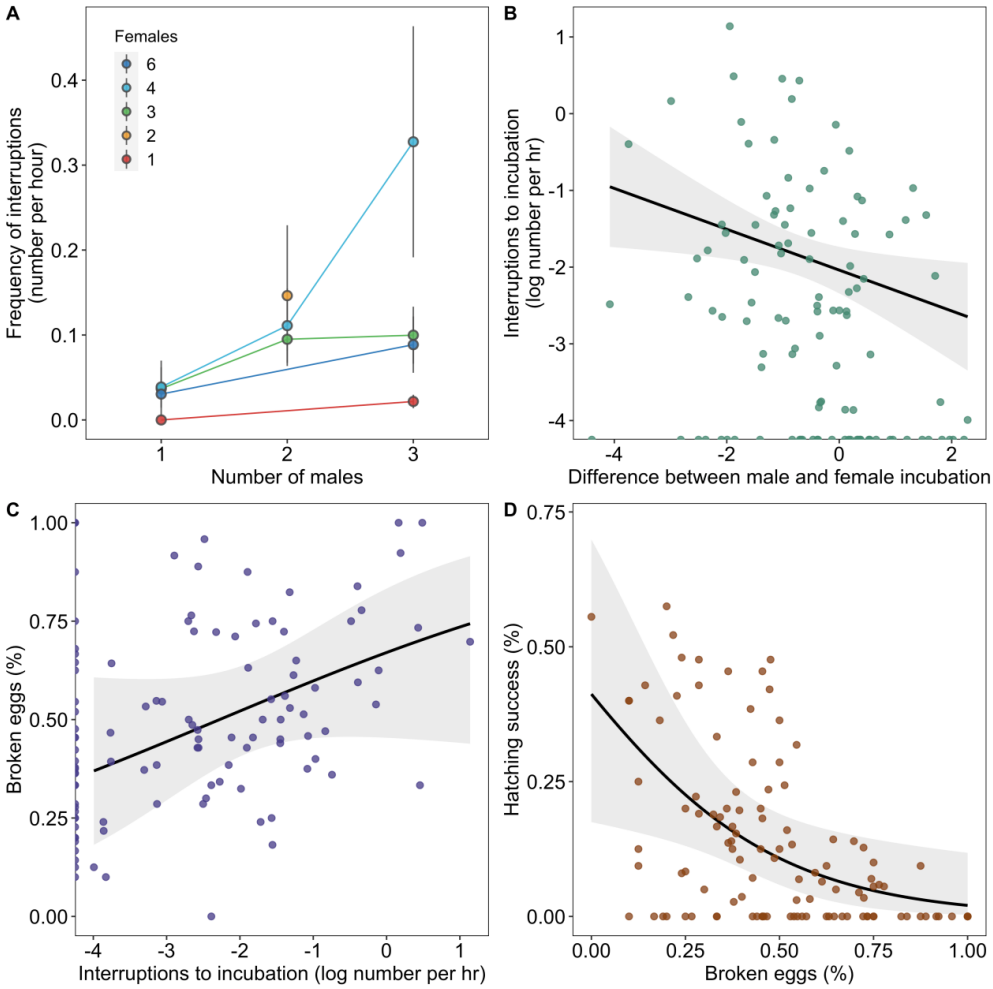


Figure 4: Coordination over reproduction changed with group complexity. (A) The probability of interruption during incubation increased with the numbers of males in groups, especially when there were intermediate numbers of females. Means ± SE are plotted. (B) Interruptions to incubations were related to the disparity in the amount of time males and females invested in incubation. (C) Interruptions to incubation were associated with more broken eggs, which decreased hatching success (D). Regression lines from glms (B & C = binomial; D = Poisson) with 95% confidence intervals. Infinite values (log(0)) in B (x axis) and C (y axis) are shown along the axis.

Star Methods

Experimental Model and Subject Details

Study population

The research was conducted on two populations. The experiments manipulating group complexity were conduced on a captive population of ostriches kept at Oudtshoorn Research Farm, South Africa (33° 38' 21.5"S, 22° 15' 17.4"E) from 2012 to 2018. Natural variation in group complexity was examined in a wild population of ostriches in Karoo National Park, South Africa (32°19'49.27"S, 22°29'59.99"E) in 2014 (8-9th November) and 2018 (17-19th November).

Method Details

Natural variation in group complexity

Natural variation in the complexity of breeding groups (group size, number of males and number of females) was examined using published literature [27–29], and directly estimated by conducting transects along the roads of the south eastern part of Karoo National Park. Each transect was carried out 2 to 3 times. Ostriches were typically observed in clearly defined groups, judged by their coordinated movement and close proximity to each other (< ~100m). In a few instances, individuals were separated by more than 100m and in these situations they were observed until it was clear whether they were part of the group or moving separately. The location of groups and single individuals was recorded using GPS on an iPhone 4 in 2018. In 2014 it was not possible to take GPS coordinates, but locations were marked on a map of the park. Additional information such as whether individuals were sexually mature (immature females = no or very few white wing feathers; immature males = mix of brown and black body plumage) was also recorded. Only one group of four (three males, one female) immature individuals, approximately two years old, and only one group of seven immature individuals (sexes unclear), approximately 1 years old, were observed in 2018. Figure 1B includes the two-year-old group, but not the one-year-old group.

Experimental design

We experimentally manipulated the complexity of 118 groups of breeding ostriches involving 309 adult ostriches (145 males and 164 females), over a seven year period (16-18 groups per year: Table S14). Groups were kept in fenced areas (range: 2400 and 70600 m^2, median = 4700 m^2) of Karoo habitat at Oudtshoorn Research Farm [39]. The number of males in groups ranged from 1 to 3 and the number of females ranged from 1 to 6. Due to limitations in the number of birds accessible for our experiments, and other experiments being conducted on the same

population, not all combinations of male and female group sizes were possible. All individuals in the Oudtshoorn population were individually identifiable by coloured and numbered neck tags.

The breeding season was typically from May to December every year. During the first ~5 months of the season, eggs were collected to measure reproductive success independently from the effects of incubation behaviour. During the last ~2 months, eggs were left in nests and incubation behaviour was monitored to examine patterns of reproductive success when individuals had to care for offspring. Reproductive success was measured as the number of eggs and number of chicks produced by groups. During the breeding season ostriches received a balanced ostrich breeder diet (90 to 120 g protein, 7.5 to 10.5 MJ metabolizable energy, 26 g calcium and 6 g phosphorus per kg feed) and ad-libitum water.

Measuring reproductive success in the absence of offspring care

To measure reproductive success independently of incubation behaviour, eggs were collected from nests twice a day and artificially incubated. Eggs were marked according to the time of day, date and group of origin, and placed under UV lights for 20 minutes for disinfection. As eggs were incubated in batches starting once a week, eggs were stored prior to incubation for 1 to 6 days under conditions known to maintain hatching success [40]: Eggs were kept on turning trays (two daily 180° rotations) in a cold room (17°C) with relative humidity between 80% and 90%. Hereafter eggs were transferred to artificial incubators set at 36.2°C with a relative humidity of 24%. Eggs in the incubator were automatically turned 60° around their long axis every hour. Eggs were set horizontally for the first 21 days of incubation and then turned vertically with their air sac on top for the rest of the hatching period. Eggs were inspected daily for signs of pipping from day 39 of incubation. The period of incubation in ostriches is ~42 days. Individual reproductive success was estimated as the number of eggs and chicks produced by groups divided by the total number of same sex individuals within groups, as we were interested in the average reproductive returns for individuals in groups with different complexities, irrespective of between individual variation in reproductive success within groups.

Measuring reproductive success when groups cared for offspring

Nests were checked daily and new eggs were marked with the date and an egg identification number. The absence and presence of previously laid eggs was recorded to track the fate of each egg. During this period, the incubation behaviour of individuals was monitored by conducting ~3 hour observations at least three times a week using binoculars (10 x 40) and a telescope (12-36 x 50). The observer sat camouflaged in a 10-meter-tall observation tower in the middle of the field site. Groups were observed for between 47 and 91 hours. The identity of each incubating individual, as well as the start and end of incubation, were recorded. When incubation was

interrupted by other individuals in the group, the time of the interruption and the identity and sex of the interrupting individual was recorded. The consequences of interruptions varied in severity from individuals returning to nests within seconds to ceasing incubation for that observation period. To avoid including minor disturbances in our measure of the number of interruptions, we only included those events that resulted in the incubating individual not returning to the nest within one minute.

From 2012 to 2014, hatching success was measured by allowing groups to naturally incubate eggs to completion. If no eggs were observed hatching in groups after 50 days of incubation, they were removed and checked for developing embryos. From 2015 onwards, changes in legislation to reduce the spread of avian flu meant that contact between adults and chicks had to be minimised. Consequently, eggs were removed from nests just before hatching (~40 days after the onset of incubation) and placed in artificial incubators to determine hatching success. Individual reproductive success was estimated in the same way as when offspring care was removed: the number of eggs and chicks produced by groups divided by the total number of same sex individuals within groups.

Quantification and Statistical Analyses

General approach

Data were analysed in R [41] using Bayesian Linear Mixed Models (BLMM) with Markov chain Monte Carlo (MCMC) estimation in the package MCMCglmm [42]. Default fixed effect priors were used (independent normal priors with zero mean and large variance (10^{10})) and for random effects inverse gamma priors were used unless otherwise specified (V = 1, nu = 0.002). Each analysis was run for 1100000 iterations with a burn-in of 100000 and a thinning interval of 1000. Convergence was checked by running models three times and examining the overlap of traces, levels of autocorrelation, and testing with Gelman and Rubin's convergence diagnostic (potential scale reduction factors <1.1)[43].

Parameter estimates for fixed effects are reported from models that included all terms of the same order and lower. For example, all main effect estimates are from models where all other main effects are included, all estimates of two-way interactions are from models that included all two-way interactions and main effects, and so forth. Quadratic effects were tested in models including main effects and effects of the same order (other quadratic effects and two-way interactions). The length of time groups were monitored varied across years (no care range = 153-189 hours; care range = 24-62 hours). This was accounted for by including a fixed effect of the amount of time groups were monitored. All continuous explanatory variables were z transformed using the

scale() function in R. Explanatory variables that were proportions were logit transformed using the logit() function in R and count variables were log transformed. Curvilinear effects of continuous explanatory variables were modelled using the quadratics of the z transformed values computed before running the models.

Fixed effects were considered significant when 95% credible intervals (CIs) did not overlap with 0 and pMCMC were less than 0.05 (pMCMC = proportion of iterations above or below a test value correcting for the finite sample size of posterior samples). By default MCMCglmm reports parameter estimates for fixed factors as differences from the global intercept. This does not allow absolute estimates and 95% CIs for all factor levels to be estimated or custom hypothesis tests of differences between factor levels. Consequently, we removed the global intercept from all models and present absolute estimates for factor levels. Differences between factor levels were estimated by subtracting the posterior samples from one level from the second level and calculating the posterior mode, 95% CI and pMCMC.

Random effects were used to model the non-independence of data arising from multiple data points per individual per group, per enclosure and per year. Random effect estimates presented in tables are from models that included the highest order fixed effect terms. To estimate the magnitude of random effects we calculated the percentage of the total random effect variance explained by each random term on the expected data scale (I2%: $(V_i/V_{total}) * 100$)[44]. To obtained estimates of I2 on the expected scale from binomial models the distribution variance for the logit link function was included in the denominator ($V_i/V_{total} + \pi$^3/2) $* 100$).

Specific analyses

1. Testing how group complexity and the need for offspring care influences male and female reproductive success

The effect of group complexity on the number of eggs individuals produced was modelled using a BLMM with a Poisson error distribution. The need for offspring care (2 level factor: no care vs care), sex of adult (2 level factor: male, female), number of males (continuous), the number of females (continuous) and the time groups were monitored were entered as fixed effects, and year, enclosure and group were included as random effects. The effects of group complexity on the number of eggs produced per adult bird with and without the need for offspring care, were estimated by fitting three-way interactions between care, sex and the number of males and females in groups (R code: M1). The number of chicks produced per individual was modelled in exactly the same way (R code: M2).

2. Testing how the benefits of cooperative offspring care vary with group complexity The effect of group complexity on the proportion of time groups protected nests was modelled using a BLMM with a binomial error distribution. The response variable was the number of observation minutes birds were sitting on nests versus the number of observation minutes nests were exposed. This accounts for variation across years in observation effort. The number of males and females in groups were included as fixed effects and year and enclosure were random effects (R code: M3). The effect of the proportion of time nests were protected on hatching success was modelled using a BLMM with a binomial error distribution of the number of eggs hatched per individual (total number of chicks produced by groups / number of same sex individuals) versus the number of eggs that did not hatch per individual (total number of eggs that failed to hatch / number of same sex individuals). The proportion of time nests were protected, the number of males and females in groups and the amount of time groups were monitored were included as fixed effects, and year and enclosure were included as random effects (R code: M4).

Typically groups only had one active nest, but in a few cases a second and a third nest were occasionally used. The amount of time groups protected their nests was calculated by summing data across all nests (total time nests were protected versus total time nests were exposed). Data were summed across nests to facilitate comparisons with the egg and chick data, which were recorded at the level of the group (e.g. total number of eggs and chicks groups produced by each group), not at the level of each nest. To check if the number of nests groups used influenced the time nests were protected and hatching success, we included the number of nests (continuous) as a fixed effect in models (R code: M3 & M4). The number of nests did not have a significant effect in any of our analyses (Tables S4 & S5).

3. Testing how individual investment in cooperative care varies with group complexity The effect of group complexity on the time individuals invested in incubation was modelled using a BLMM with a binomial error distribution. The response variable was the number of observation minutes an individual was observed sitting versus the number of minutes it was not sitting, which accounts for variation in the amount of time individuals were observed. Sex and the number of males and females in groups were included as fixed effects and year, enclosure, group and individual identity were entered as random effects (R code: M5). For this analysis only data on primary nests were included as attendance at secondary and tertiary nests was sporadic, and the presence of secondary and tertiary nests did not influence the total amount of time groups protected their nests (Tables S4 & S5).

4. Testing how coordination over reproduction changes with group complexity The effect of group complexity on the number of interruptions to incubation was modelled using a BLMM

with a Poisson error distribution. The response variable was the total number of interruptions observed across all observations. The amount of time groups were observed, the amount of time individuals were sitting on nests and the number of males and females in groups were included as fixed effects, and year and enclosure were included as random effects (R code: M6). The effect of the disparity in the time males and females invested in incubation on the number of interruptions was modelled in the same way, but an extra fixed effect of the difference in the proportion of time males and females spent incubating was included (R code: M7).

4. Testing how coordination over reproduction changes with group complexity The effect of group complexity on the number of interruptions to incubation was modelled using a BLMM with a Poisson error distribution. The response variable was the total number of interruptions observed across all observations divided by the number of hours groups were observed (this was multiplied by 100 and rounded to whole numbers as MCMCglmm requires count data to be whole numbers). The number of males and females in groups were included as fixed effects, and year and enclosure were included as random effects (R code: M6). We removed five enclosure-by-year records where no incubation was observed as this removes the possibility for interruption. The effect of the disparity in the time males and females invested in incubation on the number of interruptions was modelled in the same way, but an extra fixed effect of the difference in the proportion of time males and females spent incubating was included (R code: M7).

5. Testing how coordination over reproduction influences reproductive success The effect of interruptions on the proportion of eggs broken in nests was modelled using a BLMM with a binomial error distribution. The response variable was the number of eggs broken versus the number of eggs not broken. The number of interruptions to incubation and the amount of time groups were observed were included as fixed effects, and the year and enclosure were included as random effects (R code: M8). The same model setup was used to test how the disparity in the amount of time males and females invested in incubation influenced the proportion of eggs broken, but different fixed effects were included. The number of males and females in groups, the amount of time groups were observed and the difference in the proportion of time males and females spent incubating were included as fixed effects (R code: M10). The impact of the broken eggs on the overall hatching success of groups was modelled using a BLMM with a binomial error distribution of the number of eggs hatched versus the number of eggs that did not hatch as the response variable. The proportion of eggs that were broken was included as a fixed effect, and year and enclosure were included as random effects (R code: M9).

6.Supplementary analyses We present two additional analyses in the supplementary materials (Figure S1. Table S12 & S13) that are not discussed in the main text, but may provide useful information to some readers. These analyses examine the effects of group complexity on the total number of eggs (R code: M11) and chicks (R code: M12) produced by groups as opposed to the per individual measures of reproductive success presented in the main text.

References

1. Emlen, S.T. (1982). The Evolution of Helping. I. An Ecological Constraints Model. The American Naturalist *119*, 29–39.

2. Hatchwell, B.J., and Komdeur, J. (2000). Ecological constraints, life history traits and the evolution of cooperative breeding. Animal Behaviour *59*, 1079–1086.

3. Koenig, W.D., and Dickinson, J.L. eds. (2016). Cooperative Breeding in Vertebrates: Studies of Ecology, Evolution, and Behavior (Cambridge: Cambridge University Press).

4. Rubenstein, D.R., and Abbot, P. eds. (2017). Comparative Social Evolution (Cambridge: Cambridge University Press).

5. Davies, N.B. (1992). Dunnock Behaviour and Social Evolution (Oxford University Press).

6. Davies, N.B., Hartley, I.R., Hatchwell, B.J., Desrochers, A., and Skeer, J. (1995). The polygynandrous mating system of the alpine accentor, Prunella collaris. I. Ecological causes and reproductive conflicts. Animal Behaviour, 769–788.

7. Lukas, D., and Clutton-Brock, T. (2018). Social complexity and kinship in animal societies. Ecology Letters *21*, 1129–1134.

8. Alexander, R.D. (1974). The Evolution of Social Behavior. Annual Review of Ecology and Systematics *5*, 325–383.

9. Jetz, W., and Rubenstein, D.R. (2011). Environmental Uncertainty and the Global Biogeography of Cooperative Breeding in Birds. Current Biology *21*, 72–78.

10. Cornwallis, C.K., Botero, C.A., Rubenstein, D.R., Downing, P.A., West, S.A., and Griffin, A.S. (2017). Cooperation facilitates the colonization of harsh environments. Nature Ecology & Evolution *1*, 1–10.

11. Lukas, D., and Clutton-Brock, T. (2017). Climate and the distribution of cooperative breeding in mammals. Royal Society Open Science *4*, 160897.

12. Martin, J.S., Ringen, E.J., Duda, P., and Jaeggi, A.V. (2020). Harsh environments promote alloparental care across human societies. Proceedings of the Royal Society B: Biological Sciences *287*, 20200758.

13. Vehrencamp, S.L. (1977). Relative Fecundity and Parental Effort in Communally Nesting Anis, Crotophaga sulcirostris. Science *197*, 403–405.

14. Hellmann, J.K., Ligocki, I.Y., O'Connor, C.M., Reddon, A.R., Garvy, K.A., Marsh-Rollo, S.E., Gibbs, H.L., Balshine, S., and Hamilton, I.M. (2015). Reproductive sharing in relation to group and colony-level attributes in a cooperative breeding fish. Proceedings of the Royal Society B: Biological Sciences *282*, 20150954.

15. Markham, A.C., Gesquiere, L.R., Alberts, S.C., and Altmann, J. (2015). Optimal group size in a highly social mammal. Proceedings of the National Academy of Sciences of the United States of America *112*, 14882–14887.

16. Papageorgiou, D., and Farine, D.R. (2020). Group size and composition influence collective movement in a highly social terrestrial bird. eLife *9*, e59902.

17. Davies, N.B., and Houston, A.I. (1986). Reproductive Success of Dunnocks, Prunella modularis, in a Variable Mating System. II. Conflicts of Interest Among Breeding Adults. Journal of Animal Ecology *55*, 139–154.

18. Santos, E.S.A., Santos, L.L.S., Lagisz, M., and Nakagawa, S. (2015). Conflict and cooperation over sex: The consequences of social and genetic polyandry for reproductive success in dunnocks. The Journal of Animal Ecology *84*, 1509–1519.

19. Ferrari, M., Lindholm, A.K., and König, B. (2018). Fitness Consequences of Female Alternative Reproductive Tactics in House Mice (Mus musculus domesticus). The American Naturalist *193*, 106–124.

20. Davies, N.B. (1985). Cooperation and conflict among dunnocks, Prunella modularis, in a variable mating system. Animal Behaviour, 628–648.

21. Riehl, C. (2011). Living with strangers: Direct benefits favour non-kin cooperation in a communally nesting bird. Proceedings of the Royal Society B: Biological Sciences *278*, 1728–1735.

22. Dickinson, J.L., and Hatchwell, B.J. (2004). Fitness consequences of helping. In Ecology and Evolution of Cooperative Breeding in Birds, W. D. Koenig and J. L. Dickinson, eds. (Cambridge University Press), pp. 48–66.

23. Cockburn, A., Sims, R.A., Osmond, H.L., Green, D.J., Double, M.C., and Mulder, R.A. (2008). Can we measure the benefits of help in cooperatively breeding birds: The case of superb fairy-wrens Malurus cyaneus? Journal of Animal Ecology *77*, 430–438.

24. Schoepf, I., and Schradin, C. (2012). Better off alone! Reproductive competition and ecological constraints determine sociality in the African striped mouse (Rhabdomys pumilio). Journal of Animal Ecology *81*, 649–656.

25. Downing, P.A., Griffin, A.S., and Cornwallis, C.K. (2020). The Benefits of Help in Cooperative Birds: Nonexistent or Difficult to Detect? The American Naturalist *195*, 1085–1091.

26. Sauer, E.G.F., and Sauer, E.M. (1966). Social Behaviour of the South African Ostrich, Struthio Camelus Australis. Ostrich *37*, 183–191.

27. Bertram, B.C.R. (1992). The ostrich communal nesting system (Princeton, N.J: Princeton University Press).

28. Kimwele, C.N., and Graves, J.A. (2003). A molecular genetic analysis of the communal nesting of the ostrich (Struthio camelus). Molecular Ecology *12*, 229–236.

29. Magige, F.J., Stokke, B.G., Sortland, R., and Røskaft, E. (2009). Breeding biology of ostriches (*Struthio Camelus*) in the Serengeti ecosystem, Tanzania. African Journal of Ecology *47*, 400–408.

30. Deeming, D.C. (1996). Production, fertility and hatchability of ostrich (Struthio camelus) eggs on a farm in the United Kingdom. Animal Science *63*, 329–336.

31. Magige, F.J., Moe, B., and Røskaft, E. (2008). The white colour of the Ostrich (Struthio camelus) egg is a trade-off between predation and overheating. Journal of Ornithology *149*, 323–328.

32. Løvlie, H., and Pizzari, T. (2007). Sex in the Morning or in the Evening? Females Adjust Daily Mating Patterns to the Intensity of Sexual Harassment. The American Naturalist *170*, E1–E13.

33. Holland, B., and Rice, W.R. (1998). Perspective: Chase-Away Sexual Selection: Antagonistic Seduction Versus Resistance. Evolution *52*, 1–7.

34. Hauber, M.E., and Lacey, E.A. (2005). Bateman's Principle in Cooperatively Breeding Vertebrates: The Effects of Non-breeding Alloparents on Variability in Female and Male Reproductive Success. Integrative and Comparative Biology *45*, 903–914.

35. Williams, G.C. (1966). Adaptation and Natural Selection Reprint edition. (Princeton, NJ: Princeton University Press).

36. Hardin, G. (1968). The Tragedy of the Commons. Science *162*, 1243–1248.

37. Rankin, D.J., Dieckmann, U., and Kokko, H. (2011). Sexual Conflict and the Tragedy of the Commons. The American Naturalist *177*, 780–791.

38. Galliard, J.-F.L., Fitze, P.S., Ferrière, R., and Clobert, J. (2005). Sex ratio bias, male aggression, and population collapse in lizards. Proceedings of the National Academy of Sciences *102*, 18231–18236.

39. Cloete, S., Brand, M., Hoffman, L., and Muller, M. (2008). Live weight and reproduction performance of Zimbabwean Blue and South African Black ostriches. South African Journal of Animal Science *38*, 65–73.

40. Brand, Z., Cloete, S.W.P., Brown, C.R., and Malecki, I.A. (2008). Systematic factors that affect ostrich egg incubation traits. South African Journal of Animal Science *38*, 315–325.

41. Team, R.C. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

42. Hadfield, J.D. (2010). MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm *R* Package. Journal of Statistical Software *33*.

43. Brooks, S.P., and Gelman, A. (1998). General Methods for Monitoring Convergence of Iterative Simulations. Journal of Computational and Graphical Statistics *7*, 434.

44. Villemereuil, P. de, Schielzeth, H., Nakagawa, S., and Morrissey, M. (2016). General Methods for Evolutionary Quantitative Genetic Inference from Generalized Mixed Models. Genetics *204*, 1281–1294.

Supplementary Material: Cooperation and competition over reproduction shape the complexity of cooperative breeding groups

Julian Melgar[1], Mads F. Schou[1], Maud Bonato[2], Zanell Brand[3], Anel Engelbrecht[3], Schalk Cloete[4,3] & Charlie K. Cornwallis[1]

[1] Department of Biology, Lund University, Lund, Sweden [2] Kynsna Elephant Park, Kynsna, South Africa [3] Directorate Animal Sciences, Western Cape Department of Agriculture, Elsenburg, South Africa [4] Department of Animal Sciences, University of Stellenbosch, Matieland, South Africa

*Corresponding author: Email: julian.melgar@biol.lu.se (mailto:julian.melgar@biol.lu.se), charlie.cornwallis@biol.lu.se (mailto:charlie.cornwallis@biol.lu.se)

This file includes: Figures S1 to S2; Tables S1 to S14

Supplementary Figures

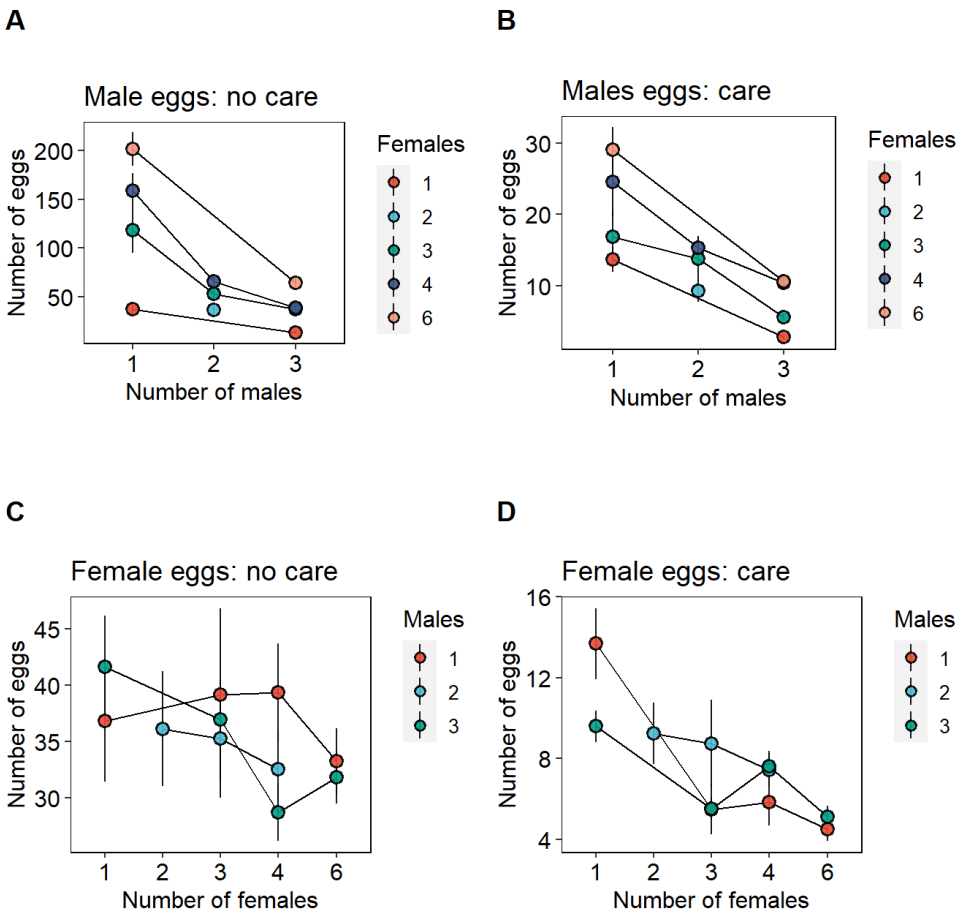


Figure S1: The effect of group complexity and offspring care on the number of eggs produced per individual.

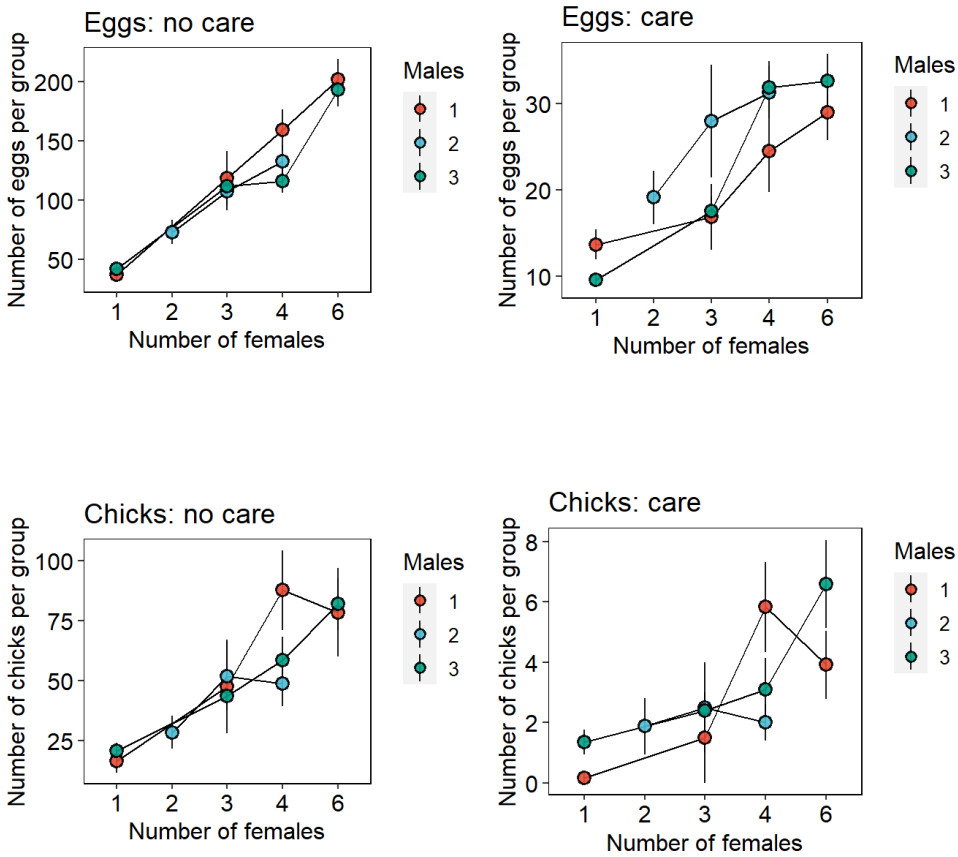


Figure S2: The total reproductive output of groups in relation to group complexity and offspring care.

Supplementary Tables

Table S1: The composition of groups observed in karoo national park

Lattitude	Longitude	Year	Date	Group_size	Females	Males	Age	Chicks	Age_chicks
-32.357	22.538	2018	17-Nov	3	2	1	Adult	0	NA
-32.342	22.536	2018	17-Nov	5	3	2	Adult	0	NA
-32.339	22.520	2018	17-Nov	2	0	2	Adult	0	NA
-32.334	22.517	2018	17-Nov	1	0	1	Adult	0	NA
-32.331	22.513	2018	17-Nov	8	3	5	Adult	0	NA
-32.349	22.487	2018	18-Nov	1	1	0	Adult	0	NA
-32.365	22.493	2018	18-Nov	3	2	1	Adult	0	NA
-32.377	22.458	2018	18-Nov	11	6	5	Adult	0	NA
-32.371	22.440	2018	18-Nov	3	2	1	Adult	0	NA
-32.400	22.446	2018	18-Nov	3	1	2	Adult	0	NA
-32.363	22.450	2018	18-Nov	3	3	0	Adult	0	NA
-32.363	22.433	2018	18-Nov	7	NA	NA	Subadult	0	NA
-32.365	22.437	2018	18-Nov	1	0	1	Adult	0	NA
-32.348	22.439	2018	18-Nov	1	0	1	Adult	0	NA
-32.353	22.433	2018	18-Nov	4	1	3	Subadult	0	NA
-32.337	22.416	2018	18-Nov	2	0	1	Adult	0	NA
-32.338	22.410	2018	18-Nov	1	0	1	Adult	0	NA
-32.324	22.404	2018	18-Nov	3	1	2	Adult	0	NA
-32.316	22.314	2018	18-Nov	2	2	0	Adult	0	NA
-32.277	22.401	2018	18-Nov	2	1	1	Adult	0	NA
-32.277	22.466	2018	18-Nov	3	2	1	Adult	0	NA
-32.287	22.477	2018	18-Nov	5	1	4	Adult	0	NA
-32.338	22.486	2018	18-Nov	6	4	2	Adult	0	NA
-32.332	22.506	2018	18-Nov	1	1	0	Adult	0	NA
-32.340	22.521	2018	18-Nov	4	2	2	Adult	0	NA
-32.328	22.532	2018	18-Nov	6	4	2	Adult	0	NA
-32.321	22.536	2018	18-Nov	4	3	1	Adult	0	NA
-32.315	22.539	2018	18-Nov	3	2	1	Adult	0	NA
-32.315	22.547	2018	18-Nov	2	2	0	Adult	0	NA
-32.301	22.553	2018	18-Nov	6	3	3	Adult	0	NA
-32.319	22.557	2018	18-Nov	7	4	3	Adult	0	NA
-32.358	22.467	2018	19-Nov	2	0	2	Adult	0	NA
-32.377	22.441	2018	19-Nov	1	0	1	Adult	0	NA

Lattitude	Longitude	Year	Date	Group_size	Females	Males	Age	Chicks	Age_chicks
-32.377	22.440	2018	19-Nov	1	0	1	Adult	0	NA
-32.374	22.441	2018	19-Nov	5	2	3	Adult	0	NA
-32.372	22.450	2018	19-Nov	3	3	0	Adult	0	NA
-32.358	22.438	2018	19-Nov	1	0	1	Adult	0	NA
-32.327	22.408	2018	19-Nov	3	0	4	Adult	0	NA
-32.332	22.400	2018	19-Nov	1	0	1	Adult	0	NA
-32.325	22.401	2018	19-Nov	3	1	2	Adult	0	NA
-32.317	22.386	2018	19-Nov	2	0	2	Adult	0	NA
-32.291	22.384	2018	19-Nov	1	0	1	Adult	0	NA
-32.278	22.422	2018	19-Nov	3	0	3	Adult	0	NA
-32.268	22.429	2018	19-Nov	2	1	1	Adult	0	NA
-32.261	22.429	2018	19-Nov	2	0	2	Adult	0	NA
-32.278	22.420	2018	19-Nov	4	2	2	Adult	0	NA
-32.259	22.450	2018	19-Nov	2	2	0	Adult	0	NA
-32.291	22.455	2018	19-Nov	3	2	1	Adult	0	NA
-32.303	22.440	2018	19-Nov	2	2	0	Adult	0	NA
-32.333	22.514	2018	19-Nov	2	1	1	Adult	0	NA
-32.325	22.529	2018	19-Nov	3	1	2	Adult	0	NA
-32.340	22.565	2018	19-Nov	6	2	4	Adult	0	NA
-32.314	22.548	2018	19-Nov	17	5	12	Adult	0	NA
-32.302	22.453	2018	19-Nov	4	2	3	Adult	0	NA
-32.312	22.556	2018	19-Nov	18	12	6	Adult	0	NA
-32.325	22.563	2018	19-Nov	11	9	2	Adult	0	NA
-32.312	22.562	2018	19-Nov	3	3	0	Adult	0	NA
-32.324	22.552	2018	19-Nov	8	6	2	Adult	0	NA
-32.330	22.551	2018	19-Nov	6	4	2	Adult	0	NA
-32.326	22.545	2018	19-Nov	1	1	0	Adult	0	NA
-32.329	22.534	2018	19-Nov	5	3	2	Adult	0	NA
-32.331	22.536	2018	19-Nov	2	1	1	Adult	0	NA
-32.338	22.538	2018	19-Nov	4	1	3	Adult	0	NA
-32.334	22.531	2018	19-Nov	3	3	0	Adult	0	NA
-32.328	22.532	2018	19-Nov	2	1	1	Adult	0	NA
-32.343	22.521	2018	19-Nov	2	1	1	Adult	0	NA
-32.356	22.538	2018	19-Nov	4	1	3	Adult	0	NA
NA	NA	2014	08-Nov	2	1	1	Adult	0	NA
NA	NA	2014	08-Nov	2	1	1	Adult	0	NA

Lattitude	Longitude	Year	Date	Group_size	Females	Males	Age	Chicks	Age_chicks
NA	NA	2014	08-Nov	2	1	1	Adult	0	NA
NA	NA	2014	08-Nov	1	0	1	Adult	0	NA
NA	NA	2014	08-Nov	2	0	2	Adult	0	NA
NA	NA	2014	08-Nov	6	3	3	Adult	0	NA
NA	NA	2014	08-Nov	6	1	5	Adult	0	NA
NA	NA	2014	08-Nov	5	3	2	Adult	0	NA
NA	NA	2014	08-Nov	10	4	3	Adult	3	6-8 months
NA	NA	2014	08-Nov	2	1	1	Adult	0	NA
NA	NA	2014	08-Nov	1	0	1	Adult	0	NA
NA	NA	2014	08-Nov	5	1	4	Adult	0	NA
NA	NA	2014	08-Nov	4	2	2	Adult	0	NA
NA	NA	2014	08-Nov	4	1	3	Adult	0	NA
NA	NA	2014	08-Nov	6	3	3	Adult	0	NA
NA	NA	2014	08-Nov	3	2	1	Adult	0	NA
NA	NA	2014	08-Nov	2	2	0	Adult	0	NA
NA	NA	2014	08-Nov	15	12	3	Adult	0	NA
NA	NA	2014	08-Nov	12	9	3	Adult	0	NA
NA	NA	2014	08-Nov	2	1	1	Adult	0	NA
NA	NA	2014	08-Nov	8	4	4	Adult	0	NA
NA	NA	2014	08-Nov	2	1	1	Adult	0	NA
NA	NA	2014	08-Nov	4	3	1	Adult	0	NA
NA	NA	2014	08-Nov	3	1	2	Adult	0	NA
NA	NA	2014	08-Nov	1	0	1	Adult	0	NA
NA	NA	2014	08-Nov	3	3	0	Adult	0	NA
NA	NA	2014	08-Nov	1	0	1	Adult	0	NA
NA	NA	2014	08-Nov	2	1	1	Adult	0	NA
NA	NA	2014	09-Nov	2	1	1	Adult	0	NA
NA	NA	2014	09-Nov	6	4	2	Adult	0	NA
NA	NA	2014	09-Nov	2	0	2	Adult	0	NA
NA	NA	2014	09-Nov	3	3	0	Adult	0	NA
NA	NA	2014	09-Nov	2	2	0	Adult	0	NA
NA	NA	2014	09-Nov	1	1	0	Adult	0	NA
NA	NA	2014	09-Nov	4	2	2	Adult	0	NA
NA	NA	2014	09-Nov	1	0	1	Adult	0	NA
NA	NA	2014	09-Nov	6	4	2	Adult	0	NA
NA	NA	2014	09-Nov	2	2	0	Adult	0	NA

Lattitude	Longitude	Year	Date	Group_size	Females	Males	Age	Chicks	Age_chicks
NA	NA	2014	09-Nov	1	0	1	Adult	0	NA
NA	NA	2014	09-Nov	2	1	1	Adult	0	NA
NA	NA	2014	09-Nov	2	1	1	Adult	0	NA
NA	NA	2014	09-Nov	7	1	6	Adult	0	NA
NA	NA	2014	09-Nov	2	0	2	Adult	0	NA

Table S2: The effect of the number of males and females on the number of eggs produced by individuals

Fixed Effects	Posterior Mode (CI)	pMCMC
Females No care	5.41 (3.63 , 6.86)	**0.001**
Females Care	5.22 (2.97 , 6.18)	**0.001**
Males No care	5.74 (3.94 , 7.17)	**0.001**
Males Care	5.57 (3.32 , 6.54)	**0.001**
Time monitored (days Z) No care	-1.9 (-3.28 , -0.14)	**0.014**
Time monitored (days Z) Care	3.15 (1.12 , 4.28)	**0.001**
Females No care: Number females	-0.09 (-0.15 , 0)	0.064
Females Care: Number females	-0.22 (-0.34 , -0.15)	**0.001**
Males No care: Number females	0.5 (0.43 , 0.58)	**0.001**
Males Care: Number females	0.47 (0.36 , 0.54)	**0.001**
Females No care: Number males	-0.02 (-0.08 , 0.07)	0.822
Females Care: Number males	0.04 (-0.04 , 0.16)	0.334
Males No care: Number males	-0.46 (-0.53 , -0.4)	**0.001**
Males Care: Number males	-0.43 (-0.49 , -0.32)	**0.001**
Females No care: Number females2	0.01 (-0.11 , 0.07)	0.794
Females Care: Number females2	0.23 (0.11 , 0.35)	**0.002**
Males No care: Number females2	-0.2 (-0.29 , -0.1)	**0.001**
Males Care: Number females2	0.08 (-0.06 , 0.17)	0.288
Females No care: Number males2	0.02 (-0.12 , 0.16)	0.75
Females Care: Number males2	-0.05 (-0.25 , 0.12)	0.468
Males No care: Number males2	0.13 (0 , 0.26)	0.072
Males Care: Number males2	-0.04 (-0.18 , 0.13)	0.786
Females No care: Number females:Number males	0.01 (-0.07 , 0.06)	0.928
Females Care: Number females:Number males	-0.01 (-0.07 , 0.1)	0.808
Males No care: Number females:Number males	0 (-0.06 , 0.07)	0.808
Males Care: Number females:Number males	-0.02 (-0.1 , 0.07)	0.772
Females No care: Number males:Number females2	0.02 (-0.03 , 0.1)	0.312
Females Care: Number males:Number females2	-0.05 (-0.15 , 0.04)	0.248

Fixed Effects	Posterior Mode (CI)	pMCMC
Males No care: Number males:Number females2	0.01 (-0.04 , 0.09)	0.418
Males Care: Number males:Number females2	-0.09 (-0.16 , 0)	0.062
Females No care: Number females:Number males2	-0.09 (-0.27 , 0.21)	0.822
Females Care: Number females:Number males2	-0.08 (-0.42 , 0.18)	0.38
Males No care: Number females:Number males2	0 (-0.17 , 0.28)	0.656
Males Care: Number females:Number males2	-0.01 (-0.27 , 0.25)	0.968
Females No care: Number females vs Females Care: Number females	0.17 (0.09 , 0.26)	**0.001**
Males No care: Number females vs Males Care: Number females	0.05 (-0.01 , 0.14)	0.154
Females No care: Number males vs Females Care: Number males	-0.07 (-0.15 , 0.04)	0.224
Males No care: Number males vs Males Care: Number males	-0.06 (-0.14 , 0)	0.06
Females No care: Number females2 vs Females Care: Number females2	-0.23 (-0.33 , -0.15)	**0.001**
Males No care: Number females2 vs Males Care: Number females2	-0.28 (-0.35 , -0.19)	**0.001**
Females No care: Number males2 vs Females Care: Number males2	0.1 (-0.08 , 0.22)	0.266
Males No care: Number males2 vs Males Care: Number males2	0.12 (0.01 , 0.25)	**0.012**
Females No care: Number females:Number males vs Females Care: Number females:Number males	-0.02 (-0.09 , 0.06)	0.594
Males No care: Number females:Number males vs Males Care: Number females:Number males	0.01 (-0.04 , 0.08)	0.528
Females No care: Number males:Number females2 vs Females Care: Number males:Number females2	0.1 (0 , 0.17)	0.054
Males No care: Number males:Number females2 vs Males Care: Number males:Number females2	0.11 (0.05 , 0.17)	**0.004**
Females No care: Number females:Number males2 vs Females Care: Number females:Number males2	0.13 (-0.13 , 0.38)	0.41
Males No care: Number females:Number males2 vs Males Care: Number females:Number males2	0.1 (-0.15 , 0.28)	0.594
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.261 (0.046 , 2.207)	83.549 (64.113 , 98.431)
Camp	0.002 (0 , 0.026)	1.667 (0.013 , 5.429)
Group	0.081 (0.056 , 0.113)	14.364 (1.53 , 31.992)
Residual	0.001 (0 , 0.006)	0.42 (0.008 , 1.245)

Table S3: The effect of the number of males and females on the number of chicks produced by individuals

Fixed Effects	Posterior Mode (CI)	pMCMC
Females No care	2.65 (1.89 , 3.65)	**0.001**
Females Care	0.83 (-0.62 , 1.72)	0.266
Males No care	3.02 (2.27 , 4.02)	**0.001**
Males Care	0.89 (-0.1 , 2.16)	0.072
Time monitored (days Z) No care	-0.23 (-1.16 , 0.57)	0.442
Time monitored (days Z) Care	1.35 (0.39 , 2.73)	**0.016**
Females No care: Number females	-0.14 (-0.25 , 0.1)	0.37
Females Care: Number females	-0.11 (-0.4 , 0.18)	0.394
Males No care: Number females	0.48 (0.33 , 0.69)	**0.001**
Males Care: Number females	0.84 (0.64 , 1.19)	**0.001**
Females No care: Number males	0.06 (-0.09 , 0.25)	0.422
Females Care: Number males	0.41 (0.11 , 0.75)	**0.014**
Males No care: Number males	-0.38 (-0.57 , -0.23)	**0.001**
Males Care: Number males	-0.32 (-0.54 , -0.1)	**0.002**
Females No care: Number females2	0.13 (-0.12 , 0.28)	0.286
Females Care: Number females2	0.43 (0.04 , 0.68)	**0.016**
Males No care: Number females2	-0.08 (-0.26 , 0.15)	0.468
Males Care: Number females2	-0.04 (-0.31 , 0.3)	0.956
Females No care: Number males2	0.06 (-0.27 , 0.38)	0.672
Females Care: Number males2	0.07 (-0.47 , 0.68)	0.738
Males No care: Number males2	0.06 (-0.14 , 0.49)	0.376
Males Care: Number males2	0.27 (-0.2 , 0.84)	0.286
Females No care: Number females:Number males	0 (-0.19 , 0.12)	0.716
Females Care: Number females:Number males	-0.3 (-0.51 , 0.08)	0.134
Males No care: Number females:Number males	-0.01 (-0.18 , 0.12)	0.736
Males Care: Number females:Number males	-0.06 (-0.29 , 0.17)	0.698
Females No care: Number males:Number females2	0.05 (-0.07 , 0.27)	0.266
Females Care: Number males:Number females2	0.38 (0.07 , 0.77)	**0.018**
Males No care: Number males:Number females2	0.06 (-0.09 , 0.25)	0.326
Males Care: Number males:Number females2	0.43 (0.11 , 0.74)	**0.01**
Females No care: Number females:Number males2	-0.02 (-0.56 , 0.53)	0.948
Females Care: Number females:Number males2	0.54 (-0.46 , 1.94)	0.208
Males No care: Number females:Number males2	0.04 (-0.49 , 0.58)	0.798
Males Care: Number females:Number males2	0.71 (-0.3 , 1.61)	0.162
Females No care: Number females vs Females Care: Number females	0.09 (-0.2 , 0.3)	0.708

Fixed Effects	Posterior Mode (CI)	pMCMC
Males No care: Number females vs Males Care: Number females	-0.42 (-0.57 , -0.14)	**0.002**
Females No care: Number males vs Females Care: Number males	-0.29 (-0.63 , -0.09)	**0.008**
Males No care: Number males vs Males Care: Number males	-0.08 (-0.22 , 0.08)	0.364
Females No care: Number females2 vs Females Care: Number females2	-0.36 (-0.53 , -0.02)	**0.036**
Males No care: Number females2 vs Males Care: Number females2	-0.05 (-0.33 , 0.18)	0.604
Females No care: Number males2 vs Females Care: Number males2	-0.03 (-0.59 , 0.43)	0.912
Males No care: Number males2 vs Males Care: Number males2	-0.11 (-0.54 , 0.29)	0.508
Females No care: Number females:Number males vs Females Care: Number females:Number males	0.2 (-0.07 , 0.45)	0.128
Males No care: Number females:Number males vs Males Care: Number females:Number males	0.04 (-0.15 , 0.23)	0.848
Females No care: Number males:Number females2 vs Females Care: Number males:Number females2	-0.39 (-0.68 , -0.02)	**0.046**
Males No care: Number males:Number females2 vs Males Care: Number males:Number females2	-0.25 (-0.62 , -0.07)	**0.006**
Females No care: Number females:Number males2 vs Females Care: Number females:Number males2	-0.77 (-1.88 , 0.33)	0.18
Males No care: Number females:Number males2 vs Males Care: Number females:Number males2	-0.68 (-1.55 , 0.16)	0.14

Random Effects	Posterior Mode (CI)	I2 % (CI)
Year	0.003 (0 , 0.148)	4.624 (0.02 , 17.172)
Camp	0.002 (0 , 0.193)	8.449 (0.042 , 23.461)
Group	0.62 (0.44 , 0.881)	86.664 (69.201 , 99.494)
Residual	0.001 (0 , 0.005)	0.263 (0.031 , 0.765)

Table S4: The effect of the number of males and females on the time nests are protected

Fixed Effects	Posterior Mode (CI)	pMCMC
Intercept	-0.47 (-1.38 , 0.15)	0.142
Number nests	0.32 (-0.07 , 0.68)	0.11
Number males	0.63 (0.24 , 1.03)	**0.001**
Number females	0.87 (0.51 , 1.32)	**0.001**
Number males2	-0.91 (-1.36 , 0.06)	0.098
Number females2	-0.23 (-0.69 , 0.17)	0.178
Number males:Number females	-0.33 (-0.72 , -0.04)	**0.03**
Number males:Number females2	0.24 (-0.12 , 0.58)	0.172
Number females:Number males2	0.49 (-0.7 , 1.57)	0.424
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.244 (0.09 , 1.706)	15.819 (2.627 , 33.913)
Camp	0.23 (0.076 , 0.721)	8.312 (1.808 , 17.647)
Residual	2.878 (2.209 , 3.966)	75.869 (57.281 , 91.246)

Table S5: The effect of nest protection on hatching success

Fixed Effects	Posterior Mode (CI)	pMCMC
Intercept	-2.33 (-3.25 , -1.67)	**0.001**
Nest protection (% time)	0.28 (0.05 , 0.61)	**0.014**
Time monitored (days Z)	-0.07 (-0.87 , 0.53)	0.668
Number nests	0.26 (-0.22 , 0.51)	0.342
Number males	0.01 (-0.36 , 0.44)	0.814
Number females	0 (-0.37 , 0.55)	0.808
Number males2	0.37 (-0.28 , 1.14)	0.294
Number females2	0.03 (-0.54 , 0.47)	0.936
Number males:Number females	-0.17 (-0.46 , 0.28)	0.654
Number males:Number females2	0.58 (0.08 , 0.98)	**0.01**
Number females:Number males2	0.85 (-0.42 , 2.2)	0.18
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.29 (0.082 , 1.725)	20.61 (2.607 , 44.704)
Camp	0.223 (0.104 , 1.135)	18.365 (3.251 , 37.883)
Residual	1.614 (0.767 , 2.728)	61.025 (38.286 , 86.02)

Table S6: The effect of the number of males and females on the amount of time individuals spend incubating

Fixed Effects	Posterior Mode (CI)	pMCMC
Females	-4.27 (-4.98 , -3.42)	**0.001**
Males	-6.24 (-7.09 , -5.35)	**0.001**
Females: Number males	0.51 (-0.01 , 0.85)	**0.042**
Males: Number males	-0.9 (-1.74 , -0.52)	**0.002**
Females: Number females	-0.38 (-0.89 , 0.09)	0.164
Males: Number females	0.52 (-0.04 , 1.05)	0.07
Females: Number males2	-0.92 (-1.86 , -0.12)	**0.024**
Males: Number males2	-0.59 (-1.52 , 0.81)	0.488
Females: Number females2	-0.29 (-0.71 , 0.47)	0.716
Males: Number females2	-0.12 (-0.9 , 0.29)	0.466
Females: Number males:Number females	-0.33 (-0.71 , 0.23)	0.278
Males: Number males:Number females	-0.49 (-1.02 , 0.21)	0.13
Females: Number males: Number females2	0.26 (-0.27 , 0.76)	0.336
Males: Number males:Number females2	0.11 (-0.45 , 0.89)	0.658
Females: Number females:Number males2	0.14 (-0.88 , 2.23)	0.416
Males: Number females:Number males2	-0.36 (-1.69 , 1.91)	0.984
Females: Number males vs Males: Number males	1.57 (0.85 , 2.2)	**0.001**
Females: Number females vs Males: Number females	-0.93 (-1.46 , -0.2)	**0.018**
Females: Number males2 vs Males: Number males2	-0.54 (-1.92 , 0.78)	0.412
Females: Number females2 vs Males: Number females2	0.23 (-0.67 , 0.8)	0.768
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.251 (0.071 , 1.64)	3.407 (0.453 , 8.981)
Camp	0.273 (0.092 , 1.111)	2.839 (0.568 , 6.448)
Group	0.667 (0.124 , 1.842)	5.155 (0.632 , 10.743)
ID	2.773 (0.912 , 5.113)	18.218 (5.923 , 29.61)
Residual	12.008 (9.596 , 14.386)	70.381 (57.995 , 83.759)

Table S7: The effect of the number of males and females on interruptions to incubation

Fixed Effects	Posterior Mode (CI)	pMCMC
Intercept	0.94 (-0.1 , 1.79)	0.06
Number males	0.78 (0.28 , 1.17)	**0.001**
Number females	0.33 (0.05 , 0.86)	**0.036**
Number males2	-0.55 (-1.51 , -0.09)	**0.038**
Number females2	-0.48 (-0.97 , -0.08)	**0.028**
Number males:Number females	-0.19 (-0.61 , 0.33)	0.528
Number males:Number females2	0.31 (-0.19 , 1.48)	0.162

Fixed Effects	Posterior Mode (CI)	pMCMC
Number females:Number males2	0.97 (-0.13 , 3.38)	0.072
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.005 (0 , 1.067)	7.325 (0.009 , 27.964)
Camp	0.005 (0 , 0.674)	4.5 (0.008 , 18.797)
Residual	2.672 (1.678 , 4.164)	88.175 (62.618 , 99.961)

Table S8: Effect of the disparity in incubation on the number of interruptions

Fixed Effects	Posterior Mode (CI)	pMCMC
Intercept	-0.01 (-1.21 , 0.94)	0.978
Difference in incubation	-0.49 (-0.71 , -0.25)	**0.002**
Time observed (hrs Z)	0.01 (-1.23 , 0.66)	0.786
Time incubating (hrs Z)	0.57 (0.2 , 0.94)	**0.002**
Number males	0.55 (0.21 , 0.91)	**0.004**
Number females	0.42 (0.06 , 0.84)	**0.02**
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.517 (0.127 , 4.653)	40.43 (8.719 , 76.352)
Camp	0.004 (0 , 0.772)	8.727 (0.008 , 24.881)
Residual	1.221 (0.767 , 2.143)	50.843 (18.074 , 81.877)

Table S9: The effect of interruptions on the % of eggs broken

Fixed Effects	Posterior Mode (CI)	pMCMC
Intercept	-0.22 (-0.9 , 0.4)	0.358
Time observed (hrs Z)	-0.07 (-0.67 , 0.38)	0.572
Number interruptions (log)	0.22 (0.05 , 0.45)	**0.028**
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.237 (0.092 , 1.427)	30.967 (9.008 , 59.391)
Camp	0.149 (0.079 , 0.49)	16.101 (4.803 , 31.147)
Residual	0.705 (0.412 , 1.067)	52.933 (26.051 , 73.831)

Table S10: The effect of the % of eggs broken on hatching success

Fixed Effects	Posterior Mode (CI)	pMCMC
Intercept	-2.5 (-3.12 , -1.91)	**0.001**
Broken eggs (%)	-0.98 (-1.17 , -0.59)	**0.001**
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.152 (0.055 , 0.944)	19.531 (5.112 , 42.539)
Camp	0.236 (0.091 , 0.823)	21.531 (4.971 , 40.399)
Residual	0.902 (0.523 , 1.635)	58.938 (34.885 , 80.786)

Table S11: The effect of the disparity in incubation between males and females on % of eggs broken

Fixed Effects	Posterior Mode (CI)	pMCMC
Intercept	-0.09 (-0.74 , 0.42)	0.72
Difference in incubation	-0.13 (-0.32 , 0)	**0.046**
Time monitored (days Z))	0.37 (-0.24 , 0.79)	0.192
Number males	0.02 (-0.16 , 0.29)	0.664
Number females	0.04 (-0.23 , 0.27)	0.746
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.236 (0.057 , 1.085)	26.197 (5.831 , 53.037)
Camp	0.178 (0.088 , 0.536)	19.088 (5.035 , 34.604)
Residual	0.692 (0.476 , 1.149)	54.715 (33.381 , 78.49)

TableS12: The effect of the number of males and females on the number of eggs produced by groups

Fixed Effects	Posterior Mode (CI)	pMCMC
No care	4.8 (4.26 , 5.52)	**0.001**
Care	4.34 (3.65 , 5.01)	**0.001**
Time monitored (days Z) No care	-0.21 (-1 , 0.32)	0.316
Time monitored (days Z) Care	1.44 (0.74 , 2.16)	**0.001**
No care: Number females	0.52 (0.46 , 0.6)	**0.001**
Care: Number females	0.44 (0.35 , 0.53)	**0.001**
No care: Number males	0.02 (-0.06 , 0.08)	0.848
Care: Number males	0.08 (-0.01 , 0.15)	0.068
No care: Number females2	-0.19 (-0.26 , -0.1)	**0.001**
Care: Number females2	0.03 (-0.07 , 0.12)	0.668
No care: Number males2	-0.01 (-0.12 , 0.15)	0.84
Care: Number males2	-0.11 (-0.28 , 0.03)	0.122
No care: Number females:Number males	0 (-0.07 , 0.06)	0.778
Care: Number females:Number males	-0.02 (-0.08 , 0.07)	0.916
No care: Number males:Number females2	0.03 (-0.05 , 0.08)	0.476
Care: Number males:Number females2	-0.06 (-0.14 , 0.02)	0.132
No care: Number females:Number males2	0.09 (-0.15 , 0.31)	0.61
Care: Number females:Number males2	-0.04 (-0.31 , 0.19)	0.704
No care: Number females vs Care: Number females	0.09 (-0.01 , 0.19)	0.054
No care: Number males vs Care: Number males	-0.07 (-0.16 , 0.04)	0.198
No care: Number females2 vs Care: Number females2	-0.21 (-0.34 , -0.1)	**0.002**
No care: Number males2 vs Care: Number males2	0.13 (-0.06 , 0.36)	0.19
No care: Number females:Number males vs Care: Number females:Number males	0 (-0.1 , 0.1)	0.898
No care: Number males:Number females2 vs Care: Number males:Number females2	0.07 (-0.01 , 0.19)	0.106

Fixed Effects	Posterior Mode (CI)	pMCMC
No care: Number females:Number males2 vs Care: Number females:Number males2	0.07 (-0.22 , 0.43)	0.502
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.002 (0 , 0.057)	10.096 (0.248 , 32.368)
Camp	0.001 (0 , 0.017)	4.387 (0.1 , 12.278)
Residual	0.103 (0.085 , 0.143)	85.517 (61.757 , 99.02)

Table S13: The effect of the number of males and females on the number of chicks produced by groups

Fixed Effects	Posterior Mode (CI)	pMCMC
No care	4.2 (3.2 , 5.01)	**0.001**
Care	1.38 (0.14 , 2.55)	**0.02**
Time monitored (days Z) No care	-0.55 (-1.36 , 0.42)	0.25
Time monitored (days Z) Care	0.91 (-0.33 , 2.04)	0.136
No care: Number females	0.52 (0.36 , 0.74)	**0.001**
Care: Number females	0.73 (0.45 , 0.94)	**0.001**
No care: Number males	0.08 (-0.1 , 0.27)	0.358
Care: Number males	0.23 (0.03 , 0.47)	**0.04**
No care: Number females2	-0.03 (-0.28 , 0.12)	0.442
Care: Number females2	0.04 (-0.24 , 0.31)	0.87
No care: Number males2	0.11 (-0.3 , 0.37)	0.808
Care: Number males2	-0.06 (-0.39 , 0.55)	0.792
No care: Number females:Number males	0 (-0.19 , 0.16)	0.998
Care: Number females:Number males	-0.09 (-0.36 , 0.12)	0.288
No care: Number males:Number females2	0.12 (-0.08 , 0.28)	0.368
Care: Number males:Number females2	0.36 (0.08 , 0.83)	**0.006**
No care: Number females:Number males2	0.1 (-0.57 , 0.63)	0.828
Care: Number females:Number males2	0.65 (-0.13 , 1.75)	0.082
No care: Number females vs Care: Number females	-0.14 (-0.43 , 0.13)	0.318
No care: Number males vs Care: Number males	-0.13 (-0.42 , 0.11)	0.302
No care: Number females2 vs Care: Number females2	-0.04 (-0.42 , 0.25)	0.574
No care: Number males2 vs Care: Number males2	-0.03 (-0.59 , 0.56)	0.932
No care: Number females:Number males vs Care: Number females:Number males	0.14 (-0.16 , 0.45)	0.412
No care: Number males:Number females2 vs Care: Number males:Number females2	-0.4 (-0.76 , 0.05)	0.072
No care: Number females:Number males2 vs Care: Number females:Number males2	-0.34 (-1.83 , 0.28)	0.208
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.001 (0 , 0.051)	1.488 (0.025 , 5.699)
Camp	0.041 (0.001 , 0.212)	10.227 (0.067 , 22.909)
Residual	0.789 (0.546 , 0.991)	88.285 (75.232 , 99.798)

Table S14: Sample size of experiment and summary statistics of reproductive success and incubation

	2012 (N=17)	2013 (N=18)	2014 (N=19)	2015 (N=13)	2016 (N=16)	2017 (N=14)	2018 (N=16)	Total (N=113)
Number_of_females								
1	2 (11.8%)	2 (11.1%)	2 (10.5%)	4 (30.8%)	4 (25.0%)	4 (28.6%)	4 (25.0%)	22 (19.5%)
2	2 (11.8%)	2 (11.1%)	4 (21.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	8 (7.1%)
3	5 (29.4%)	6 (33.3%)	4 (21.1%)	4 (30.8%)	4 (25.0%)	3 (21.4%)	4 (25.0%)	30 (26.5%)
4	8 (47.1%)	8 (44.4%)	9 (47.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	25 (22.1%)
6	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (38.5%)	8 (50.0%)	7 (50.0%)	8 (50.0%)	28 (24.8%)
Number_of_males								
1	5 (29.4%)	6 (33.3%)	6 (31.6%)	2 (15.4%)	3 (18.8%)	3 (21.4%)	4 (25.0%)	29 (25.7%)
2	7 (41.2%)	7 (38.9%)	7 (36.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	21 (18.6%)
3	5 (29.4%)	5 (27.8%)	6 (31.6%)	11 (84.6%)	13 (81.2%)	11 (78.6%)	12 (75.0%)	63 (55.8%)
Number_of_eggs_laid								
Mean (SD)	25.706 (9.068)	28.667 (11.632)	20.421 (9.593)	23.538 (15.103)	22.500 (10.764)	25.786 (16.101)	15.562 (7.642)	23.159 (11.885)
Range	9.000 - 42.000	8.000 - 47.000	9.000 - 40.000	8.000 - 63.000	10.000 - 43.000	8.000 - 61.000	5.000 - 29.000	5.000 - 63.000
Number_of_chicks_hatched								
Mean (SD)	2.941 (2.989)	2.333 (2.590)	2.000 (3.528)	5.692 (6.575)	3.625 (2.604)	3.643 (4.618)	2.000 (2.989)	3.053 (3.852)
Range	0.000 - 9.000	0.000 - 7.000	0.000 - 10.000	0.000 - 23.000	0.000 - 10.000	0.000 - 12.000	0.000 - 12.000	0.000 - 23.000
Number_of_broken_eggs								
Mean (SD)	13.765 (7.989)	16.611 (8.919)	12.947 (8.134)	8.462 (4.858)	10.750 (8.970)	12.714 (7.518)	6.250 (5.871)	11.850 (8.172)
Range	0.000 - 30.000	1.000 - 34.000	3.000 - 35.000	4.000 - 19.000	1.000 - 32.000	1.000 - 24.000	1.000 - 21.000	0.000 - 35.000
Incubation_time_in_minutes								
Mean (SD)	992.000 (780.623)	1329.222 (860.343)	1894.211 (930.376)	2498.692 (1426.348)	1793.312 (808.881)	1501.000 (1053.690)	2042.250 (750.026)	1695.982 (1024.630)
Range	0.000 - 2283.000	0.000 - 2590.000	273.000 - 3342.000	325.000 - 3905.000	0.000 - 3250.000	27.000 - 2855.000	508.000 - 3100.000	0.000 - 3905.000

Paper II

Cheating triggers tragedy of the commons, group size attenuates it

Julian Melgar[1],+, Mads F. Schou[1], Maud Bonato[2], Zanell Brand[3], Anel Engelbrecht[3], Schalk Cloete[4],[3], Pfunzo T. Muvhali[4], Bengt Hansson1 & Charlie K. Cornwallis[1],+

[1] Department of Biology, Lund University, Lund, Sweden [2] Kynsna Elephant Park, Kynsna, South Africa [3] Directorate Animal Sciences, Western Cape Department of Agriculture, Elsenburg, South Africa [4] Department of Animal Sciences, University of Stellenbosch, Matieland, South Africa

+Corresponding authors.

Email: julian.melgar@biol.lu.se, charlie.cornwallis@biol.lu.se

Abstract

Cooperative societies frequently contain cheats that reap the rewards provided by others without contributing anything. Cheating is predicted to lead to the demise of cooperative groups, and yet cooperation is widespread in animals. Under what conditions does cheating occur without causing the breakdown of cooperation? Here we test the idea that in complex social groups cheating is more likely to occur because cheats are harder to detect and the costs of cheating are shared among more cooperators. We investigated this by experimentally manipulating group size in a species renowned for having reproductive cheats, the cooperatively breeding ostrich, *Struthio camelus*. Ostrich groups nurture a common nest where multiple individuals contribute offspring, but not all individuals contribute to parental care (incubation). We found that while the opportunities for cheating are greater in larger groups with more cooperators, cheats maximize their reproductive success in small groups. However, in small groups cooperators react strongly to cheats by reducing incubation effort, leading to a collapse of reproduction. In contrast, in large groups, individuals appeared buffered from the reproductive costs of cheating by the presence of many cooperators. Our results suggest that the emergence of cheats in social groups contributes to group complexity. Small groups are destabilized by cheating while the impact on individuals in large groups is offset by the presence of more cooperators.

Introduction

Cooperation is widespread across the tree of life and has allowed a wide range of organisms to expand into new niches and reach new adaptive peaks (Archibald, 2014; Bourke, 2011; Maynard Smith & Eors, 1995; Rubenstein & Abbot, 2017). Although individuals clearly benefit from cooperating, they are also vulnerable to cheats that increase their fitness by exploiting the resources provided by cooperators (Ghoul et al., 2014; Michod & Herron, 2006; Wade & Breden, 1980). The persistence of cooperative groups therefore requires that the effects of cheating are minimized in order to prevent a "tragedy of the commons" (Hardin, 1968; Rankin et al., 2007).

A number of mechanisms have evolved in animal societies to prevent cheating. For instance, individuals have been shown to punish cheats by evicting them from social groups, by physically harassing them, or by destroying their offspring (Fischer et al., 2014; Foster & Ratnieks, 2000, 2001; Mulder & Langmore, 1993; Ratnieks & Visscher, 1989; Reeve & Nonacs, 1992; Travisano & Velicer, 2004; but see Riehl & Frederickson, 2016). Although research has focused on the ways cheating is controlled, cheating still occurs frequently in nature and we know less about the

circumstances where cheating can proliferate (West et al., 2021). What factors influence the prevalence of cheating and are the effects on cooperators mitigated in any way?

One factor predicted to influence the likelihood of detecting cheats (Alencar et al., 2008; Fischer et al., 2014), and determine the strength of selection for controlling cheating (Brännström et al., 2011; Brown, 1982; Shen et al., 2014) is the complexity of social groups. In small groups, the behavior of individuals may be more easily tracked allowing cheats to be identified, and consequently punished (high costs to cheating) (Alencar et al., 2008; Fischer et al., 2014). Also, the resources cheats can exploit may be limited in small groups due to the presence of fewer cooperators (low benefits of cheating) (Gore et al., 2009; Ross-Gillespie et al., 2007). In contrast, in larger groups the cost of cheating to cooperators may be buffered by the presence of other cooperators (Brown, 1982; Gore et al., 2009; Ross-Gillespie et al., 2007), and cheats are likely to be harder to detect, eluding punishment (Alencar et al., 2008; Fischer et al., 2014). Consequently, cheating is expected to be more likely to occur in larger groups where both the ability, and strength of selection, to control cheating is weaker (Brown, 1982; Gore et al., 2009; Ross-Gillespie et al., 2007). This prediction has been challenging to test because it requires experimentally manipulating group complexity, and the opportunities for cheating, while monitoring the behavior and reproductive success of individuals in detail.

Here we use a unique system, the cooperatively breeding ostrich, *Struthio camelus*, and experimentally manipulate the numbers of male and female breeders to mimic natural variation in group complexity (see paper 1, Sauer & Sauer, 1966; Bertram, 1992; Magige et al., 2009). Ostriches typically breed in groups of unrelated individuals that nest communally and cooperate over incubation (Bertram, 1992; Kimwele & Graves, 2003; Magige et al., 2009). Despite the cooperative nature of their breeding system, ostriches are renowned for being reproductive cheats: females frequently lay eggs in nests but never help with incubation, a behaviour that we define as "cheating" (Bertram, 1979; Kimwele & Graves, 2003). These features of the ostrich breeding system make them ideal to study the emergence of female cheating and the impact cheats have on the reproductive success of cooperators in groups that vary in complexity. We identified cheats using a combination of parentage data and observations of copulation and incubation behaviour (see methods for details). Leading to the classification of two different types of reproductive females: cheats and cooperators. Using this data we tested how the number of females in groups influences rates of cheating and cooperation; quantified the expected reproductive payoffs of cooperating and cheating; examined how cooperators responded to the presence of cheats; and analyzed the consequences of cheating for the breeding success of groups of different complexity.

Results

Opportunities for cheating and cooperation vary with group complexity

We found that out of 107 breeding groups, 32 (30%) had at least one reproductive female that did not contribute to collective incubation (i.e. a cheat). Of these, 23 groups had one cheat, seven had two, and two had three cheats (Figure 1A). The opportunities for both cheating and cooperating increased with the number of females in groups (Figure 1B. Frequency of cheats: Number of females: posterior mode (PM) and credible interval (CI) = 0.25 (0.01 , 0.43), pMCMC = 0.04; Frequency of cooperators: Number of females: PM (CI) = 0.76 (0.6 , 0.99), pMCMC = 0.001. Table S1). In small groups (< 3 females) rates of cooperation were extremely high, with over 90% of females contributing to incubation and cheating being almost non-existent (Figures 1C & 1D). However, as group sizes became larger, the rate of increase in number of cooperators slowed down (Figures 1C. Number of cooperators: Number of females^2: PM (CI) = 0.2 (0.03 , 0.39), pMCMC = 0.02. Table S1), whereas the rates of cheating accelerated (Figures 1D. Number of cheats: Number of females^2: PM (CI) = -1.08 (-1.69 , -0.78), pMCMC = 0.001. Table S1). Consequently, larger groups had more cheats and more cooperators, but the proportion of cheats relative to cooperators increased with the number of females in groups.

The expected reproductive payoffs of cheating are particularly high in small groups

One explanation for the persistence of cooperative groups in the face of cheating is that cheats have lower reproductive success than cooperators (Riehl & Frederickson, 2016; but see Riehl & Strong, 2019; Deng et al., 2015). We tested this idea by estimating the expected reproductive payoffs of cooperating and cheating, measured as the number of offspring groups produced multiplied by the estimated individual share (see Methods for details). We found that, across all group sizes, the expected reproductive

payoffs for cheats were at least as high as they were for cooperators (Cheats vs Cooperators in groups with cheats: PM (CI) = 0.06 (-0.29 , 0.47), pMCMC = 0.606; Cheats : vs Cooperators in groups without cheats: PM (CI) = 0.19 (-0.18 , 0.56), pMCMC = 0.388. Table S2).

The expected reproductive success of cheats was particularly high in small groups (Figure 2. Cheats : number of cooperators^2: PM (CI) = 0.34 (-0.04 , 0.78), pMCMC = 0.074. Table S2. See also Table S3). Similar patterns were evident for cooperators

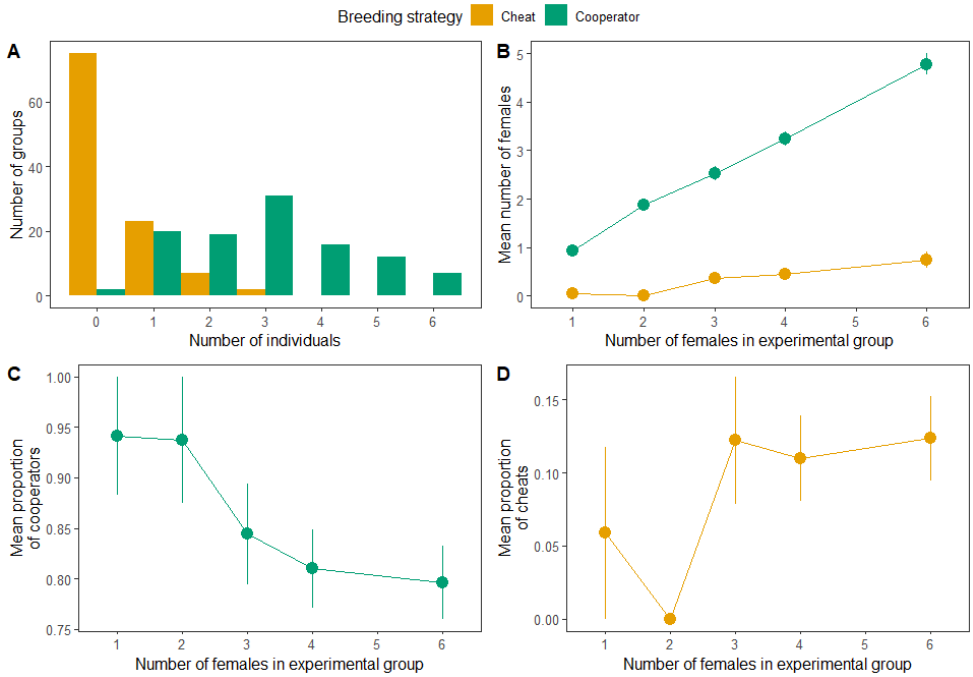


Figure 1: The occurrence of cheats and cooperators in experimentally established breeding groups of ostriches. A) The frequency of groups with a given number of cooperators (green) and cheats (orange). B) The mean number of cooperators (green) and cheats (orange) across groups with different numbers of females. C) The mean proportion of cooperators across groups with different numbers of females. D) The mean proportion of cheats across groups with different numbers of females. Points and error bars in B-D show mean +/- 1 standard error.

in the absence of cheats (Figure 2. Cooperators in groups without cheats : Number of cooperators: PM (CI) = -0.27 (-0.43 , -0.15), pMCMC = 0.001. Table S2). However, this was not the case for cooperators with a cheat in their group, suggesting that cheating

was costly for cooperators in small groups (Cooperators in groups with cheats : Number of cooperators: PM (CI) = 0.02 (-0.31 , 0.21), pMCMC = 0.764. Table S2). As groups increased in size, these effects disappeared and cheats and cooperators, both with and without cheats in their groups, had similar expected reproductive success (Figure 2). These effects did not appear to be due to individual differences between cheats and cooperators. Across successive breeding attempts there was little repeatability in the breeding roles that individuals adopted, suggesting that cheating is dependent on circumstance rather than inherent differences between individuals (variation in cheating explained by individual, % (CI) = 1.842 (0 , 14.592). TableS3).

While the costs of cheating for cooperators appear to be mitigated by being in larger groups (Figure 2), the detrimental effects of cheats likely pose a problem for the

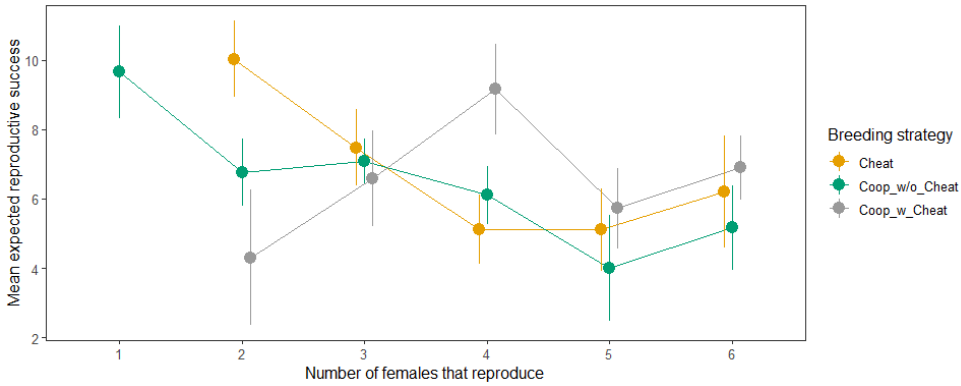


Figure 2: The reproductive returns and costs of cheating. The effect of the number reproductive females (cheats + cooperators) on the mean reproductive success of cheats (orange), cooperators in groups without cheats (green) and cooperators in groups with cheats (grey). Points and error bars show mean +/- 1 standard error.

stability of small breeding groups. Small groups are nevertheless frequently observed in the wild, and given that cheats benefit more from being in small groups, how is cheating controlled under these circumstances?

Cooperators adjust their incubation effort in response to cheats in small groups

In groups without cheats, cooperators decreased their investment in incubation as the number of cooperators increased (Figure 3A. Number of female cooperators: PM (CI) = -0.43 (-0.71 , -0.16), pMCMC = 0.008. Table S5). This is in line with our previous findings that showed cooperative incubation enables individuals to reduce their work load while increasing the amount of time nests are protected (see paper 1). However, in groups with cheats, investment in incubation did not decline linearly as the number of cooperators increased (Figure 3A. Number of female cooperators: PM (CI) = -0.13 (-0.51 , 0.27), pMCMC = 0.566. Table S5). Instead, incubation effort by cooperators was generally lower in groups with cheats (Cooperators in groups without cheats vs cooperators in groups with cheats: PM (CI) = 0.78 (0.17 , 1.15), pMCMC = 0.01. Table S5). This was particularly pronounced in small groups, causing the relationship between incubation effort and number of cooperators to be relatively flat in groups with cheats (Figure 3A).

Cooperators with the most to lose respond most strongly to cheats

We examined whether the response to the presence of cheats was similar across all individuals within groups, or whether individuals that had the most to lose were more sensitive to cheats. It is possible that individuals only contribute to cooperative

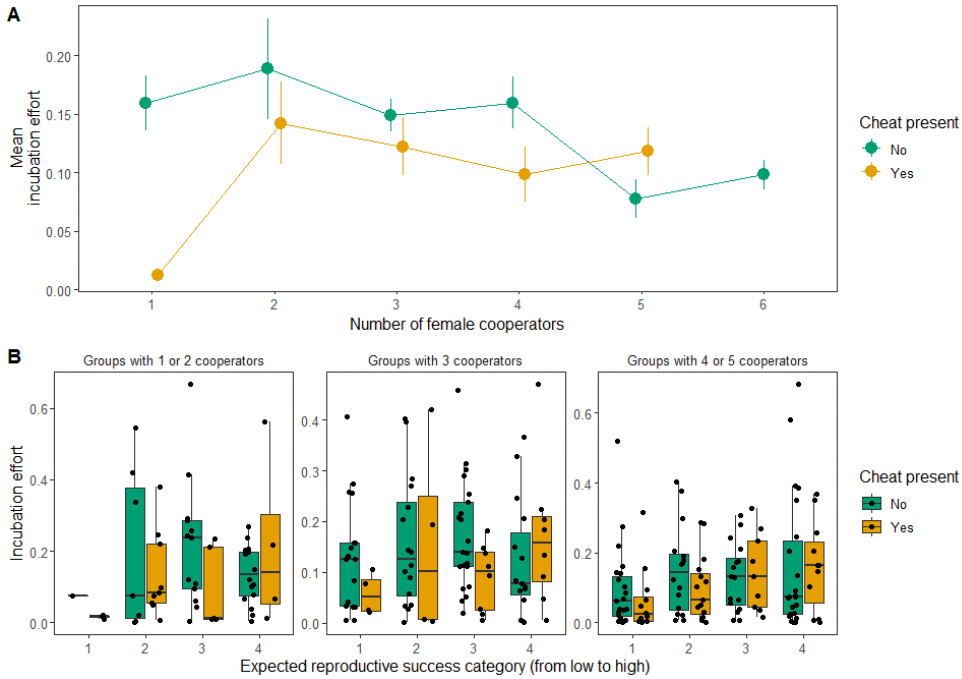


Figure 3: The effect of cheats on the incubation effort of cooperators. A) Mean individual incubation effort in relation to the number of female cooperators in groups, where cheats were present (orange) and absent (green). B) The effect of the presence of cheats on the incubation effort of individuals in relation to their expected reproductive success (1=low to 4=high) in different group sizes. Points and error bars in A show mean +/- 1 standard error.

incubation when their expected reproductive returns exceed a certain threshold (Sachs et al., 2004; Smith et al., 2014). The presence of cheats may thus influence individuals that are close to this threshold more than those that perceive their reproductive success to be higher. We investigated this by examining the relationship between expected reproductive success and incubation effort in the presence and absence of cheats.

In groups where cheats were present, incubation effort was tailored to expected reproductive success (PM (CI) = 0.29 (0.04 , 0.7), pMCMC = 0.03. Table S5). This was not the case for groups without cheats. When cheats were absent, individuals contributed to cooperative incubation irrespective of their potential reproductive success (PM (CI) = -0.01 (-0.26 , 0.21), pMCMC = 0.908. Table S5). The difference in the relationship between incubation effort and expected reproductive success in groups with and without cheats was, at least partly, driven by the fact that individuals with low expected reproductive success appeared to reduce their incubation effort in the presence of cheats across all group sizes (figure 3B).

Cheats drive a tragedy of the commons in small groups, but cooperation in large groups attenuates it

It has previously been shown in ostriches that the more time eggs are protected, the higher the hatching success of groups (see paper 1). A reduction in the amount of time individuals incubate in the presence of cheats may therefore compromise the reproductive success of groups. We found that in group with cheats, hatching success increased with the number of females that contributed to incubation (Figure 4. PM (CI) = 1.09 (0.21 , 1.84), pMCMC = 0.006. Table S6). This was not the case in groups without cheats (Figure 4. PM (CI) = 0.36 (-0.18 , 0.86), pMCMC = 0.158. Table S6). These differences were due to hatching success being compromised in small groups when cheats were present, but not when cheats were absent. For example, the presence of cheats resulted in complete hatching failure in groups with a single incubating female. This did not happen in groups without cheats, nor in larger groups (Figure 4). Together, these results suggest that the reduction in incubation effort triggered by the presence of cheats drives a tragedy of the commons in small, but not large, groups.

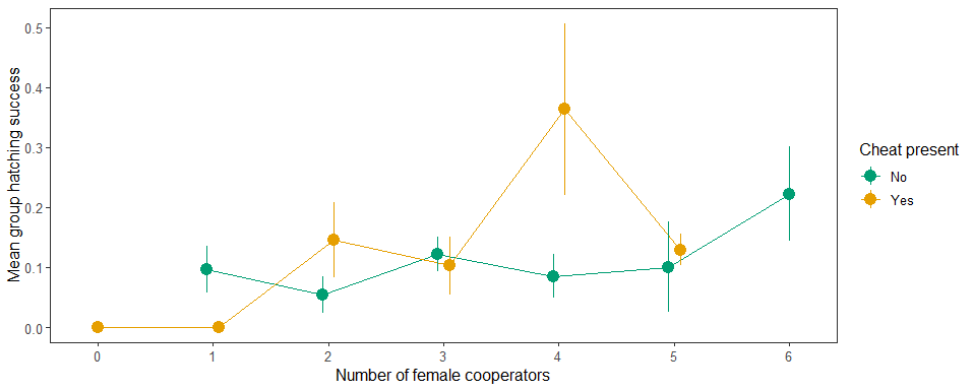


Figure 4: Cheating and the reproductive success of groups. The effect of number of female cooperators on the hatching success of eggs in groups with (orange) and without (green) cheats. In groups without female cooperators, incubation was taken care of by males only. Groups where no incubation was recorded were excluded from the data. Points and error bars show mean +/- 1 standard error.

Discussion

Our results show that the opportunities for cheating increase as groups become larger and contain more cooperators, whereas cheats are virtually absent in small groups (Figure 1). These results are in line with the prediction that the common goods provided by cooperators in larger groups allow cheating to proliferate (Gore

et al., 2009; Ross-Gillespie et al., 2007). However, when looking at the effects of breeding group size (i.e. the number of females that contribute to incubation) on individual expected reproductive success, we found that cheats maximize their reproductive success in small groups (Figure 2). Why don't we then see more cheats in small groups? The answer is that cheating in small groups elicits strong reductions in incubation by cooperators (Figure 3A) leading to breeding failure for all members of the group (Figure 4). This tragedy of the commons, appears attenuated in larger groups by more individuals cooperating where the costs of cheating to individual reproductive success are more diffuse.

Our results suggest that the presence of cheats induces individuals to calculate their potential returns from reproductive events, and adjust their cooperative behavior accordingly. It is unclear why cheats induce individuals to become calculating about their reproductive payoffs. However, understanding the mechanisms that individuals use to estimate their reproductive success could help clarify this question. Our experiments were not designed to reveal such mechanisms, but some insight was provided by the presence of females in groups that did not copulate or incubate (classified as "other", non-breeding individuals). In the absence of cheats, an increasing number of such non-breeding females had an inhibitory effect on the incubation behavior of cooperators (PM (CI) = -0.44 (-0.65 , -0.25), pMCMC = 0.001. Table S4). This suggests that individuals use a simple "rule of thumb" of the number of individuals within the group not contributing to incubation to adjust their investment in cooperation, rather than calculating the expected reproductive success of others (e.g. through mating behavior). Such simple rules have also been found to be used to coordinate cooperation in another communal breeding bird, the Greater Ani, *Crotophaga major* (Riehl, 2010).

The question still remains of why individuals should desert incubation when they find themselves in small groups with a cheat. It is likely that cooperators have laid eggs in the deserted nest and that they will suffer the loss of this investment when the breeding attempt is aborted. It would, however, be fallacious to expect that individuals base their decisions on past investment, the so called 'Concorde fallacy' (Curio, 1987; Dawkins & Carlisle, 1976). Decisions should instead be weighed against future benefits (Dawkins & Carlisle, 1976; Sargent & Gross, 1985). The relative costs and benefits for cooperators of abandoning eggs versus raising the offspring of cheats is thus likely to depend on how current and future reproduction investment is traded-off (McNamara et al., 2004; Nielsen et al., 2006; Williams, 1966).

Ostriches are long lived, reaching well over 30 years in captivity, and breed frequently (Bertram, 1992). The chance of finding future reproductive opportunities is therefore likely to be high. Moreover, the costs of incubation are likely to be significant, as it takes 42 days for ostrich eggs to hatch, and ostriches have to face the challenges of incubating in habitats where predation risk and thermal stress is high (Bertram, 1992; Magige et al., 2009; Schou et al., 2021). It may in fact be

extremely difficult for single individuals, or small groups, to successfully complete incubation as temperatures often exceed 40°C in many parts of their geographical range, which increases the need of both egg protection (see paper 4 and Bertram, 1992), and thermoregulation by adults (see paper 4 and Bertram (1992), Schou et al. (2021)). Given that eggs in a nest guarded by a small group may be unlikely to hatch, abandoning such nests will come at a relatively small cost to individuals.

In summary, these results provide experimental evidence that under certain conditions (small groups) the response to cheating by cooperators results in a tragedy of the commons. In turn, this may nullify the benefits of being a cheat in small groups, and make it unlikely for cheating to proliferate. Under other circumstances (big groups), the negative effect of cheats on individuals is buffered by the presence of other cooperators, and cheating becomes a viable, and more frequent, strategy. This buffering effect of cooperation also suggests that the need for having mechanisms to control cheats may be reduced in big groups. The fact that cheats and cooperators can coexist in big groups, with apparently little conflict, makes such groups complex beyond their sheer size (Lukas & Clutton-Brock, 2018). Understanding the coexistence of two different breeding strategies in ostrich groups can help us shed light on the much bigger question about how animal societies have reached their diversity and complexity (Veit, 2019).

Methods

Study population

The research was conducted on a captive population of ostriches kept in fenced areas (range: 2400 and 70600 m2, median = 4700 m2) of Karoo habitat at Oudtshoorn Research Farm, South Africa (33° 38’ 21.5“S, 22° 15’ 17.4”E). The experiments involved 118 breeding groups monitored from 2012 to 2018 involving 147 males and 170 females. All individuals were individually identifiable by coloured neck tags.

Experimental design

We manipulated the complexity of ostrich groups by experimentally establishing groups with different numbers of males (1 to 3) and females (1 to 6) across a seven-year period (16-18 groups per year). Due to limitations in the number of birds accessible for our experiments, and other experiments being conducted on the same population, not all combinations of male and female group sizes were possible. The final sample size of the experiment was slightly reduced for the following reasons: Individual ostriches were removed from the study due to casualties, injuries or

aggressive behaviour. Entire groups were excluded if any of their members was removed, and not replaced, less than two weeks before the start of the observations of incubation behaviour, as such a removal was likely to cause a disruption in breeding behaviour. Groups were also excluded from the study if no incubation or copulation behaviour was observed. This resulted in a final sample size of 107 groups with 143 males and 162 females, many of which were included in the study in more than one year. The breeding season was typically from May to December. During the first ~5 months of the season, eggs were collected twice a day and incubated artificially. During the last ~2 months eggs were left in nests and the data for this study was collected. During the breeding season, ostriches received a balanced ostrich breeder diet (90 to 120 g protein, 7.5 to 10.5 MJ metabolizable energy, 26 g calcium and 6 g phosphorus per kg feed) and ad-libitum water.

Monitoring incubation and copulation behaviour

Copulation frequency, copulation success and incubation behaviour (start time, duration and end time) were monitored by conducting ~3 hour observations at least three times a week using binoculars (10 x 40) and a telescope (20-60 x 80). The observer sat camouflaged in a 10-meter-tall observation tower in the middle of the field site. Each group was observed for between 47 and 91 hours. Individual incubation effort was estimated as the proportion of time that each individual spent incubating (total amount of time each individual was observed incubating divided by the total observation time).

Expected reproductive success

There was a correlation between copulation success and reproductive success (Figure S1. Pearson's correlation test: $r(157) = 0.51$, $p < 0.001$). We estimated this correlation using parentage and copulation success data from the three initial years of our study (2012-2014). The parentage data was obtained from blood and tissue samples collected from adults and their offspring. From these samples, seven highly polymorphic tracts of repetitive DNA (microsatellites) were amplified using Phusion Blood Direct PCR Kit (Thermo ScientificTM) and fluorescently labelled primers. These seven microsatellites have previously been used to assign parentage in ostriches with high confidence (Bonato, 2009). After DNA-amplification, the amplicons were separated by size using capillary electrophoresis. Microsatellite scoring was then performed visually using the software Geneious 10.2.3. Finally, a parentage analysis was run in the software Cervus 3.0.7.

The expected reproductive success of every individual was measured as the number of eggs produced by their group multiplied by the percentage of copulations each individual obtained (number of successful copulations gained by an individual divided by the total number of successful copulations observed in that individual's

breeding group). This allowed us to estimate individual reproductive success using some of the main cues that ostriches are likely to use when estimating their own reproductive success.
For figure 3B the distribution of expected reproductive success across the whole population was used to create four categories of expected reproductive success. Individuals that had an expected reproductive success below the first quantile of the distribution were assigned reproductive success category 1, individuals between the first quantile and the median (including the first quantile) were assigned category 2, individuals between the median and the third quartile (including the median) category 3, and individuals on and above the third quartile were assigned category 4.

Hatching success

Nests were checked daily and new eggs were marked with the date and an egg identification number. The absence and presence of previously laid eggs was recorded to track the fate of each egg. Between 2012 and 2014, hatching success was measured by allowing groups to naturally incubate eggs to completion. If no eggs were observed hatching in groups after 50 days, they were removed and checked for developing embryos. In 2015, changes to legislation to reduce the spread of bird flu meant that contact between adults and chicks had to be minimised. Consequently, between 2015 and 2018, eggs were removed from nests just before hatching (~40 days after the onset of incubation) and placed in artificial incubators to estimate hatching success.

Quantification and statistical Analyses

General approach

Data were analysed in R using Bayesian Linear Mixed Models (BLMM) with Markov chain Monte Carlo (MCMC) estimation in the package MCMCglmm. Default fixed effect priors were used (independent normal priors with zero mean and large variance (10^{10})) and for random effects inverse gamma priors were used. Apart from in binary models. For binary models fixed effect priors were specified as mu = 0, V = 1 + π2 / 3 (relatively flat on the logit scale), residual variance is not identifiable and was fixed at 1, and for random effects parameter expanded priors were used (V = 1, nu = 0.002, alpha.mu = 0, alpha.V = 1000) (Hadfield, 2021). Each analysis was run for 1100000 iterations with a burn-in of 100000 and a thinning interval of 1000. Convergence was checked by running models three times and examining the overlap of traces, levels of autocorrelation, and testing with Gelman and Rubin's convergence diagnostic (potential scale reduction factors <1.1).

Parameter estimates for fixed effects are reported from models that included all terms of the same order and lower. For example, all main effect estimates are from models where all other main effects are included, all estimates of two-way interactions are from models that included all two-way interactions and main effects, and so forth. Quadratic effects were tested in models including main effects and effects of the same order (other quadratic effects and two-way interactions). All continuous explanatory variables were z transformed using the scale() function in R. Curvilinear effects of continuous explanatory variables were modelled using the quadratics of the z transformed values computed before running the models.

Fixed effects were considered significant when 95% credible intervals (CIs) did not overlap with 0 and pMCMC were less than 0.05 (pMCMC = proportion of iterations above or below a test value correcting for the finite sample size of posterior samples). By default MCMCglmm reports parameter estimates for fixed factors as differences from the global intercept. This does not allow absolute estimates and 95% CIs for all factor levels to be estimated or custom hypothesis tests of differences between factor levels. Consequently, we removed the global intercept from all models (with one exception, M2b, specified in the section below) and present absolute estimates for factor levels. Differences between factor levels were estimated by subtracting the posterior samples from one level from the second level and calculating the posterior mode, 95% CI and pMCMC. The non-independence of data arising from multiple data points per individual, per group, per enclosure (used repeatedly across years), and per year was modelled using random effects. To estimate the magnitude of random effects we calculated the percentage of variation explained by each random term (I2%: (Vi/Vtotal)x100) (Villemereuil et al., 2016) after accounting for variation attributable to fixed effects. To obtain estimates of I2 on the expected scale from binomial models the distribution variance for the link function (logit) was included in the denominator (Vi/(Vtotal + $\pi^3/2$) x100). Random effect estimates are reported from models that included the highest order fixed effect terms.

Specific analyses

1. Testing how female group size influences frequencies of female cheats and cooperators.

The effect of group size on the frequency of female cheats and cooperators was modelled using a BLMM with a Poisson error distribution. The response variable was the frequency of a given breeding strategy in each breeding group. Breeding strategy (2 level factor: Cooperator or Cheat), number of males (continuous), number of females (continuous) were entered as fixed effects, and year, enclosure and group were included as random effects. The effects of female group size on the frequency of cheats and cooperators were estimated by fitting two-way interactions between breeding strategy and number of females (R code: M1).

2. Testing how group composition influences expected reproductive success.

The effect of group composition on the expected reproductive success of cheats and cooperators was modelled using a BLMM with a Poisson error distribution. The response variable was individual expected reproductive success. Number of males, number of female cooperators and number of non-reproductive females (continuous, referred to as "Other"). To test the presence of cheats in the group a new 3 level factor was created (referred to as breeding status): a) cheat, b) cooperator in a group with cheat(s), and c) cooperator in a group without cheat(s). Due to the fact the vast majority (72%) of the groups that had a cheat only had one cheat, we used the presence or absence of cheat in a given group to create this variable, rather than as number of cheats in the group. Year, enclosure, group and individual were included as random effects. The effects of number of female cooperators on reproductive success was estimated by fitting a two-way interaction between breeding status and number of female cooperators (R code: M2).

2b. Testing how group composition influences expected reproductive success of cheats.

To examine whether the effect of number of female cooperators on the reproductive success of cheats was influenced by other terms in the model, we modelled this effect using a BLMM with a Poisson error distribution including only cheats. The response variable was thus the expected reproductive success of cheats. Number of non-reproductive females, number of female cooperators and number of males were entered as fixed effects. Since none of the explanatory variables was a factor, the global intercept was left in the model. Year, enclosure, group and individual were included as random effects (R code: M2c).

3. Testing repeatability of cheating across years.

We tested the repeatability of cheating behaviour across years using a BLMM with binary error distribution of the probability of cheating as the response variable (1 if an individual was a cheat in a given year, and 0 if she was not a cheat). Number of males and females in the group, as well as age were included as fixed effects. Year, enclosure, group and individual were entered as random effects. Repeatability of cheating was then estimated by the percentage of variation explained by individual (R code: M2Ch).

4. Testing how group composition and expected reproductive success influences individual incubation effort.

The effect of group composition and expected reproductive success on the time individuals invested in incubation was modelled using a BLMM with a binomial error distribution. The response variable was the number of observation minutes an individual was observed sitting versus the number of minutes it was not sitting,

which accounts for variation in the amount of time individuals were observed. The presence of cheats in the group (2 level factor: Cheat present-Yes or Cheat present-No), number of female cooperators, expected reproductive success, number of non-reproductive females and number of males were included as fixed effects. Year, enclosure, group and individual were entered as random effects. The effects of number of female cooperators on the incubation effort of cooperators in groups with and without cheats was estimated by fitting a two-way interaction between presence of cheats in the group and number of female cooperators. In a similar way, the effect of expected reproductive success was modelled fitting a two-was interaction between presence of cheats in the group and expected reproductive success (R code: M4).

5. Testing how group composition influences hatching success.

The effect of group composition on hatching success was modelled using a BLMM with a binomial error distribution. The response variable was the number of eggs that hatched versus the number of eggs that did not hatch. Presence of cheats, number of female cooperators, number of non-reproductive females and number of males were included as fixed effects. Year, enclosure and group were included as random effects. The effects of number of female cooperators on hatching success in group with and without cheats was estimated fitting a two-way interaction between presence of cheats and number of female cooperators. (R code: M8).

References

Alencar, A., Deoliveirasiqueira, J., & Yamamoto, M. (2008). Does group size matter? Cheating and cooperation in brazilian school children☆. *Evolution and Human Behavior*, *29*(1), 42–48. https://doi.org/10.1016/j.evolhumbehav.2007.09.001

Archibald, J. (2014). *One plus one equals one: Symbiosis and the evolution of complex life*. Oxford University Press.

Bertram, B. C. R. (1992). *The ostrich communal nesting system:* Princeton University Press. https://doi.org/10.2307/j.ctt7ztm99

Bertram, B. C. R. (1979). Ostriches recognise their own eggs and discard others. *Nature*, *279*(5710), 233–234. https://doi.org/10.1038/279233a0

Bonato, M. (2009). *Mate choice and immunocompetence in ostriches (struthio camelus)* [Thesis, Stellenbosch : University of Stellenbosch]. https://scholar.sun.ac.za:443/handle/10019.1/1257

Bourke, A. F. G. (2011). *Principles of social evolution*. Oxford University Press. https://oxford.universitypressscholarship.com/view/10.1093/acprof:oso/9780199231157.001.0001/acprof-9780199231157

Brännström, Å., Gross, T., Blasius, B., & Dieckmann, U. (2011). Consequences of fluctuating group size for the evolution of cooperation. *Journal of Mathematical Biology*, *63*(2), 263–281. https://doi.org/10.1007/s00285-010-0367-3

Brown, J. L. (1982). Optimal group size in territorial animals. *Journal of Theoretical Biology*, *95*(4), 793–810. https://doi.org/10.1016/0022-5193(82)90354-X

Curio, E. (1987). Animal decision-making and the "concorde fallacy". *Trends in Ecology & Evolution*, *2*(6), 148–152. https://doi.org/10.1016/0169-5347(87)90064-4

Dawkins, R., & Carlisle, T. R. (1976). Parental investment, mate desertion and a fallacy. *Nature*, *262*(5564), 131–133. https://doi.org/10.1038/262131a0

Deng, C., Slamti, L., Raymond, B., Liu, G., Lemy, C., Gominet, M., Yang, J., Wang, H., Peng, Q., Zhang, J., Lereclus, D., & Song, F. (2015). Division of labour and terminal differentiation in a novel bacillus thuringiensis strain. *The ISME Journal*, *9*(2), 286–296. https://doi.org/10.1038/ismej.2014.122

Fischer, S., Zöttl, M., Groenewoud, F., & Taborsky, B. (2014). Group-size-dependent punishment of idle subordinates in a cooperative breeder where helpers pay to stay. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1789), 20140184. https://doi.org/10.1098/rspb.2014.0184

Foster, K. R., & Ratnieks, F. L. W. (2000). Facultative worker policing in a wasp. *Nature*, *407*(6805), 692–693. https://doi.org/10.1038/35037665

Foster, K. R., & Ratnieks, F. L. W. (2001). Convergent evolution of worker policing by egg eating in the honeybee and common wasp. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *268*(1463), 169–174. https://doi.org/10.1098/rspb.2000.1346

Ghoul, M., Griffin, A. S., & West, S. A. (2014). Toward an evolutionary definition of cheating. *Evolution*, *68*(2), 318–331. https://doi.org/https://doi.org/10.1111/evo.12266

Gore, J., Youk, H., & Oudenaarden, A. van. (2009). Snowdrift game dynamics and facultative cheating in yeast. *Nature*, *459*(7244), 253–256. https://doi.org/10.1038/nature07921

Hadfield, J. D. (2021). *MCMCglmm course notes*. https://cran.r-project.org/web/packages/MCMCglmm/vignettes/CourseNotes.pdf

Hardin, G. (1968). The tragedy of the commons. *Science*, *162*(3859), 1243–1248. https://doi.org/10.1126/science.162.3859.1243

Kimwele, C. N., & Graves, J. A. (2003). A molecular genetic analysis of the communal nesting of the ostrich (struthio camelus). *Molecular Ecology*, *12*(1), 229–236. https://doi.org/https://doi.org/10.1046/j.1365-294X.2003.01727.x

Lukas, D., & Clutton-Brock, T. (2018). Social complexity and kinship in animal societies. *Ecology Letters*, *21*(8), 1129–1134. https://doi.org/https://doi.org/10.1111/ele.13079

Magige, F. J., Stokke, B. G., Sortland, R., & Røskaft, E. (2009). Breeding biology of ostriches (struthio camelus) in the serengeti ecosystem, tanzania. *African Journal of Ecology*, *47*(3), 400–408. https://doi.org/https://doi.org/10.1111/j.1365-2028.2008.01002.x

Maynard Smith, J., & Eors, S. (1995). *The major transitions in evolution*. Oxford University Press.

McNamara, J. M., Barta, Z., & Houston, A. I. (2004). Variation in behaviour promotes cooperation in the prisoner's dilemma game. *Nature*, *428*(6984), 745–748. https://doi.org/10.1038/nature02432

Michod, R. E., & Herron, M. D. (2006). Cooperation and conflict during evolutionary transitions in individuality. *Journal of Evolutionary Biology*, *19*(5), 1406–1409. https://doi.org/10.1111/j.1420-9101.2006.01142.x

Mulder, R. A., & Langmore, N. E. (1993). Dominant males punish helpers for temporary defection in superb fairy-wrens. *Animal Behaviour*, *45*(4), 830–833. https://doi.org/10.1006/anbe.1993.1100

Nielsen, C. R., Parker, P. G., & Gates, R. J. (2006). Intraspecific nest parasitism of cavity-nesting wood ducks: Costs and benefits to hosts and parasites. *Animal Behaviour*, *72*(4), 917–926. https://doi.org/10.1016/j.anbehav.2006.03.004

Rankin, D. J., Bargum, K., & Kokko, H. (2007). The tragedy of the commons in evolutionary biology. *Trends in Ecology & Evolution*, *22*(12), 643–651. https://doi.org/10.1016/j.tree.2007.07.009

Ratnieks, F. L. W., & Visscher, P. K. (1989). Worker policing in the honeybee. *Nature*, *342*(6251), 796–797. https://doi.org/10.1038/342796a0

Reeve, H. K., & Nonacs, P. (1992). Social contracts in wasp societies. *Nature*, *359*(6398), 823–825. https://doi.org/10.1038/359823a0

Riehl, C. (2010). A simple rule reduces costs of extragroup parasitism in a communally breeding bird. *Current Biology*, *20*(20), 1830–1833. https://doi.org/10.1016/j.cub.2010.09.005

Riehl, C., & Frederickson, M. E. (2016). Cheating and punishment in cooperative animal societies. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1687), 20150090. https://doi.org/10.1098/rstb.2015.0090

Riehl, C., & Strong, M. J. (2019). Social parasitism as an alternative reproductive tactic in a cooperatively breeding cuckoo. *Nature*, *567*(7746), 96–99. https://doi.org/10.1038/s41586-019-0981-1

Ross-Gillespie, A., Gardner, A., West, S. A., & Griffin, A. S. (2007). Frequency dependence and cooperation: Theory and a test with bacteria. *The American Naturalist*, *170*(3), 331–342. https://doi.org/10.1086/519860

Rubenstein, D. R., & Abbot, P. (Eds.). (2017). *Comparative social evolution*. Cambridge University Press. https://doi.org/10.1017/9781107338319

Sachs, J. L., Mueller, U. G., Wilcox, T. P., & Bull, J. J. (2004). The evolution of cooperation. *The Quarterly Review of Biology*, *79*(2), 135–160. https://doi.org/10.1086/383541

Sargent, R. C., & Gross, M. R. (1985). Parental investment decision rules and the concorde fallacy. *Behavioral Ecology and Sociobiology*, *17*(1), 43–45. https://doi.org/10.1007/BF00299427

Sauer, E. G. F., & Sauer, E. M. (1966). Social behaviour of the south african ostrich, struthio camelus australis. *Ostrich*, *37*(sup1), 183–191. https://doi.org/10.1080/00306525.1966.9639797

Schou, M. F., Bonato, M., Engelbrecht, A., Brand, Z., Svensson, E. I., 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*(1), 666. https://doi.org/10.1038/s41467-021-20937-7

Shen, S.-F., Akçay, E., & Rubenstein, D. R. (2014). Group size and social conflict in complex societies. *The American Naturalist*, *183*(2), 301–310. https://doi.org/10.1086/674378

Smith, J., Dyken, J. D. V., & Velicer, G. J. (2014). Nonadaptive processes can create the appearance of facultative cheating in microbes. *Evolution*, *68*(3), 816–826. https://doi.org/https://doi.org/10.1111/evo.12306

Travisano, M., & Velicer, G. J. (2004). Strategies of microbial cheater control. *Trends in Microbiology*, *12*(2), 72–78. https://doi.org/10.1016/j.tim.2003.12.009

Veit, W. (2019). Evolution of multicellularity: Cheating done right. *Biology & Philosophy*, *34*(3), 34. https://doi.org/10.1007/s10539-019-9688-9

Wade, M. J., & Breden, F. (1980). The evolution of cheating and selfish behavior. *Behavioral Ecology and Sociobiology*, *7*(3), 167–172. https://doi.org/10.1007/BF00299360

West, S. A., Cooper, G. A., Ghoul, M. B., & Griffin, A. S. (2021). Ten recent insights for our understanding of cooperation. *Nature Ecology & Evolution*, 1–12. https://doi.org/10.1038/s41559-020-01384-x

Williams, G. C. (1966). Natural selection, the costs of reproduction, and a refinement of lack's principle. *The American Naturalist*, *100*(916),

Supplementary Material:
Cheating triggers tragedy of the commons, group size attenuates it

Julian Melgar[1+], Mads F. Schou[1], Maud Bonato[2], Zanell Brand[3], Anel Engelbrecht[3], Schalk Cloete[4,3], Pfunzo T. Muvhali[4], Bengt Hansson[1] & Charlie K. Cornwallis[1+]

[1] Department of Biology, Lund University, Lund, Sweden [2] Kynsna Elephant Park, Kynsna, South Africa [3] Directorate Animal Sciences, Western Cape Department of Agriculture, Elsenburg, South Africa [4] Department of Animal Sciences, University of Stellenbosch, Matieland, South Africa

[+]Corresponding authors: Email: julian.melgar@biol.lu.se (mailto:julian.melgar@biol.lu.se), charlie.cornwallis@biol.lu.se (mailto:charlie.cornwallis@biol.lu.se)

This file includes: Figure S1; Tables S1 to S6

Supplementary Figures

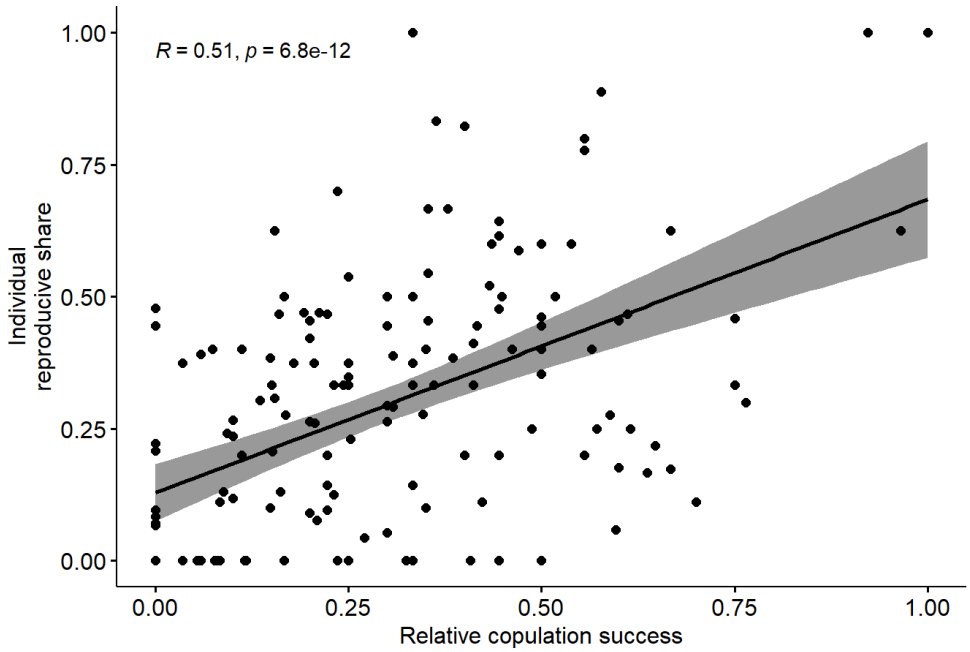


Figure S1. Correlation between female individual reproductive share (the proportion of offspring tha individuals mothered in their breeding group) and relative individual copulation success (the proportion of successful copulations, out of the group total, that individuals gained). The regression line and correlation statistics were calculated using Spearman correlation. Grey area shows the 95% confidence intervales. Each data point is a single individual. Groups in which no copulations were observed and/or no parentage was assigned are excluded from the analyses. Groups with only one individual of the sex being analyzed were also excluded from the analyses.

Supplementary Tables

Table S1. Effect of group size on frequencies of cooperators and cheats.

Fixed Effects	**Posterior Mode (CI)**	**pMCMC**
Cheats : ZNumber of males	-0.01 (-0.23 , 0.17)	0.656
Cooperators : ZNumber of males	0.06 (-0.11 , 0.22)	0.44
Cheats : ZNumber of females	0.25 (0.01 , 0.43)	**0.04**
Cooperators : ZNumber of females	0.76 (0.6 , 0.99)	**0.001**
Cheats : ZNumber of females^2	-1.08 (-1.69 , -0.78)	**0.001**
Cooperators : ZNumber of females^2	0.2 (0.03 , 0.39)	**0.02**
Cheats : ZNumber of females vs Cooperators : ZNumber of females	-0.54 (-0.87 , -0.29)	**0.001**
Cheats : ZNumber of females^2 vs Cooperators : ZNumber of females^2	-1.42 (-1.86 , -1.04)	**0.001**
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.139 (0.018 , 0.797)	88.246 (58.906 , 99.714)
Camp	0.001 (0 , 0.02)	2.909 (0.031 , 10.512)
Group	0.001 (0 , 0.013)	2.037 (0.048 , 7.09)
Residual	0.002 (0 , 0.061)	6.808 (0.062 , 30.068)

Table S2. Effect of group composition on expected reproductive success.

Fixed Effects	**Posterior Mode (CI)**	**pMCMC**
Cheats	1.83 (1.36 , 2.17)	**0.001**
Cooperators in groups without cheats	1.53 (1.28 , 1.84)	**0.001**
Cooperators in groups with cheats	1.67 (1.33 , 1.93)	**0.001**
ZNumber of other females	0.02 (-0.08 , 0.13)	0.646
ZNumber of males	0.04 (-0.08 , 0.15)	0.624
Cheats : ZNumber of cooperators	0.11 (-0.28 , 0.39)	0.62
Cooperators in groups without cheats : ZNumber of cooperators	-0.27 (-0.43 , -0.15)	**0.001**
Cooperators in groups with cheats : ZNumber of cooperators	0.02 (-0.31 , 0.21)	0.764
Cheats : Znumber of cooperators^2	0.34 (-0.04 , 0.78)	0.074
Cooperators in groups without cheats : ZNumber of cooperators^2	0.07 (-0.05 , 0.19)	0.204

Fixed Effects	Posterior Mode (CI)	pMCMC
Cooperators in groups with cheats : ZNumber of cooperators^2	-0.02 (-0.43 , 0.22)	0.576
Cheats vs Cooperators in groups without cheats	0.19 (-0.18 , 0.56)	0.388
Cheats vs Cooperators in groups with cheats	0.06 (-0.29 , 0.47)	0.606
Cooperators in groups without cheats vs Cooperators in groups with cheats	-0.06 (-0.34 , 0.18)	0.582
Cheats : ZNumber of cooperators vs Cooperators in groups without cheats : ZNumber of cooperators	0.38 (0.01 , 0.73)	0.05
Cheats : ZNumber of cooperators vs Cooperators in groups with cheats : ZNumber of cooperators	0.11 (-0.27 , 0.51)	0.544
Cooperators in groups without cheats : ZNumber of cooperators vs Cooperators in groups with cheats : ZNumber of cooperators	-0.29 (-0.53 , 0.02)	0.096
Cheats : Znumber of cooperators^2 vs Cooperators in groups with cheats : ZNumber of cooperators^2	0.45 (-0.05 , 0.92)	0.08
Cooperators in groups without cheats : ZNumber of cooperators^2 vs Cooperators in groups with cheats : ZNumber of cooperators^2	0.21 (-0.16 , 0.49)	0.298
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.05 (0.003 , 0.359)	13.562 (0.469 , 33.783)
Camp	0.001 (0 , 0.029)	0.931 (0.033 , 3.275)
Group	0.001 (0 , 0.022)	0.77 (0.018 , 2.49)
Individual	0.002 (0 , 0.208)	10.363 (0.041 , 24.932)
Residual	0.598 (0.425 , 0.807)	74.375 (51.241 , 94.113)

Table S3. Effect of group composition on the expected reproductive success of cheats.

Fixed Effects	**Posterior Mode (CI)**	**pMCMC**
Intercept	1.84 (1.44 , 2.13)	**0.001**
ZNumber of other females	-0.18 (-0.59 , 0.1)	0.16
ZNumber of female cooperators	-0.01 (-0.37 , 0.21)	0.638
ZNumber of males	0.05 (-0.16 , 0.27)	0.598
ZNumber of female cooperators^2	0.34 (0.1 , 0.61)	**0.008**
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.002 (0 , 0.152)	14.807 (0.08 , 53.482)
Camp	0.004 (0 , 0.223)	26.963 (0.143 , 76.326)
Group	0.002 (0 , 0.15)	19.977 (0.121 , 72.097)
Individual	0.002 (0 , 0.124)	15.798 (0.082 , 58.585)
Residual	0.002 (0 , 0.165)	22.455 (0.161 , 75.209)

Table S4. Effect of group structure and age on individual probability of cheating.

Fixed Effects	**Posterior Mode (CI)**	**pMCMC**
Intercept	-510.02 (-803.34 , -201.43)	**0.001**
Znumber of males	-41.43 (-141.46 , 20.41)	0.184
Znumber of females	24.2 (-86.85 , 129.11)	0.664
Zage	-36.29 (-116.88 , 47.66)	0.362
ZNumber of males^2	316.19 (77.32 , 677.33)	**0.004**
ZNumber of females^2	-92.26 (-379.97 , 127.79)	0.422
Zage^2	12.13 (-70.71 , 111.39)	0.684
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	-362.624 (0 , 63855.426)	3.796 (0 , 21.283)
Camp	450.816 (0.001 , 125218.058)	11.067 (0 , 36.546)
Group	-49.54 (0 , 35387.275)	1.912 (0 , 13.066)
Individual	-34.971 (0 , 34109.397)	1.842 (0 , 14.592)
Residual	174869.04 (56148.441 , 420763.388)	81.384 (55.082 , 100)

Table S5. Effect of group composition and expected reproductive success on incubation effort.

Fixed Effects	**Posterior Mode (CI)**	**pMCMC**
Female cheats in camp - No	-2.24 (-2.58 , -1.96)	**0.001**
Female cheats in camp - Yes	-2.89 (-3.38 , -2.48)	**0.001**
Female cheats in camp - No : ZNumber of other females	-0.44 (-0.65 , -0.25)	**0.001**
Female cheats in camp - Yes : ZNumber of other females	-0.4 (-1.16 , 0.22)	0.228
Female cheats in camp - No : Znumber of males	0.06 (-0.25 , 0.26)	0.898
Female cheats in camp - Yes : Znumber of males	-0.12 (-0.38 , 0.25)	0.618
Female cheats in camp - No : ZExpected reproductive success	-0.01 (-0.26 , 0.21)	0.908
Female cheats in camp - Yes : ZExpected reproductive success	0.29 (0.04 , 0.7)	**0.03**
Female cheats in camp - No : ZNumber of female cooperators	-0.43 (-0.71 , -0.16)	**0.008**

Fixed Effects	Posterior Mode (CI)	pMCMC
Female cheats in camp - Yes : ZNumber of female cooperators	-0.13 (-0.51 , 0.27)	0.566
Female cheats in camp - No : ZExpected reproductive success : ZNumber of female cooperators	0.1 (-0.1 , 0.26)	0.386
Female cheats in camp - Yes : ZExpected reproductive success : ZNumber of female cooperators	0.13 (-0.2 , 0.55)	0.452
Female cheats in camp - No vs Female cheats in camp - Yes	0.78 (0.17 , 1.15)	**0.01**
Female cheats in camp - No : ZExpected reproductive success vs Female cheats in camp - Yes : ZExpected reproductive success	-0.34 (-0.8 , 0.02)	0.066
Female cheats in camp - No : ZNumber of female cooperators vs Female cheats in camp - Yes : ZNumber of female cooperators	-0.33 (-0.81 , 0.11)	0.162
Female cheats in camp - No : ZExpected reproductive success : ZNumber of female cooperators vs Female cheats in camp - Yes : ZExpected reproductive success : ZNumber of female cooperators	0 (-0.5 , 0.34)	0.768
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.004 (0 , 0.175)	1.72 (0.007 , 6.582)
Camp	0.002 (0 , 0.114)	1.288 (0.012 , 4.47)
Group	0.001 (0 , 0.133)	1.332 (0.009 , 5.294)
Individual	0.881 (0.414 , 1.321)	34.672 (19.322 , 49.197)
Residual	1.481 (1.124 , 1.859)	60.986 (45.168 , 76.042)

Table S6. Effect of group composition on hatching success.

Fixed Effects	Posterior Mode (CI)	pMCMC
Female cheat in camp - No	-3.29 (-3.77 , -2.44)	**0.001**
Female cheat in camp - Yes	-2.7 (-3.66 , -2.03)	**0.001**
ZNumber of other females	0 (-0.41 , 0.42)	0.948
ZNumber of males	0.11 (-0.23 , 0.66)	0.416
Female cheat in camp - No : ZNumber of female cooperators	0.36 (-0.18 , 0.86)	0.158
Female cheat in camp - Yes : ZNumber of female cooperators	1.09 (0.21 , 1.84)	**0.006**

Fixed Effects	Posterior Mode (CI)	pMCMC
Female cheat in camp - No : ZNumber of female cooperators^2	0.13 (-0.33 , 0.59)	0.566
Female cheat in camp - Yes : ZNumber of female cooperators^2	-0.86 (-2.53 , -0.1)	**0.044**
Female cheat in camp - No vs Female cheat in camp - Yes	-0.24 (-1.25 , 0.59)	0.488
Female cheat in camp - No : ZNumber of female cooperators vs Female cheat in camp - Yes : ZNumber of female cooperators	-0.41 (-1.64 , 0.36)	0.234
Female cheat in camp - No : ZNumber of female cooperators^2 vs Female cheat in camp - Yes : ZNumber of female cooperators^2	1.03 (0.09 , 2.71)	**0.034**
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.007 (0 , 0.642)	3.831 (0.005 , 17.842)
Camp	0.003 (0 , 0.771)	5.595 (0.008 , 22.552)
Group	0.025 (0 , 4.045)	48.262 (0.005 , 98.108)
Residual	0.021 (0 , 3.888)	42.313 (0.012 , 97.289)

Paper III

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-021-20937-7 OPEN

Extreme temperatures compromise male and female fertility in a large desert bird

Mads F. Schou[1✉], Maud Bonato[2], Anel Engelbrecht[3], Zanell Brand[3], Erik I. Svensson[1], Julian Melgar[1], Pfunzo T. Muvhali[2], Schalk W. P. Cloete[2,3] & Charlie K. Cornwallis[1]

Temperature has a crucial influence on the places where species can survive and reproduce. Past research has primarily focused on survival, making it unclear if temperature fluctuations constrain reproductive success, and if so whether populations harbour the potential to respond to climatic shifts. Here, using two decades of data from a large experimental breeding programme of the iconic ostrich (*Struthio camelus*) in South Africa, we show that the number of eggs females laid and the number of sperm males produced were highly sensitive to natural temperature extremes (ranging from −5 °C to 45 °C). This resulted in reductions in reproductive success of up to 44% with 5 °C deviations from their thermal optimum. In contrast, gamete quality was largely unaffected by temperature. Extreme temperatures also did not expose trade-offs between gametic traits. Instead, some females appeared to invest more in reproducing at high temperatures, which may facilitate responses to climate change. These results show that the robustness of fertility to temperature fluctuations, and not just temperature increases, is a critical aspect of species persistence in regions predicted to undergo the greatest change in climate volatility.

[1] Department of Biology, Lund University, Lund, Sweden. [2] Department of Animal Sciences, University of Stellenbosch, Matieland, South Africa. [3] Directorate Animal Sciences, Western Cape Department of Agriculture, Elsenburg, South Africa. ✉email: mads.schou@biol.lu.se

The range of temperatures that organisms can tolerate has a crucial influence on their distributions across space and time[1–3]. Our current understanding of thermal tolerance largely comes from studies examining how high temperatures affect survival[4–7]. However, it has recently been argued that because reproductive failure often occurs well before death, temperature effects on fertility (thermal fertility limits) may be more important in determining species responses to environmental change[8–12]. Characterizing how natural temperature fluctuations affect investment in fertility traits, such as the number and viability of eggs and sperm, and the impact this has on reproductive success is therefore crucially important, especially as climatic variation is expected to increase globally[13,14]. Do extreme temperatures have damaging effects on different fertility traits and if so, is there the potential for selection to increase resilience to changing climates?

Responses to selection for coping with more extreme and unpredictable temperatures relies on individuals varying in their thermal resilience[15]. One factor that can influence individual variation in thermal resilience is how reproductive and somatic investment are managed under thermal stress. For example, temperature extremes may lead to high physiological demand to protect essential organismal functions that reduce investment in reproduction[1,16,17]. Reduced reproductive investment can in turn generate trade-offs between different fertility traits that limit responses to selection for increased resilience to temperature change. However, whether temperature extremes expose such reproductive trade-offs, and the extent to which individuals vary in their prioritization of investment across different fertility traits, is unclear.

Research on the effects of natural temperature variation on reproduction in non-domesticated endotherms has primarily been on temperate species[18–33]. However, temperature unpredictability is greatest in tropical and sub-tropical regions and climate modelling shows this will increase in the future[13,34]. The reproductive performance of species living in such regions may also be particularly sensitive to the effects of climatic fluctuations, as they often have prolonged breeding seasons that increase their risk of exposure to shifts in environmental conditions. Furthermore, because temperate species typically have short breeding seasons, timed to the seasonal appearance of food (phenology), there has been a focus on whether advancing spring temperatures reduce breeding success through phenological mismatches[18–28,35,36]. Consequently, more information is needed on the effects of ecologically relevant temperatures on investment in the traits directly related to fertility, such as the production and viability of eggs and sperm.

Here we examine how temperature fluctuations over a 20-year period affect multiple fertility traits in the world's largest bird, the ostrich (*Struthio camelus*), which reproduce throughout the year in tropical and sub-tropical regions (Fig. 1)[37–39]. Individually marked birds ($n = 1299$, Supplementary Table 1) were studied in the Klein Karoo region of South Africa where temperatures during the reproductive cycle ranged from −5 to 45 °C. Data on the fertility of females and males was obtained by collecting eggs daily from captive pairs, and by collecting natural ejaculates from captive solitary males. All pairs and solitary males used for sperm collection were kept in separate fenced enclosures of natural Karoo scrub exposed to natural weather conditions (Fig. 1a). Data were matched with onsite temperature records to investigate: (1) how thermal fluctuations shape investment in gametic traits (number of eggs and sperm, egg mass and sperm viability) and reproductive success (hatching success and offspring numbers), (2) individual variation in the resilience of fertility to temperature change, and (3) whether extreme temperatures cause trade-offs in investment across gametic traits.

Results

Is fertility compromised by hot and cold temperatures? The number of eggs females laid and the number of sperm males ejaculated were significantly reduced by both increases and decreases in ambient temperature (Fig. 2a, b). The effects of temperature were not immediate, but resulted from a critical thermal window 2–4 days before laying and ejaculation (Supplementary Figs. 1 and 2; see the subsection "Time lag effects of

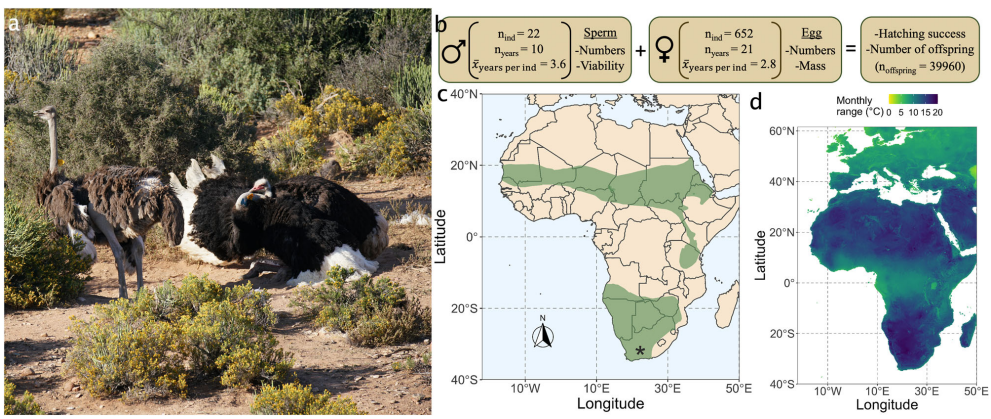


Fig. 1 Ostriches (*Struthio camelus*) cope with large thermal fluctuations in their native habitat, reproducing successfully across Africa from the Western Cape to the deserts of Southern and Northern Africa. a Courtship by a male ostrich (right) towards a female (left) in one of the enclosures ($n = 197$) at the study site used to keep a single breeding pair (photo: CKC). **b** Data structure of fertility traits obtained from 1998 to 2018 at the study site of Oudtshoorn Research Farm in the arid Klein Karoo region of South Africa. Sperm viability data was not available for all of the solitary males where measures of sperm numbers were obtained (sperm viability: $n_{ind} = 18$, $n_{years} = 7$, $\bar{x}_{years\ per\ ind} = 2.7$). See also Supplementary Table 1 for detailed overview of sample sizes. **c** Geographic range (green) of the ostrich[93] with the study site marked by an asterisk. **d** Monthly temperature range was calculated by estimating the range of temperatures of each month and then calculating the mean of this across all months[94].

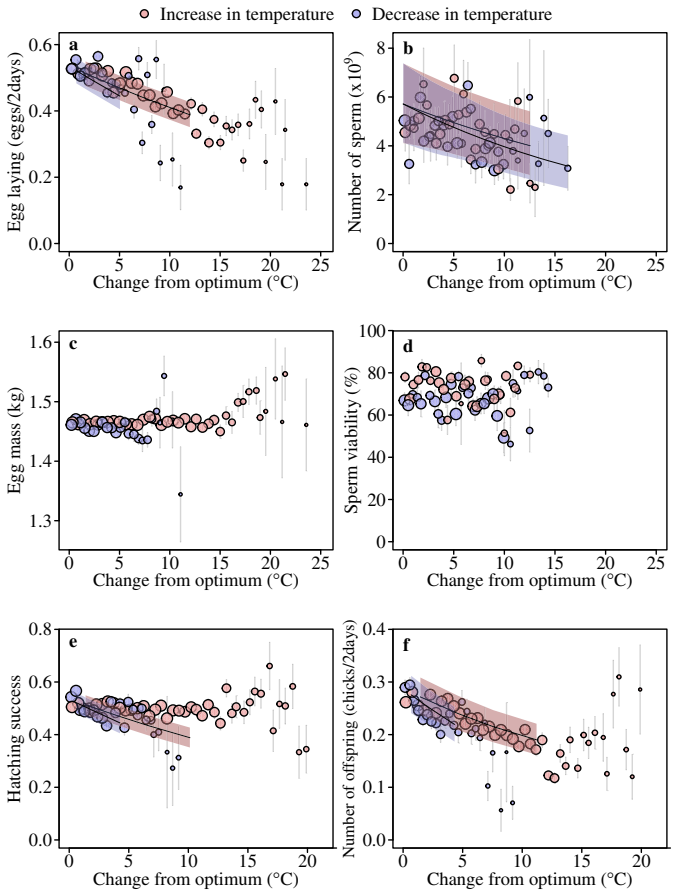


Fig. 2 Temperature extremes compromise male ($n = 22$) and female ($n = 652$) fertility. Female egg laying rate (**a**) and number of sperm ejaculated by males (**b**) were both highly sensitive to increases and decreases in temperature. Female (**c**: egg mass) and male (**d**: sperm viability) gamete quality were generally more resistant to temperature change. Hatching success (**e**), which is influenced by the egg mass[41,42], sperm numbers and sperm viability, was also less affected by temperature change. The number of offspring (**f**) is a product of hatching success and rates of egg laying and was influenced by changes in temperature that occured during egg laying. Ostrich females can only lay an egg every other day and we therefore used number of eggs or chicks per number of two-day intervals (eggs/2 days or chicks/2 days) (see the subsection "Time lag effects of temperature on gametes" in "Methods" section). The range of temperatures that sperm traits were measured at differed from the other traits, because it was not possible to collect sperm across all years (Supplementary Table 1). Fitted lines and 95% credible intervals (shaded area) from the primary set of models are shown for traits significantly affected by temperature (Supplementary Tables 2–7). For binomial models the fitted lines span the modelled binned temperature classes making them robust to outliers. Points are averages with standard errors binned according to the temperature variable. Point size illustrates relative number of observations. Source data are provided as a Source Data file.

temperature on gametes" in "Methods" section). During this critical thermal window, egg laying rate peaked at 20 °C (Supplementary Fig. 3), dropping by 15% and 18% when temperatures increased and decreased by 5 °C, respectively (Fig. 2a; Table 1, Supplementary Table 2). Similar reductions were seen in the number of sperm males ejaculated (19% with 5 °C increases and decreases from the optimum; Fig. 2b; Table 1, Supplementary Table 3), but the thermal optimum appeared to be slightly higher than for egg laying, peaking at ~26 °C (Supplementary Fig. 4). While this may indicate there is the potential for conflict over the thermal optima of males and females, this dataset was not designed to test this (see the subsection "thermal stress index" in "Methods" section). It is also likely that both 20 and 26 °C are within the thermal neutral zone (TNZ), which although not explicitly known for ostriches, spans from 10–15 to 30 °C in the closest relative, the emu (*Dromaius novaehollandiae*)[40].

Fluctuations in temperature had much less of an effect on gamete viability than on the number of gametes. The mass of eggs females produced only decreased by 0.7% when temperatures fell from 20 to 15 °C and were unaffected by increases in temperature (Fig. 2c; Table 1; Supplementary Table 4). Similarly, the viability of sperm (viable sperm: normal morphology, intact membrane and eosin impermeable) males produced was robust to temperature fluctuations, with no consistent change with increases or

Table 1 Individual variation in the resilience of fertility to temperature change.

Trait	Fixed effects (CI)			Repeatability (CI)			PSlopeVar (CI)
	Intercept	Slopes		Intercept	Slopes		
		$T_{heat\ stress}$	$T_{cold\ stress}$		$T_{heat\ stress}$	$T_{cold\ stress}$	
Egg laying	0.32 (0.18,0.49)	−1.68 (−1.90, −1.50)***	−2.10 (−2.43, −1.80)***	0.27 (0.21,0.33)	0.24 (0.17,0.32)	0.18 (0.06,0.34)	0.16 (0.14,0.18)
Egg mass	1.43 (1.42,1.45)	0 (−0.01,0.02)	−0.05 (−0.07,−0.03)***	0.62 (0.59,0.65)	0.52 (0.48,0.57)	0.47 (0.41,0.53)	0.03 (0.03,0.04)
Number of sperm	10.46 (10.16,10.79)	−0.67 (−1.30, −0.02)*	−0.67 (−1.13, −0.23)**	0.23 (0.13,0.39)	0.47 (0.13,0.84)	0.57 ((0.24,0.79)	0.07 (0.04,0.13)
Sperm viability	−1.91 (−2.19,−1.62)	0.13 (−0.24,0.42)	−0.21 (−0.55,0.11)	0.54 (0.35,0.74)	0.61 (0.38,0.81)	0.60 (0.36,0.81)	0.1 (0.05,0.17)

We quantified the differences between individuals relative to within and between individual variation (repeatability) for fertility at intermediate temperatures (intercept) and for the change in fertility with increasing and decreasing temperatures (slopes). Estimates and credible intervals (CI) were extracted from the second set of MCMCglmm models including individual by year slopes. See Supplementary Tables 8–11 for model details including estimates of repeatability on the expected scale and variance of fixed effects. PSlopeVar = ratio of the slope variance to the total phenotypic variance.
*pMCMC < 0.05, **pMCMC < 0.01, ***pMCMC < 0.001.

decreases in temperature (Fig. 2d; Table 1; Supplementary Table 5).

Do changes in fertility traits matter for reproductive success? The effect of temperature on reproductive success (number of offspring) is a product of changes in egg laying rates and the probability that eggs hatch. Hatching success is in turn influenced by the fertilizing ability of males, which depends on the numbers and viability of sperm inseminated, and egg viability, which is linked to egg mass[41,42]. The potential effects of ambient temperatures during incubation on hatching success were removed by artificially incubating eggs using an on-site hatchery. Hatching success was significantly affected by the temperature birds experienced prior to laying: hatching success was reduced by 4–7% with 5 °C increases and decreases from 20 °C (Fig. 2e; $T_{heat\ stress}$ (credible interval, CI) = −0.26 (−0.43, −0.09), pMCMC = 0.002; $T_{cold\ stress}$ (CI) = −0.57 (−0.98, −0.01), pMCMC = 0.028; Supplementary Table 6). Combined with changes in egg laying rates, this resulted in the total number of offspring decreasing by 28% with 5 °C increases, and 44% with 5 °C decreases in temperature from 20 °C (Fig. 2f; $T_{cold\ stress}$ (CI) = −2.10 (−2.57, −1.60), pMCMC = 0.001; $T_{heat\ stress}$ (CI) = −1.42 (−1.61, −1.21), pMCMC = 0.001; Supplementary Table 7). Reproductive success can also be reduced if individuals die from temperature-related stress during the breeding season, but during the 21 years of experimental breeding only six adult deaths (0.5%) related to overheating were recorded. These results suggest that the negative effects of temperature fluctuations on reproductive success arise through the cumulative, detrimental effects on egg and sperm production under both low and high temperatures. It is also worth noting that these effects may be even more pronounced in wild populations where access to food and water is likely to be more restricted.

Do individuals vary in how resilient their fertility is to temperature change? There was substantial variation among females in how resilient their laying rates were to temperature change. Differences between individual females explained 24% of variation in the rate of decline in egg laying when temperatures increased, and 18% of variation when temperatures decreased (Table 1). Similarly, some males were much more resilient to temperature change than others, as indicated by the number of sperm they ejaculated (Table 1). When temperatures increased, 47% of variation in the decline in sperm numbers was explained by differences between males, and 57% when temperature decreased. We examined the robustness of these results using character state models where values of a trait are correlated between different temperature categories (cold (<17.7 °C), hot (>28.7 °C) and benign): correlations lower than one indicate variation between individuals in their response to temperature change[43]. These analyses confirmed that there were substantial differences among males and females in their responses to temperature change (Supplementary Tables 12 and 13).

Females were extremely consistent in their egg mass, which was relatively unaffected by temperature change (PSlopeVar: 0.03, Table 1). While average egg mass ranged from 1.41 to 1.68 kg among females, the most extreme change in egg mass of a female from 20 to 25 °C was an increase of just 0.015 kg. Despite this, a relatively large proportion of the variation in egg mass change was explained by differences between females, around 50%. Such consistent differences among females is in accordance with research on other bird species where egg mass is variable in populations, but highly consistent within individuals[44]. For males, the pattern was similar with around 60% of variation in the change in sperm viability with temperature being explained

by differences between males (Table 1). That said, character state models showed only a weak correlation between measures of sperm viability at benign versus cold and hot temperatures, suggesting that data from extreme temperatures may inflate the estimation of between individual differences (Supplementary Table 15). Taken together, these results show that when temperatures increase and decrease, individual females and males vary substantially in the number and viability of eggs and sperm they produce. The efficacy of selection to promote thermal tolerance is therefore unlikely to be limited by a lack of variation between individuals.

Is the resilience of fertility to temperature change compromised by trade-offs between traits? When individuals are exposed to temperature extremes, simultaneous investment in multiple traits may not be possible. The resulting trade-offs can take two forms. First, negative correlations between fertility traits may occur at extreme temperatures because physiological stress limits the resources individuals have to invest across reproductive traits. Second, there may be negative correlations in the degree of change across traits (thermal resilience) rather than absolute trait values. For example, investment in the maintenance of one trait may come at the expense of maintenance of other traits.

We found no evidence of any negative correlations between any fertility traits within or among individuals at any temperature (Fig. 3; Supplementary Table 18). This shows that the number of eggs females produce and the number of sperm males ejaculate is not traded-off against egg mass or sperm viability in either hot or cold periods. Instead, correlations between traits within females were generally significantly positive, indicating that investment in the number and mass of eggs are up and down regulated together (Fig. 3; Supplementary Table 18). Furthermore, among individuals there was a significant positive relationship between change in egg laying rates and change in egg mass as temperatures increased (Fig. 3). This is contrary to the idea that temperature stress induces trade-offs between fertility traits. Instead, this suggests that some females respond to higher temperatures by producing more eggs that are also heavier, compared to other females.

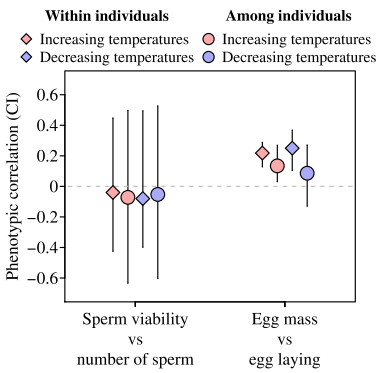


Fig. 3 Correlated changes in the number and quality of gametes as temperatures increased and decreased. The number of eggs and sperm females ($n = 652$) and males ($n = 18$) produced was not traded-off against egg mass and sperm viability as temperatures changed (see also Supplementary Table 18). This was consistent within and among individuals. Changes in egg-laying rates were positively correlated to egg mass as temperatures increased both within and among females (credible interval (CI) of phenotypic correlation excluded zero). Source data are provided as a Source Data file.

Discussion

It has been argued that to understand how species are affected by environmental change, it is crucial to broaden the current focus on lethal limits to include thermal fertility limits[9]. Our results provide support for this proposition, as only six adults (0.5%) died from thermal stress, whereas there were dramatic reductions of 28–44% in reproductive success with 5 °C deviations from their thermal optimum. Although increased climatic change has brought into focus the effect of rising temperatures on survival and population persistence[34], our results show that cooler, as well as hotter, temperatures may pose a challenge for species.

Much of the classical life-history research on birds has focused on the seasonal appearance of food as a factor limiting breeding success[45,46], whereas the direct effects of temperature on reproduction have remained more unclear (but see Hurley et al.[11]). In ectotherms, extreme temperatures have been shown to reduce both the number and the quality of gametes individuals produce[9,47,48] and similar effects have been found in domestic chickens, domestic mammals and laboratory mice[49–52]. Such concordant effects of heat stress on different gametic traits suggests that high temperatures may lead to a general degradation of reproductive function. While our results show that heat and cold stress compromise reproductive success, this was not because of consistent detrimental effects across all traits, but rather specific responses of traits to temperature change: Sperm viability and egg mass did not decline even under the most extreme thermal stress, whereas the number of gametes individuals produced was highly sensitive to temperature change.

One potential reason for why the numbers and quality of gametes differ in their response to temperature change is that they are under different mechanistic control. Reductions in sperm and oocyte production caused by heat stress have been shown in mammals to occur due to decreases in testosterone in males and changes in luteinizing hormone in females[50,51]. General physiological changes due to temperature stress may therefore reduce rates of gametogenesis[9]. In contrast, changes in sperm and follicular function have previously been linked to processes, such as DNA damage[9,49–51], and may be somewhat shielded from physiological stress by the follicle/testes–blood barriers[53]. Alternatively, the limited effects of temperature on sperm viability and egg mass may be due to reduced sensitivity to physiological stress, consistent with early life-history models[54], or other measures of gametic performance may be required to detect the effects of temperature on gamete quality. For example, the biochemical composition of eggs can vary independently of egg mass and can influence offspring fitness[55,56]. The differences in the response of gametic traits to temperature change highlights the importance of understanding reproductive mechanisms when predicting outcomes of environmental change, and has important implications for how thermal fertility limits are studied.

The evolution of increased thermal tolerance is key to the persistence of populations as environments change and become more unpredictable[1,15]. Our results show that ostrich populations harbour individual variation in resilience to temperature change that may facilitate responses to shifting climates. However, this raises the question of why some individuals are more susceptible to temperature change than others? Given the fitness benefits of increased thermal tolerance, why has selection not eliminated variation within populations[57]? One possibility is that there are alternative strategies to cope with temperature change during reproduction. If thermal tolerance is costly, tolerant individuals that reproduce across a wide range of temperatures (generalists)

may have comparable fitness to individuals that only reproduce under specific thermal conditions (specialists), if they have lower reproductive success per breeding attempt[1,58–60]. We found no support for this idea, and if anything the opposite was true: Certain females appeared to specialize in reproducing at higher temperatures by increasing both the number and mass of eggs they laid, with no apparent reductions in egg mass at other times. It is possible that the ability of females to increase laying rates without compromising egg mass under extreme temperatures is facilitated by their unique life-history characteristics, including laying extremely small eggs relative to their body size. Whether certain life-history characteristics increase or decrease the vulnerability of species to climate change is unclear and clearly warrants further investigation.

Another possibility is that variation in thermal tolerance is maintained due to alternative breeding strategies. Ostriches have an extremely flexible breeding system, reproducing in both pairs and cooperative groups[37–39]. Cooperative breeding in birds has been shown to be a successful strategy for coping with high and fluctuating temperatures where breeding in pairs often fails[61–63]. In this study, it was necessary to restrict breeding opportunities to pairs to gain detailed measures of individual reproductive success. It is therefore possible that the sensitivity of individuals to temperature change may be alleviated by the buffering effects of sociality when opportunities to breed in groups arise[64–67].

This study shows thermal stress is an important factor that can limit reproductive success (see also Nord and Nilsson[68] and Walsh et al.[9]), even in species, such as the ostrich, that are well adapted to survive in extreme thermal environments. To explain the past and predict the future effects of climate change, it is crucial to quantify the effects of temperature on the fertility in species inhabiting different biogeographical zones and with different breeding biology. The extent to which the results of this study can be generalized remains to be established, given that little is known about temperature-dependent fertility in other tropical and sub-tropical species. However, the challenges faced by endotherms in arid, tropical and sub-tropical regions are clear and have already led to the collapse of entire bird communities[34]. A key feature of climate change highlighted by our results is that both hot and cold temperatures likely pose a challenge for species, providing an illustration of why temperature fluctuations, and not just temperature increases, are critical to study.

Methods

Study site and population. The study site is situated at the Oudtshoorn Research Farm in the arid Klein Karoo of South Africa (GPS: 33°38′21.5″S, 22°15′17.4″E). The ostriches used in this study are derived from 139 founding individuals, consisting of individuals classified into one of two subpopulations with the popularized names South African Blacks (*S. camelus*) or Zimbabwean Blues (*S.c. australis*). From 1998 to 2018 the reproduction of captive breeding pairs ($n_{females} = 756$, $n_{males} = 701$) was monitored in 197 enclosures of ~0.25 ha of natural Karoo habitat[69]. A male and a female ostrich were assigned to each enclosure in May/June each year and kept together until the end of the breeding season in December/January. Male–female combinations were established to prevent inbreeding and where possible, generate new combinations each year. From 2008 to 2018 the fertility of males ($n = 22$) kept in solitary enclosures (20 m × 17 m) and trained to ejaculate into an artificial cloaca using a dummy female was monitored (method developed by Rybnik et al.[70]). Ostriches received a diet designed for breeding individuals (90–120 g protein, 7.5–10.5 MJ metabolizable energy, 26 g calcium and 6 g phosphorus per kg feed) and water ad libitum. Levels of dietary protein and energy were reduced across years to lower feed costs, which had negligible effects on fertility[71,72]. Maximum daily temperature records were obtained from a local weather station 600 m from the study site. Ethical clearance was obtained from the Western Cape Department of Agriculture (DECRA R12/48).

Reproductive data

Female gametic traits. Pairs were checked twice a day and any eggs were collected and weighed using an electronic balance (Mercer). This gave us an estimate of the daily changes in quantity and mass of female gametes, that could be directly compared to daily temperatures. In two years the laying season was extended beyond February until April. All data from these months were removed to ensure data were consistent with other years. We also removed data from pairs where the male or female was replaced during the breeding season, which occurred sometimes when individuals were injured or died. Data on the rate of egg laying from these replacement pairs indicated that acclimation to enclosures and new partners takes ~45 days (Supplementary Fig. 5). Based on this information we removed data from the first 45 days from each season. Two-year-old females had substantially lower reproductive success than older breeders (Supplementary Fig. 6, see also Cloete et al.[69]) so these were removed from the data. Pairs that spent fewer than 200 days in their enclosure in a given year were removed so that data were consistent across pairs and years. Finally, pairs that laid fewer than 10 eggs per year were removed to avoid including incompatible pairings and individuals not in breeding condition, which reduced the total number of females in the analyses to 652.

Male gametic traits. For males, the ability to deliver high quantities of sperm of high quality is crucial for fertilization success[73–75]. We obtained natural ejaculates from solitary males kept in individual enclosures and estimated the number of sperm and sperm viability. Semen collections were performed three to five times a week and after periods of sexual rest the first three ejaculates collected were discarded. From the resulting set of ejaculates we kept data on the first ejaculate collected each day, typically obtained in the morning, from each individual. Sperm concentration was measured with a spectrophotometer in 20 µL semen diluted 1:400 (v/v) with a phosphate buffered saline solution containing 10% formalin. The number of sperm was estimated as the product of sperm concentration and ejaculate volume, which we estimated using an automatic pipette. Sperm viability was estimated by inspecting 500 sperm stained with nigrosin-eosin, and characterizing a sperm as viable if the morphology was normal (complete unit of tail, midpiece and slightly curved head)[76], the membrane was intact and eosin impermeable[77]. Only males from which we were able to obtain at least five ejaculates were included in the analysis to avoid including males not accustomed to the ejaculation collection process. Subsets of these data have previously been used to test effects of season, age and collectio[78–80].

Hatching success and number of offspring. Hatching success reflects the product of both male and female gametic traits as well as the quality of incubation. To control incubation effects, eggs were artificially incubated in an on-site hatchery until hatching. Eggs were stored (1–6 days) at 17 °C and 80–90% humidity with two daily rotations through a 180° angle until eggs were moved to incubators once a week. Eggs were incubated at 36.2 °C and 24% humidity with hourly rotation on their long axis through a 60° angle for the first 35 days and then switched to a hatcher set at 36 °C and 24% humidity for the remaining 7 days[81]. This dataset was subject to the same filtering procedure as the female egg traits.

Statistical analyses

Time lag effects of temperature on gametes. The time period where different traits are influenced by fluctuations in ambient temperatures (i.e. the critical thermal window) is unknown. We therefore estimated the sensitivity of each trait to different sliding thermal windows preceding gamete production using general linear models (GLMs), where different thermal windows were entered as predictors of gametic traits at the population level. A window size of 3 days was chosen and one day steps were examined from 7 days before to 5 days after egg laying. We chose a window size of 3 days to capture immediate temperature fluctuations, while minimizing the effects of seasonal trends that occurred with larger windows. This also enabled us to avoid missing daily extreme events that occurred with smaller windows. Supplementary one-day and two-day window analyses supported this decision, as three sequential one-day windows (or two overlapping two-day windows) were particular important predictors of egg-laying (Supplementary Fig. 7). The thermal windows after egg laying served as controls, as we did not expect any predictive power apart from the autocorrelation in temperature. In each window, the average daily maximum temperature (AVG-T_{MAX}) was modelled as a quadratic effect. To identify the critical thermal window, we compared the models using Akaike information criterion (AIC) or QAIC (Quasi-AIC) to account for the overdispersion common to logistic regressions. The maximum egg-laying rate is one egg every 2 days. We therefore modelled the probability of laying as the number of 2-day intervals with (eggs/2 days) and without eggs using a Binomial error distribution, which was necessary to correctly model the variance in successes (our response ranged from 0 to 1 whereas eggs per day ranged from 0 to 0.5). Model comparison with QAIC showed that the critical thermal window was 2–4 days before egg-laying (Supplementary Fig. 1). Interestingly 2 days is also the time it takes for eggs to travel down the oviduct[82,83]. Egg mass was modelled using a Gaussian error distribution and the ranking of AIC was very sensitive to small model adjustments and extreme temperatures, reflecting the generally low effect of temperature on this trait (Fig. 1 and see the section "Discussion"). Visual inspection revealed a consistent trend of increasing egg mass at extreme high temperatures but not at intermediate to high temperatures (Fig. 1). To reduce the influence of these extreme data points, without removing the entire trend of what may be a true biological signal we removed the 0.5% hottest and the 0.5% coldest records in this particular analysis. Several thermal windows prior to egg-laying appeared to predict egg mass equally well, but we proceeded with 0–2 days before egg laying as

the critical window for this trait due to its proximity to day of laying (Supplementary Fig. 1). For both hatching success (Binomial error distribution: number hatched vs. number not hatched) and the number of offspring (Binomial error distribution: 2-day intervals with chicks vs. 2 days without chicks, chicks/2 days) we used 0–4 days before egg laying as the critical thermal window as this included all days used as predictors for egg mass and egg laying rate. In birds, spermatogenesis is believed to range from 11 to 15 days[84], and we therefore tested thermal windows from 15 days before to 5 days after ejaculation. The critical thermal window for the number of sperm (Poisson distribution) was 2–4 days before ejaculation, and while sperm viability (Binomial error distribution: number alive vs. number dead) was also influenced by temperature during this time, the window 4–6 days before ejaculation was a better predictor (Supplementary Fig. 2). However, as results did not differ between the analyses of sperm viability detailed below (random regression and character-state models) when using 2–4 vs. 4–6 days, we used 2–4 days for consistency across traits. The critical thermal windows estimated for sperm and egg traits are specific to this study. If other species are studied it will be important to estimate these parameters using similar critical thermal window analyses from time series datasets.

Thermal stress index. For each trait we modelled the response to increases and decreases in temperature by creating cold and heat thermal stress indexes. This was done by first estimating the temperature at which trait values were maximized (thermal optimum), and secondly by calculating decreases ($T_{\text{cold stress}}$) and increases ($T_{\text{heat stress}}$) away from this optimum. Using GLMs we modelled the change in number of sperm and eggs produced as a response to AVG-T_{MAX} (linear and quadratic terms) of the critical thermal window, and extracted the parametric vertex as the thermal optimum (rounded to closest degree Celsius). For egg laying the optimum was estimated as AVG-$T_{\text{MAX}} = 20$ °C (Supplementary Fig. 3), which also reflects the centre of the TNZ of the emu[40] (unknown for the ostrich). For the number of sperm ejaculated the optimal temperature was estimated to be 26 °C (Supplementary Fig. 4). As a result, $T_{\text{heat stress}}$ for females was from 20 to 45 °C and for males it was from 26 to 45 °C. $T_{\text{cold stress}}$ was from 20 to 10 °C for females and from 26 to 10 °C for males. The observed difference in thermal optima between sexes is intriguing, but this dataset was not designed to robustly test for sex differences: the fitness of males and females are intertwined in the pairs and we have no direct data on how solitary male sperm performance influenced female fitness. To make the intercept of the statistical models represent the most benign temperature we subtracted the minimum stress value resulting in 0 being the new minimum (no stress) of the thermal stress index. The variance of slopes (see below) depends on the scale of the environmental parameter and we therefore standardized this by dividing by the maximum of the range resulting in 1 being the maximum deviation from 0.

Modelling resilience to temperature change using random regression models. We constructed random regression models in R v.3.6.0[85] using the Bayesian framework implemented in the R-package MCMCglmm v.2.29[86]. For both residual and random terms we used the weakly informative inverse-Gamma distribution (scale = 0.001, shape = 0.001, i.e. $V = n$, $nu = (n-1) + 0.002$ with n being the dimension of the matrix) as priors. For female gametic traits, models were run for 10,000,000 iterations of which the initial 100,000 were discarded and only every 10,000th iteration was used for estimating posterior probabilities. For male gametic traits, models were run for 3,000,000 iterations, of which the initial 30,000 were discarded and only every 3000th 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 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, correlations and repeatability measures. Models included the fixed effects of thermal stress (ranging from 0 to 1) and stress type (cold or heat). The interaction between thermal stress and stress type was modelled with a common intercept for cold stress and heat stress, as the construction of the thermal stress index dictated that these intercepts are identical.

For the three traits modelled with Binomial error distributions (egg laying, hatching success and number of offspring) data were grouped into four hot and three cold thermal stress classes, each representing the number of observations with success (e.g. 2-day intervals with egg) and the number of observations with failure (e.g. 2-day intervals without egg). For female gametic traits we included the additional fixed effects of female subpopulation (South African Blacks: 476 females, Zimbabwean Blues: 68 females or crosses: 108 females) and its interaction with the thermal stress and stress type, as well as female age and the subpopulation of the pair male. Results were highly consistent across subpopulations and we therefore report fixed effect estimates from the most numerous subpopulation (South African Blacks) for brevity. Population-specific estimates are available in the results tables provided in the supplementary information. The mass of eggs decreased with the number of days since the previous egg (Supplementary Fig. 8). This was accounted for by including days since previous egg (linear and quadratic terms, log-transformed) as a fixed effect in the egg mass model. Several sperm-characteristics may peak at an intermediate age[78], and therefore linear and quadratics effects of age were included as fixed effects in models. We accounted for environmental effects that differed across years, such as diet, by including year as a random effect. For egg-laying rates, egg mass, hatching success and offspring number, enclosure was also added as a random effect, since they were used repeatedly across years and varied in size and vegetation cover. The males used for sperm collection were kept in the same enclosures across years and therefore we did not have enclosure as random effect in analyses of sperm traits (not possible to separate individual from enclosure effects). The enclosures where males were kept for sperm collection are, however, extremely similar making it unlikely that this was a significant source of error variance.

Quantifying individual variation in resilience to temperature change. In all models the thermal stress index and type of stress (cold versus heat) was allowed to interact with ostrich ID to model the individual variance (id). This was modelled as 3 × 3 unstructured variance–covariance matrix composed of the intercept (id_{int}), slope during cold stress ($\text{id}_{\text{sl-cold}}$) and slope during heat stress ($\text{id}_{\text{sl-heat}}$). Individual repeatability (R) of trait values at the optimum temperature ($T_{\text{stress}} = 0$, 20 °C for females and 26 °C for males) was then estimated as the proportion of intercept variance that is explained by the individual variance in intercepts:

$$R_{\text{int}} = \frac{\sigma^2_{\text{id}_{\text{int}}}}{\sigma^2_{\text{id}_{\text{int}}} + \sigma^2_{\text{year}} + \sigma^2_{\text{enclosure}} + \sigma^2_{\text{res}}}. \quad (1)$$

Individual variation in the cold and heat stress slopes was used as an estimate of variation in resilience to increasing and decreasing temperatures, i.e. phenotypic plasticity. However, to estimate the repeatability of slopes for individuals (consistency of individual by environment interaction; I × E), we constructed a second set of models. In these models a second 3 × 3 unstructured variance–covariance matrix of individual by year (id-yr) combinations was added, allowing the repeatability of thermal plasticity within individuals across different breeding years to be calculated. Variance in individual slopes is on a different scale to that of intercepts, and also dependent on the scaling of the temperature index. For these reasons we followed a recently introduced practice[87,88] and estimated the repeatability of thermal slopes as the proportion of slope variance attributable to between individual variance:

$$R_{\text{sl}} = \frac{\sigma^2_{\text{id}_{\text{sl}}}}{\sigma^2_{\text{id}_{\text{sl}}} + \sigma^2_{\text{id-yr}_{\text{sl}}}}. \quad (2)$$

To quantify how much variation in each trait was explained by responses to temperature we transformed the between individual and within individual slope variance to the same scale as the intercept variances using $\sigma^2_{\text{E}} = \sigma^2_{\text{sl}} * \text{var}(x)$, where $\text{var}(x)$ is the variance of the environmental variable, the temperature index[89]. We then expressed this variation as a ratio of the total variance, including between individual and within individual intercept variance as well as year, enclosure and residual variance:

$$\text{PSlopeVar} = \frac{\sigma^2_{\text{id}_{\text{Thot}}} + \sigma^2_{\text{id-yr}_{\text{Thot}}} + \sigma^2_{\text{id}_{\text{Tcold}}} + \sigma^2_{\text{id-yr}_{\text{Tcold}}}}{\sigma^2_{\text{id}_{\text{Thot}}} + \sigma^2_{\text{id-yr}_{\text{Thot}}} + \sigma^2_{\text{id}_{\text{Tcold}}} + \sigma^2_{\text{id-yr}_{\text{Tcold}}} + \sigma^2_{\text{id}_{\text{int}}} + \sigma^2_{\text{id-yr}_{\text{int}}} + \sigma^2_{\text{year}} + \sigma^2_{\text{enclosure}} + \sigma^2_{\text{residual}}}. \quad (3)$$

It has recently been debated if the fixed effect variance (σ^2_{f}) should be included in the denominator when estimating R[90]. There are arguments for including fixed effect variance if it captures natural variation and excluding it if it represents experimental variance[91]. For full transparency we chose to report estimates of σ^2_{f} excluding variance from the thermal index ($\sigma^2_{\text{f}_{\text{-thermal stress}}}$) as this parameter has already been accounted for by the interaction with the random terms. We estimated fixed effect variance of all terms ($\sigma^2_{\text{f}_{\text{all}}}$) and of thermal stress separately ($\sigma^2_{\text{f}_{\text{thermal stress}}}$) following de Villemereuil et al. [91], such that $\sigma^2_{\text{f}_{\text{-thermal stress}}} = \sigma^2_{\text{f}_{\text{all}}} - \sigma^2_{\text{f}_{\text{thermal stress}}}$.

As egg laying, hatching success and number of offspring are modelled via a logit link function, estimates of R are calculated on the latent scale. While this scale has the benefit of fulfilling the typical assumptions of parametric analyses, it may not reflect the scale at which selection is working. Methods have therefore been developed to make inferences on the observed scale[92]. There are currently no methods to perform this transformation for a model using a logit-link function and where the number of trials varies between data points. Instead it is possible to calculate estimates of repeatability on the expected scale (corresponding to the liability scale in a threshold model) according to equations in de Villemereuil et al. [92] using the R-package QGglmm[92]. Similar methods are not available for the slope variance parameters presented below, and all estimates presented in the main document are therefore on the latent scale for consistency. Where possible, we also provide estimates on the expected scale in the supplementary material (Supplementary Tables 2–10).

Modelling resilience to temperature change using character-state models. As an alternative modelling approach to random regression, we modelled changes in each trait across three temperature categories (cold, benign and hot), using character-state models. For egg-related traits the ranges for these categories were limited by the lower number of cold compared to hot days, according to the thermal optimum cut-off used in the random regression analysis (20 °C). To avoid low replication in the cold category relative to hot days we assigned the lowest 50% of days classified as $T_{\text{cold stress}}$ as cold (<17.7 °C, $n_{\text{eggs}} = 10{,}483$), and the highest 30% of days classified as $T_{\text{heat stress}}$ as hot (>28.7 °C, $n_{\text{eggs}} = 14{,}759$), with the remainder being classified as benign ($n_{\text{eggs}} = 56{,}297$). Data on sperm traits had higher temperature

values. We therefore increased the temperature cut-offs (cold: <18.7 °C, $n_{ejaculations}$ = 319; hot: >29.7 °C, $n_{ejaculations}$ = 392 and benign $n_{ejaculations}$ = 1174). The models were constructed in MCMCglmm v.2.29[86] and followed the same general approach as the random regression models described above. The major difference was that temperature category was included as a fixed factor and the interaction between the random effect ostrich ID and temperature category was modelled as a 3 × 3 unstructured variance–covariance matrix composed of the cold, benign and hot temperature categories. We also estimated the residual variance separately for each temperature category (see Supplementary Tables 12–17 for further details on the model components).

Modelling trade-offs between traits. To quantify correlations between female gametic traits (egg mass vs. number of eggs with 0–4 days before egg laying as the critical thermal window) and between male gametic traits (sperm viability vs. number of sperm with 2–4 days before ejaculation as the critical thermal window) two-trait models were used. These were setup using MCMCglmm v.2.29[86] with the same error distributions as the single-trait models. For female gametic traits, models were run for 5,000,000 iterations of which the initial 100,000 were discarded and only every 2000th iteration was used for estimating posterior probabilities. For male gametic traits, models were run for 3,000,000 iterations, of which the initial 30,000 were discarded and only every 3000th iteration was used for estimating posterior probabilities. Each trait comparison was analysed with both random regression models and with character-state models, containing the same fixed effects as the single-trait models, but with the reserved term "trait" interacted with all fixed effect components. Models also contained the same basic random effects as the single trait models, but with the random effects and residuals estimated separately for each trait. In the random regression models the interaction between ostrich ID and thermal stress was modelled by constructing two 4 × 4 unstructured variance–covariance matrices, one for $T_{heat\ stress}$ and one for $T_{cold\ stress}$, composed of the intercept and slope for both traits. Two similar matrices were constructed for the interaction between individual by year (id-yr) records and thermal stress. Using these matrices, we extracted covariance between traits in the response to heat or cold stress among and within individuals, which was then used to estimate correlations (correlation = $covariance_{trait1,trait2}/sqrt(var_{trait1}{}^{*}var_{trait2})$). In the character-state models the interaction between ostrich ID and temperature category was modelled by constructing three 2 × 2 unstructured variance–covariance matrices composed of either the cold, benign or hot thermal category for both traits. These matrices were used to extract covariance components between traits among individuals for a given thermal category, and use these to estimate correlations. Similar matrices were also used to model the residual variance (within individuals) in the character-state models.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data that 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 these data, and so are not publicly available. Data are however available from the WCDA upon request. Source data are provided with this paper.

Code availability

The code used in this study is available as an R-file (Supplementary Code 1).

Received: 28 July 2020; Accepted: 4 January 2021;
Published online: 02 February 2021

References

1. Angilletta, M. J. *Thermal Adaptation: A Theoretical And Empirical Analysis* (Oxford University Press, 2009).
2. Chown, S. L., Sinclair, B. J., Leinaas, H. P. & Gaston, K. J. Hemispheric asymmetries in biodiversity—a serious matter for ecology. *PLoS Biol.* **2**, e406 (2004).
3. Sunday, J. M., Bates, A. E. & Dulvy, N. K. Thermal tolerance and the global redistribution of animals. *Nat. Clim. Change* **2**, 686–690 (2012).
4. Kellermann, V., van Heerwaarden, B., Sgrò, C. M. & Hoffmann, A. A. Fundamental evolutionary limits in ecological traits drive *Drosophila* species distributions. *Science* **325**, 1244–1246 (2009).
5. Araújo, M. B. et al. Heat freezes niche evolution. *Ecol. Lett.* **16**, 1206–1219 (2013).
6. García-Robledo, C., Kuprewicz, E. K., Staines, C. L., Erwin, T. L. & Kress, W. J. Limited tolerance by insects to high temperatures across tropical elevational gradients and the implications of global warming for extinction. *Proc. Natl Acad. Sci. USA* **113**, 680–685 (2016).
7. Geerts, A. N. et al. Rapid evolution of thermal tolerance in the water flea, *Daphnia*. *Nat. Clim. Change* **5**, 665–668 (2015).
8. Iossa, G. Sex-specific differences in thermal fertility limits. *Trends Ecol. Evol.* **34**, 490–492 (2019).
9. Walsh, B. S. et al. The impact of climate change on fertility. *Trends Ecol. Evol.* **34**, 249–259 (2019).
10. Vasudeva, R. et al. Adaptive thermal plasticity enhances sperm and egg performance in a model insect. *eLife* **8**, e49452 (2019).
11. Hurley, L. L., McDiarmid, C. S., Friesen, C. R., Griffith, S. C. & Rowe, M. Experimental heatwaves negatively impact sperm quality in the zebra finch. *Proc. R. Soc. B* **285**, 20172547 (2018).
12. Dahlke, F., Wohlrab, S., Butzin, M. & Pörtner, H. Thermal bottlenecks in the lifecycle define climate vulnerability of fish. *Science* **369**, 65–70 (2020).
13. Bathiany, S., Dakos, V., Scheffer, M. & Lenton, T. M. Climate models predict increasing temperature variability in poor countries. *Sci. Adv.* **4**, 1–11 (2018).
14. Vázquez, D. P., Gianoli, E., Morris, W. F. & Bozinovic, F. Ecological and evolutionary impacts of changing climatic variability. *Biol. Rev.* **92**, 22–42 (2017).
15. Chevin, L.-M., Lande, R. & Mace, G. M. Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biol.* **8**, e1000357 (2010).
16. Sgrò, C. M. & Hoffmann, A. A. Genetic correlations, tradeoffs and environmental variation. *Heredity* **93**, 241–248 (2004).
17. Wood, C. W. & Brodie, E. D. Environmental effects on the structure of the G-matrix. *Evolution* **69**, 2927–2940 (2015).
18. Brommer, J. E., Merila, J., Sheldon, B. C. & Gustavsson, L. Natural selection and genetic variation for reproductive reaction norms in a wild bird population. *Evolution* **59**, 1362–1371 (2005).
19. Brommer, J. E., Rattiste, K. & Wilson, A. J. Exploring plasticity in the wild: laying date–temperature reaction norms in the common gull *Larus canus*. *Proc. R. Soc. B* **275**, 687–693 (2008).
20. Nussey, D. H., Postma, E., Gienapp, P., Visser, M. E. & Gienapp, P. Selection on heritable phenotypic plasticity in a wild bird population. *Science* **310**, 304–306 (2005).
21. Charmantier, A. et al. Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science* **320**, 800–803 (2008).
22. Matthysen, E., Adriaensen, F. & Dhondt, A. A. Multiple responses to increasing spring temperatures in the breeding cycle of blue and great tits (*Cyanistes caeruleus*, *Parus major*). *Glob. Change Biol.* **17**, 1–16 (2011).
23. Both, C. & Visser, M. E. Adjustment to climate change is constrained by arrival date in a long-distance migrant bird. *Nature* **411**, 296–298 (2001).
24. Schiegg, K., Pasinelli, G., Walters, J. R. & Daniels, S. J. Inbreeding and experience affect response to climate change by endangered woodpeckers. *Proc. R. Soc. B* **269**, 1153–1159 (2002).
25. Wilson, S., Norris, D. R., Wilson, A. G. & Arcese, P. Breeding experience and population density affect the ability of a songbird to respond to future climate variation. *Proc. R. Soc. B* **274**, 2539–2545 (2007).
26. Dunn, P. O. & Winkler, D. W. Climate change has affected the breeding date of tree swallows throughout North America. *Proc. R. Soc. B* **266**, 2487–2490 (1999).
27. Hällfors, M. H. et al. Shifts in timing and duration of breeding for 73 boreal bird species over four decades. *Proc. Natl Acad. Sci. USA* **117**, 18557–18565 (2020).
28. Gienapp, P., Postma, E. & Visser, M. E. Why breeding time has not responded to selection for earlier breeding in a songbird population. *Evolution* **60**, 2381 (2006).
29. Järvinen, A. Global warming and egg size of birds. *Ecography* **17**, 108–110 (1994).
30. Kitaysky, A. S. & Golubova, E. G. Climate change causes contrasting trends in reproductive performance of planktivorous and piscivorous alcids. *J. Anim. Ecol.* **69**, 248–262 (2000).
31. Julliard, R., Clavel, J., Devictor, V., Jiguet, F. & Couvet, D. Spatial segregation of specialists and generalists in bird communities. *Ecol. Lett.* **9**, 1237–1244 (2006).
32. Weatherhead, P. J. Effects of climate variation on timing of nesting, reproductive success, and offspring sex ratios of red-winged blackbirds. *Oecologia* **144**, 168–175 (2005).
33. Auer, S. K. & Martin, T. E. Climate change has indirect effects on resource use and overlap among coexisting bird species with negative consequences for their reproductive success. *Glob. Change Biol.* **19**, 411–419 (2013).
34. Riddell, E. A., Iknayan, K. J., Wolf, B. O., Sinervo, B. & Beissinger, S. R. Cooling requirements fueled the collapse of a desert bird community from climate change. *Proc. Natl Acad. Sci. USA***116**, 21609–21615 (2019).
35. Visser, M. E., Van Noordwijk, A. J., Tinbergen, J. M. & Lessells, C. M. Warmer springs lead to mistimed reproduction in great tits (*Parus major*). *Proc. R. Soc. B* **265**, 1867–1870 (1998).

36. Both, C., Bouwhuis, S., Lessells, C. M. & Visser, M. E. Climate change and population declines in a long-distance migratory bird. *Nature* **441**, 81–83 (2006).
37. Magige, F. J., Stokke, B. G., Sortland, R. & Røskaft, E. Breeding biology of ostriches (*Struthio camelus*) in the Serengeti ecosystem, Tanzania. *Afr. J. Ecol.* **47**, 400–408 (2009).
38. Bertram, B. C. R. *The Ostrich Communal Nesting System* (Princeton University Press, New Jersey, 1992).
39. Kimwele, C. N. & Graves, J. A. A molecular genetic analysis of the communal nesting of the ostrich (*Struthio camelus*). *Mol. Ecol.* **12**, 229–236 (2003).
40. Maloney, S. K. Thermoregulation in ratites: a review. *Aust. J. Exp. Agric.* **48**, 1293–1301 (2008).
41. Hassan, S. M., Siam, A. A., Mady, M. E. & Cartwright, A. L. Egg storage period and weight effects on hatchability of ostrich (*Struthio camelus*) eggs. *Poult. Sci.* **84**, 1908–1912 (2005).
42. Gonzalez, A., Satterlee, D. G., Moharer, F. & Cadd, G. G. Factors affecting ostrich egg hatchability. *Poult. Sci.* **78**, 1257–1262 (1999).
43. Roff, D. A. & Wilson, A. J. Quantifying genotype-by-environment interactions in laboratory systems. In *Genotype-by-Environment Interactions and Sexual Selection* (eds. Hunt, J. & Hosken, D.) 100–136 (John Wiley & Sons, Ltd, 2014).
44. Christians, J. K. Avian egg size: variation within species and inflexibility within individuals. *Biol. Rev. Camb. Philos. Soc.* **77**, 1–26 (2002).
45. Lack, D. *The Natural Regulation of Animal Numbers* (Clarendon Press, 1954).
46. Perrins, C. M. The timing of birds' breeding seasons. *Ibis* **112**, 242–255 (1970).
47. Sales, K. et al. Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. *Nat. Commun.* **9**, 1–11 (2018).
48. McAfee, A. et al. Vulnerability of honey bee queens to heat-induced loss of fertility. *Nat. Sustain* **3**, 367–376 (2020).
49. Pérez-Crespo, M., Pintado, B. & Gutiérrez-Adán, A. Scrotal heat stress effects on sperm viability, sperm DNA integrity, and the offspring sex ratio in mice. *Mol. Reprod. Dev.* **75**, 40–47 (2008).
50. Hansen, P. J. Effects of heat stress on mammalian reproduction. *Philos. Trans. R. Soc. B* **364**, 3341–3350 (2009).
51. Moreno, R. D., Lagos-Cabre, R., Bunay, J., Urzua, N. & Bustamante-Marin, X. Molecular basis of heat stress damage in mammalian testis. In *Testis: Anatomy, Physiology and Pathology* (eds. Nemoto, Y. & Inaba, N.) 127–155 (Nova Science, 2012).
52. Karaca, A. G., Parker, H. M., Yeatman, J. B. & McDaniel, C. D. The effects of heat stress and sperm quality classification on broiler breeder male fertility and semen ion concentrations. *Br. Poult. Sci.* **43**, 621–628 (2002).
53. Mita, P., Hinton, B. T. & Dufour, J. M. The blood–testis and blood–epididymis barriers are more than just their tight junctions. *Biol. Reprod.* **84**, 851–858 (2011).
54. Smith, C. C. & Fretwell, S. D. The optimal balance between size and number of offspring. *Am. Nat.* **108**, 499–506 (1974).
55. Ojanen, M. Composition of the eggs of the great tit (*Parus major*) and pied flycatcher (*Ficedula hypoleuca*). *Ann. Zool. Fenn.* **20**, 57–63 (1983).
56. Krist, M. Egg size and offspring quality: a meta-analysis in birds. *Biol. Rev.* **86**, 692–716 (2011).
57. Falconer, D. S. & Mackay, T. F. C. *Introduction to Quantitative Genetics* (Pearson, 1996).
58. Lynch, M. & Gabriel, W. Environmental tolerance. *Am. Nat.* **129**, 283–303 (1987).
59. Gilchrist, G. W. Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. *Am. Nat.* **146**, 252–270 (1995).
60. Whitlock, M. C. The red queen beats the jack-of-all-trades: the limitations on the evolution of phenotypic plasticity and niche breadth. *Am. Nat.* **148**, S65 (1996).
61. Pen, I. & Weissing, F. J. Towards a unified theory of cooperative breeding: the role of ecology and life history re-examined. *Proc. R. Soc. B* **267**, 2411–2418 (2000).
62. Emlen, S. T. The evolution of helping. I. An ecological constraints model. *Am. Nat.* **119**, 29–39 (1982).
63. Rubenstein, D. R. Spatiotemporal environmental variation, risk aversion, and the evolution of cooperative breeding as a bet-hedging strategy. *Proc. Natl Acad. Sci. USA* **108**, 10816–10822 (2011).
64. Cornwallis, C. K. et al. Cooperation facilitates the colonization of harsh environments. *Nat. Ecol. Evol.* **1**, 0057 (2017).
65. Rubenstein, D. R. & Lovette, I. J. Temporal environmental variability drives the evolution of cooperative breeding in birds. *Curr. Biol.* **17**, 1414–1419 (2007).
66. Albright, T. P. et al. Mapping evaporative water loss in desert passerines reveals an expanding threat of lethal dehydration. *Proc. Natl Acad. Sci. USA* **114**, 201613625 (2017).
67. Vincze, O. et al. Parental cooperation in a changing climate: fluctuating environments predict shifts in care division. *Glob. Ecol. Biogeogr.* **26**, 347–358 (2017).
68. Nord, A. & Nilsson, J. Å. Heat dissipation rate constrains reproductive investment in a wild bird. *Funct. Ecol.* **33**, 250–259 (2019).
69. Cloete, S. W. P. et al. Variance components for live weight, body measurements and reproductive traits of pair-mated ostrich females. *Br. Poult. Sci.* **47**, 147–158 (2006).
70. Rybnik, P. K., Horbanczuk, J. O., Naranowicz, H., Lukaszewicz, E. & Malecki, I. A. Semen collection in the ostrich (*Struthio camelus*) using a dummy or a teaser female. *Br. Poult. Sci.* **48**, 635–643 (2007).
71. Brand, T. S., Olivier, T. R. & Gous, R. M. The response in food intake and reproductive parameters of breeding ostriches to increasing dietary energy. *South Afr. J. Anim. Sci.* **40**, 434–437 (2010).
72. Brand, T. S., Olivier, T. R. & Gous, R. M. The reproductive response of female ostriches to dietary protein. *Br. Poult. Sci.* **56**, 232–238 (2015).
73. Martin, P. A., Reimers, T. J., Lodge, J. R. & Dziuk, P. J. The effect of ratios and numbers of spermatozoa mixed from two males on proportions of offspring. *J. Reprod. Fertil.* **39**, 251–258 (1974).
74. Birkhead, T. R. & Møller, A. P. *Sperm Competition and Sexual Selection* (Academic Press, 1998).
75. Birkhead, T. R. & Biggins, J. D. Sperm competition mechanisms in birds: models and data. *Behav. Ecol.* **9**, 253–260 (1998).
76. Soley, J. T. & Roberts, J. C. Ultrastructure of ostrich (*Struthio camelus*) spermatozoa. II. Scanning electron microscopy. *Onderstepoort J. Vet. Res.* **61**, 239–246 (1994).
77. Lake, P. E. & Stewart, J. M. *Artificial Insemination in Poultry. Ministry of Agriculture Fisheries and Food, Bulletin 213* (Her Majesty's Stationery Office, 1978).
78. Bonato, M., Malecki, I. A., Rybnik-Trzaskowska, P. K., Cornwallis, C. K. & Cloete, S. W. P. Predicting ejaculate quality and libido in male ostriches: effect of season and age. *Anim. Reprod. Sci.* **151**, 49–55 (2014).
79. Bonato, M., Rybnik, P. K., Malecki, I. A., Cornwallis, C. K. & Cloete, S. W. P. Twice daily collection yields greater semen output and does not affect male libido in the ostrich. *Anim. Reprod. Sci.* **123**, 258–264 (2011).
80. Muvhali, P. T. et al. Ostrich ejaculate characteristics and male libido around equinox and solstice dates. *Trop. Anim. Health and Prod.* **52**, 2609–2619 (2020).
81. Brand, Z., Cloete, S. W. P., Brown, C. R. & Malecki, I. A. Systematic factors that affect ostrich egg incubation traits. *South Afr. J. Anim. Sci.* **38**, 315–325 (2008).
82. Bronneberg, R. G. G. et al. The relation between ultrasonographic observations in the oviduct and plasma progesterone, luteinizing hormone and estradiol during the egg laying cycle in ostriches. *Domest. Anim. Endocrinol.* **32**, 15–28 (2007).
83. Van Schalkwyk, S. J., Cloete, S. W. P. & De Kock, J. A. Repeatability and phenotypic correlations for body weight and reproduction in commercial ostrich breeding pairs. *Br. Poult. Sci.* **37**, 953–962 (1996).
84. Jones, R. C. & Lin, M. Spermatogenesis in birds. In *Oxford Reviews of Reproductive Biology*, Vol. 15 (ed. Milligan, S. R.) (Oxford University Press, 1993).
85. R Core Team. *R: A Language and Environment for Statistical Computing* (R Core Team, 2020).
86. Hadfield, J. D. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J. Stat. Softw.* **33**, 1–22 (2010).
87. Araya-Ajoy, Y. G. & Dingemanse, N. J. Repeatability, heritability, and age-dependence of seasonal plasticity in aggressiveness in a wild passerine bird. *J. Anim. Ecol.* **86**, 227–238 (2017).
88. Araya-Ajoy, Y. G., Mathot, K. J. & Dingemanse, N. J. An approach to estimate short-term, long-term and reaction norm repeatability. *Methods Ecol. Evol.* **6**, 1462–1473 (2015).
89. Scheiner, S. M. Genetics and evolution of phenotypic plasticity. *Annu. Rev. Ecol. Syst.* **24**, 35–68 (1993).
90. Wilson, A. J. Why h^2 does not always equal V_A/V_P. *J. Evol. Biol.* **21**, 647–650 (2008).
91. de Villemereuil, P., Morrissey, M. B., Nakagawa, S. & Schielzeth, H. Fixed-effect variance and the estimation of repeatabilities and heritabilities: Issues and solutions. *J. Evol. Biol.* **31**, 621–632 (2018).
92. de Villemereuil, P., Schielzeth, H., Nakagawa, S. & Morrissey, M. General methods for evolutionary quantitative genetic inference from generalized mixed models. *Genetics* **204**, 1281–1294 (2016).
93. BirdLife International. *BirdLife International and Handbook of the Birds of the World. Bird Species Distribution Maps of the World* (BirdLife International, 2019).
94. Fick, S. E. & Hijmans, R. J. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.* **37**, 4302–4315 (2017).

Acknowledgements

We are thankful to all the staff and workers at Oudtshoorn Research Farm for assistance with data collection and maintenance of the birds and to the Western Cape Government

for use of their resources. Special thanks to Naomi Oosthuizen for assistance with semen collections. The computations were performed on resources provided by SNIC through Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) under Project SNIC 2018/8-359. This research was funded by a stipend from the Carlsberg Foundation to M.F.S. and by the Swedish Research Council (grant number 2017-03880), the Knut and Alice Wallenberg Foundation (Wallenberg Academy fellowship number 2018.0138), Carl Tryggers (grant numbers 12: 92 and 19: 71) and an Oppenheimer travel exchange fund to C.K.C. E.I.S. was financially supported by a grant from the Swedish Research Council (grant number 2016-03356). The maintenance and development of the ostrich populations used in this study was funded by the Western Cape Department of Agriculture and supported by grants from the Western Cape Agricultural Research Trust (Grant number 0070/000VOLSTRUISE: (Cloete)) as well as the Technology and Human Resources for Industry programme (THRIP—Grant number TP14081390585) of the South African National Research Foundation to S.W.P.C.

Author contributions

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

Funding

Open Access funding provided by Lund University.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41467-021-20937-7.

Correspondence and requests for materials should be addressed to M.F.S.

Peer review information *Nature Communications* thanks Nicola Hemmings, Sheri Johnson, Tom Price and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary Information

Extreme temperatures compromise male and female fertility in a large desert bird

Contents

1 Supplementary Tables

1.1 Supplementary Table 1: Sample sizes within years

Yearly number of females and males and observations included in the analysis. Data of egg laying, egg mass, hatchting success and number of offspring were all obtained from the same females every year.

Year	Eggs	Egg mass records	Egg hathcing records	Females	Males (number of sperm)	Males (sperm viability)	Ejaculates (number of sperm)	Ejaculates (sperm viability)
1998	3705	3603	3303	86	0	0	0	0
1999	3506	3421	3310	90	0	0	0	0
2000	2446	2424	2378	48	0	0	0	0
2001	2972	2938	2903	53	0	0	0	0
2002	2221	2193	2123	56	0	0	0	0
2003	4530	4471	4355	102	0	0	0	0
2004	6160	6104	5988	148	0	0	0	0
2005	4702	4641	4369	131	0	0	0	0
2006	5781	5705	5462	124	0	0	0	0
2007	4570	4485	4225	95	0	0	0	0
2008	5470	5366	5024	116	4	4	37	37
2009	4444	4376	4018	100	8	8	358	352
2010	5116	5051	4928	107	7	7	379	380
2011	4147	4107	3981	90	8	7	297	232
2012	2772	2749	2642	71	10	0	79	0
2013	3595	3544	3463	82	8	5	39	16
2014	2974	2920	2880	69	8	0	209	0
2015	2860	2806	2736	65	7	0	20	0
2016	2857	2806	2681	63	10	10	245	225
2017	3164	3116	3074	64	10	8	122	58
2018	3547	3512	3463	70	0	0	0	0

1.2 Results from random regression models (Supplementary Tables 2-7)

1.2.1 Supplementary Table 2: Egg laying (random regression)

Testing the effect of thermal stress on egg laying rate using a random slope model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	0.26 (0.08,0.42)	**0.006**	-
	dam_subpopZB	-0.39 (-0.67,-0.18)	**0.001**	-
	dam_subpopCross	-0.15 (-0.36,0.04)	0.141	-
	sire_subpopZB	-0.13 (-0.24,0)	0.051	-
	sire_subpopCross	-0.09 (-0.29,0.12)	0.4	-
	dam_age_z	-0.04 (-0.07,0)	**0.034**	-
	TSIcon:StressCold	-1.97 (-2.33,-1.61)	**0.001**	-
	TSIcon:StressHeat	-1.62 (-1.81,-1.41)	**0.001**	-
	TSIcon:StressCold:dam_subpopZB	-0.37 (-1.57,0.64)	0.507	-
	TSIcon:StressHeat:dam_subpopZB	-0.37 (-0.96,0.26)	0.246	-
	TSIcon:StressCold:dam_subpopCross	-1.36 (-2.28,-0.45)	**0.004**	-
	TSIcon:StressHeat:dam_subpopCross	0.38 (-0.15,0.91)	0.164	-
Random effect var.	(Intercept)	0.564 (0.511,0.694)	-	damid
	TSIcon:StressCold	2.139 (1.247,3.32)	-	damid
	TSIcon:StressHeat	2.956 (2.32,3.53)	-	damid
	enclosure	0.179 (0.14,0.238)	-	enclosure
	year	0.092 (0.037,0.156)	-	year
	residuals	0.603 (0.579,0.641)	-	units
Correlations	TSIcon:StressCold:(Intercept).damid	0.136 (-0.08,0.361)	0.172	-
	TSIcon:StressHeat:(Intercept).damid	-0.072 (-0.208,0.044)	0.154	-
Fixed effect var.	All fixed - thermal stress	0.027 (0.013,0.06)	-	-
Expected scale	Intercept individual variance	4.967 (4.505,5.822)	-	-
	Intercept phenotypic variance	10.18 (9.519,10.717)	-	-
	Intercept repeatability	0.519 (0.463,0.556)	-	-

For fixed effects the posterior mean is reported

1.2.2 Supplementary Table 3: Number of sperm (random regression)

Testing the effect of thermal stress on number of sperm using a random slope model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	10.47 (10.17,10.84)	**0.001**	-
	poly(age, 2)1	1.62 (-2.56,6.24)	0.503	-
	poly(age, 2)2	-5.47 (-8.18,-3.22)	**0.001**	-
	TSI:StressCold	-0.63 (-1.07,-0.24)	**0.002**	-
	TSI:StressHeat	-0.67 (-1.24,-0.02)	**0.028**	-
Random effect var.	(Intercept)	0.265 (0.157,0.663)	-	name
	TSI:StressCold	0.24 (0.123,0.871)	-	name
	TSI:StressHeat	0.705 (0.231,2.878)	-	name
	year	0.049 (0.015,0.197)	-	year
	residuals	0.923 (0.86,0.987)	-	units
Correlations	TSI:StressCold:(Intercept).name	-0.162 (-0.583,0.49)	0.786	-
	TSI:StressHeat:(Intercept).name	-0.111 (-0.571,0.468)	0.921	-
Fixed effect var.	All fixed - thermal stress	0.018 (0.004,0.041)	-	-
Expected scale	Intercept individual variance	487.713 (166.046,2733.395)	-	-

For fixed effects the posterior mean is reported

1.2.3 Supplementary Table 4: Egg mass (random regression)

Testing the effect of thermal stress on egg mass using a random slope model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	1.43 (1.42,1.45)	**0.001**	-
	dam_subpopZB	0.07 (0.04,0.11)	**0.001**	-
	dam_subpopCross	0.04 (0.01,0.07)	**0.001**	-
	sire_subpopZB	0.01 (0.01,0.02)	**0.001**	-
	sire_subpopCross	0 (0,0.01)	0.404	-
	poly(days.since.prev.eggLOG_z, 2)1	-5.15 (-5.3,-5)	**0.001**	-
	poly(days.since.prev.eggLOG_z, 2)2	-1.85 (-1.98,-1.7)	**0.001**	-
	dam_age_z	0 (-0.01,0)	**0.004**	-
	TSI:StressCold	-0.05 (-0.06,-0.03)	**0.001**	-
	TSI:StressHeat	0.01 (0,0.02)	0.261	-
	TSI:StressCold:dam_subpopZB	0.02 (-0.04,0.07)	0.461	-
	TSI:StressHeat:dam_subpopZB	-0.02 (-0.05,0.01)	0.301	-
	TSI:StressCold:dam_subpopCross	-0.02 (-0.06,0.02)	0.307	-
	TSI:StressHeat:dam_subpopCross	0.01 (-0.02,0.03)	0.497	-
Random effect var.	(Intercept)	0.017 (0.015,0.019)	-	damid
	TSI:StressCold	0.021 (0.018,0.025)	-	damid
	TSI:StressHeat	0.012 (0.01,0.013)	-	damid
	enclosure	0.002 (0.002,0.003)	-	enclosure
	year	0 (0,0.001)	-	year
	residuals	0.005 (0.005,0.005)	-	units
Correlations	TSI:StressCold:(Intercept).damid	-0.082 (-0.2,-0.012)	**0.032**	-
	TSI:StressHeat:(Intercept).damid	-0.024 (-0.112,0.053)	0.463	-
Fixed effect var.	All fixed - thermal stress	0.001 (0.001,0.002)	-	-

For fixed effects the posterior mean is reported

1.2.4 Supplementary Table 5: Sperm viability (random regression)

Testing the effect of thermal stress on sperm viability using a random slope model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	-1.91 (-2.23,-1.61)	**0.001**	-
	poly(age, 2)1	-2.64 (-5.45,-0.16)	**0.044**	-
	poly(age, 2)2	1.46 (0.16,2.57)	**0.014**	-
	TSI:StressCold	-0.24 (-0.55,0.08)	0.152	-
	TSI:StressHeat	0.06 (-0.22,0.29)	0.651	-
Random effect var.	(Intercept)	0.165 (0.073,0.352)	-	name
	TSI:StressCold	0.366 (0.134,0.707)	-	name
	TSI:StressHeat	0.18 (0.083,0.441)	-	name
	year	0.032 (0.016,0.252)	-	year
	residuals	0.039 (0.034,0.043)	-	units
Correlations	TSI:StressCold:(Intercept).name	0.279 (-0.402,0.571)	0.671	-
	TSI:StressHeat:(Intercept).name	-0.267 (-0.681,0.287)	0.527	-
Fixed effect var.	All fixed - thermal stress	0.004 (0,0.021)	-	-
Expected scale	Intercept individual variance	631.663 (197.463,1409.6)	-	-

For fixed effects the posterior mean is reported

1.2.5 Supplementary Table 6: Hatching success (random regression)

Testing the effect of thermal stress on hatching success using a random slope model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	-0.04 (-0.25,0.18)	0.685	-
	dam_subpopZB	0.12 (-0.24,0.43)	0.501	-
	dam_subpopCross	0.05 (-0.24,0.32)	0.705	-
	sire_subpopZB	0.16 (-0.01,0.32)	0.069	-
	sire_subpopCross	0.45 (0.17,0.69)	**0.001**	-
	dam_age_z	-0.1 (-0.14,-0.05)	**0.001**	-
	TSIcon:StressCold	-0.57 (-0.98,0.01)	**0.028**	-
	TSIcon:StressHeat	-0.26 (-0.43,-0.09)	**0.002**	-
	TSIcon:StressCold:dam_subpopZB	-0.15 (-1.54,1.47)	0.826	-
	TSIcon:StressHeat:dam_subpopZB	-0.14 (-0.67,0.41)	0.628	-
	TSIcon:StressCold:dam_subpopCross	-1.46 (-2.73,-0.36)	**0.012**	-
	TSIcon:StressHeat:dam_subpopCross	-0.2 (-0.62,0.26)	0.382	-
Random effect var.	(Intercept)	1.164 (0.99,1.363)	-	damid
	TSIcon:StressCold	0.784 (0.299,2.943)	-	damid
	TSIcon:StressHeat	0.728 (0.376,0.977)	-	damid
	enclosure	0.341 (0.236,0.424)	-	enclosure
	year	0.134 (0.07,0.282)	-	year
	residuals	0.556 (0.519,0.61)	-	units

For fixed effects the posterior mean is reported

1.2.6 Supplementary Table 7: Number of offspring (random regression)

Testing the effect of thermal stress on number of offspring using a random slope model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	-1.25 (-1.46,-1.05)	**0.001**	-
	dam_subpopZB	-0.18 (-0.47,0.14)	0.253	-
	dam_subpopCross	-0.08 (-0.34,0.18)	0.501	-
	sire_subpopZB	0.04 (-0.11,0.18)	0.636	-
	sire_subpopCross	0.25 (0.01,0.48)	0.051	-
	dam_age_z	-0.07 (-0.12,-0.04)	**0.001**	-
	TSIcon:StressCold	-2.1 (-2.57,-1.6)	**0.001**	-
	TSIcon:StressHeat	-1.42 (-1.61,-1.21)	**0.001**	-
	TSIcon:StressCold:dam_subpopZB	-0.66 (-2.12,0.67)	0.341	-
	TSIcon:StressHeat:dam_subpopZB	-0.51 (-1.08,0.07)	0.087	-
	TSIcon:StressCold:dam_subpopCross	-1.88 (-3.01,-0.78)	**0.008**	-
	TSIcon:StressHeat:dam_subpopCross	0.04 (-0.43,0.48)	0.885	-
Random effect var.	(Intercept)	1.058 (0.884,1.23)	-	damid
	TSIcon:StressCold	2.419 (1.14,4.239)	-	damid
	TSIcon:StressHeat	1.889 (1.458,2.443)	-	damid
	enclosure	0.3 (0.224,0.383)	-	enclosure
	year	0.106 (0.047,0.212)	-	year
	residuals	0.703 (0.663,0.747)	-	units

For fixed effects the posterior mean is reported

1.3 Results from random regression models with year specific slopes (Supplementary Tables 8-11)

1.3.1 Supplementary Table 8: Egg laying (year specific random regression)

Testing the effect of thermal stress on egg laying rate using a (yearly) random slope model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	0.32 (0.18,0.49)	**0.001**	-
	dam_subpopZB	-0.39 (-0.6,-0.18)	**0.001**	-
	dam_subpopCross	-0.16 (-0.33,0.04)	0.091	-
	sire_subpopZB	-0.17 (-0.34,0)	**0.046**	-
	sire_subpopCross	-0.23 (-0.49,0.04)	0.083	-
	dam_age_z	0.01 (-0.04,0.05)	0.756	-
	TSIcon:StressCold	-2.1 (-2.43,-1.8)	**0.001**	-
	TSIcon:StressHeat	-1.68 (-1.9,-1.5)	**0.001**	-
	TSIcon:StressCold:dam_subpopZB	-0.39 (-1.24,0.62)	0.402	-
	TSIcon:StressHeat:dam_subpopZB	-0.45 (-1.01,0.16)	0.143	-
	TSIcon:StressCold:dam_subpopCross	-1.28 (-2.03,-0.58)	**0.001**	-
	TSIcon:StressHeat:dam_subpopCross	0.42 (-0.06,0.91)	0.091	-
Random effect var.	(Intercept)	0.311 (0.244,0.384)	-	damid
	TSIcon:StressCold	1.345 (0.457,2.22)	-	damid
	TSIcon:StressHeat	1.609 (1.146,2.275)	-	damid
	(Intercept)	0.484 (0.427,0.548)	-	year_damid
	TSIcon:StressCold	4.339 (3.103,7.009)	-	year_damid
	TSIcon:StressHeat	4.963 (4.411,5.794)	-	year_damid
	enclosure	0.087 (0.054,0.134)	-	enclosure
	year	0.073 (0.034,0.157)	-	year
	residuals	0.163 (0.147,0.184)	-	units
Repeatabilities	Intercept	0.275 (0.216,0.324)	-	-
	Heat stress slope	0.244 (0.174,0.322)	-	-
	Cold stress slope	0.177 (0.06,0.341)	-	-
Fixed effect var.	All fixed - thermal stress	0.036 (0.013,0.062)	-	-
Expected scale	Intercept individual variance	2.919 (2.377,3.57)	-	-
	Intercept phenotypic variance	8.463 (7.88,9.2)	-	-
	Intercept repeatability	0.354 (0.284,0.409)	-	-

For fixed effects the posterior mean is reported

1.3.2 Supplementary Table 9: Number of sperm (year specific random regression)

Testing the effect of thermal stress on number of sperm using a (yearly) random slope model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	10.45 (10.11,10.76)	**0.001**	-
	poly(age, 2)1	2.52 (-2.46,8.23)	0.374	-
	poly(age, 2)2	-6.2 (-10.37,-2.03)	**0.006**	-
	TSI:StressCold	-0.67 (-1.18,-0.23)	**0.008**	-
	TSI:StressHeat	-0.67 (-1.32,-0.07)	**0.032**	-
Random effect var.	(Intercept)	0.309 (0.131,0.658)	-	name
	TSI:StressCold	0.299 (0.131,0.982)	-	name
	TSI:StressHeat	0.762 (0.188,2.469)	-	name
	(Intercept)	0.16 (0.088,0.258)	-	year_name
	TSI:StressCold	0.286 (0.134,0.851)	-	year_name
	TSI:StressHeat	0.771 (0.18,2.15)	-	year_name
	year	0.002 (0,0.114)	-	year
	residuals	0.883 (0.812,0.933)	-	units
Repeatabilities	Intercept	0.232 (0.137,0.404)	-	-
	Heat stress slope	0.493 (0.136,0.831)	-	-
	Cold stress slope	0.414 (0.254,0.793)	-	-
Fixed effect var.	All fixed - thermal stress	0.011 (0.002,0.067)	-	-
Expected scale	Intercept individual variance	544.614 (143.844,2543.709)	-	-
	Intercept phenotypic variance	12684.275 (5969.37,40455.823)	-	-
	Intercept repeatability	0.039 (0.021,0.076)	-	-

For fixed effects the posterior mean is reported

1.3.3 Supplementary Table 10: Egg mass (year specific random regression)

Testing the effect of thermal stress on egg mass using a (yearly) random slope model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	1.43 (1.42,1.45)	**0.001**	-
	dam_subpopZB	0.09 (0.05,0.12)	**0.001**	-
	dam_subpopCross	0.05 (0.02,0.07)	**0.002**	-
	sire_subpopZB	0.02 (0,0.03)	**0.036**	-
	sire_subpopCross	0 (-0.02,0.03)	0.857	-
	poly(days.since.prev.eggLOG_z, 2)1	-5.03 (-5.16,-4.89)	**0.001**	-
	poly(days.since.prev.eggLOG_z, 2)2	-1.86 (-2,-1.72)	**0.001**	-
	dam_age_z	-0.01 (-0.01,0)	**0.02**	-
	TSI:StressCold	-0.05 (-0.07,-0.03)	**0.001**	-
	TSI:StressHeat	0 (-0.01,0.02)	0.572	-
	TSI:StressCold:dam_subpopZB	0.01 (-0.04,0.07)	0.675	-
	TSI:StressHeat:dam_subpopZB	-0.03 (-0.06,0.01)	0.152	-
	TSI:StressCold:dam_subpopCross	-0.02 (-0.07,0.02)	0.275	-
	TSI:StressHeat:dam_subpopCross	0.01 (-0.01,0.04)	0.378	-
Random effect var.	(Intercept)	0.015 (0.013,0.017)	-	damid
	TSI:StressCold	0.019 (0.015,0.022)	-	damid
	TSI:StressHeat	0.011 (0.009,0.012)	-	damid
	(Intercept)	0.004 (0.004,0.004)	-	year_damid
	TSI:StressCold	0.021 (0.018,0.024)	-	year_damid
	TSI:StressHeat	0.01 (0.009,0.011)	-	year_damid
	enclosure	0 (0,0)	-	enclosure
	year	0 (0,0.001)	-	year
	residuals	0.004 (0.004,0.005)	-	units
Repeatabilities	Intercept	0.622 (0.588,0.652)	-	-
	Heat stress slope	0.525 (0.482,0.57)	-	-
	Cold stress slope	0.474 (0.41,0.528)	-	-
Fixed effect var.	All fixed - thermal stress	0.001 (0.001,0.002)	-	-

For fixed effects the posterior mean is reported

1.3.4 Supplementary Table 11: Sperm viability (year specific random regression)

Testing the effect of thermal stress on sperm viability using a (yearly) random slope model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	-1.93 (-2.24,-1.67)	**0.001**	-
	poly(age, 2)1	-3.53 (-7.92,0.29)	0.099	-
	poly(age, 2)2	0.25 (-4.05,4.3)	0.909	-
	TSI:StressCold	-0.2 (-0.53,0.11)	0.242	-
	TSI:StressHeat	0.13 (-0.18,0.48)	0.392	-
Random effect var.	(Intercept)	0.155 (0.084,0.381)	-	name
	TSI:StressCold	0.293 (0.096,0.625)	-	name
	TSI:StressHeat	0.208 (0.1,0.581)	-	name
	(Intercept)	0.094 (0.06,0.179)	-	year_name
	TSI:StressCold	0.153 (0.091,0.341)	-	year_name
	TSI:StressHeat	0.154 (0.085,0.341)	-	year_name
	year	0.004 (0,0.159)	-	year
	residuals	0.036 (0.03,0.039)	-	units
Repeatabilities	Intercept	0.592 (0.31,0.727)	-	-
	Heat stress slope	0.652 (0.359,0.82)	-	-
	Cold stress slope	0.615 (0.364,0.815)	-	-
Fixed effect var.	All fixed - thermal stress	0.001 (0,0.04)	-	-
Expected scale	Intercept individual variance	466.637 (229.317,1463.239)	-	-
	Intercept phenotypic variance	1246.648 (718.613,2649.615)	-	-
	Intercept repeatability	0.532 (0.277,0.702)	-	-

For fixed effects the posterior mean is reported

1.4 Results from character state models (Supplementary Tables 12-17)

1.4.1 Supplementary Table 12: Egg laying (character-state)

Testing the effect of thermal stress on egg laying rate using a character-state model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	0.08 (-0.07,0.25)	0.313	-
	TSIcatCold	-0.05 (-0.14,0.03)	0.251	-
	TSIcatHot	-0.64 (-0.72,-0.56)	**0.001**	-
	dam_subpopZB	-0.45 (-0.66,-0.2)	**0.001**	-
	dam_subpopCross	-0.13 (-0.32,0.05)	0.192	-
	sire_subpopZB	-0.14 (-0.29,0)	0.055	-
	sire_subpopCross	-0.15 (-0.38,0.08)	0.212	-
	dam_age_z	-0.03 (-0.07,0.01)	0.22	-
	TSIcatCold:dam_subpopZB	-0.07 (-0.32,0.2)	0.582	-
	TSIcatHot:dam_subpopZB	-0.17 (-0.41,0.08)	0.188	-
	TSIcatCold:dam_subpopCross	-0.26 (-0.46,-0.06)	**0.016**	-
	TSIcatHot:dam_subpopCross	0.14 (-0.06,0.36)	0.204	-
Random effect var.	TSIcatBenign	0.505 (0.43,0.585)	-	damid
	TSIcatCold	0.541 (0.441,0.711)	-	damid
	TSIcatHot	0.884 (0.694,1.03)	-	damid
	enclosure	0.142 (0.098,0.189)	-	enclosure
	year	0.08 (0.041,0.18)	-	year
	TSIcatBenign	0.363 (0.335,0.399)	-	units
	TSIcatCold	0.93 (0.821,1.043)	-	units
	TSIcatHot	0.857 (0.789,0.982)	-	units
Correlations	TSIcatCold:TSIcatBenign.damid	0.849 (0.783,0.883)	**0.001**	-
	TSIcatHot:TSIcatBenign.damid	0.877 (0.834,0.915)	**0.001**	-

For fixed effects the posterior mean is reported

1.4.2 Supplementary Table 13: Number of sperm (character-state)

Testing the effect of thermal stress on number of sperm using a character-state model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	10.39 (10.06,10.74)	**0.001**	-
	TSIcatCold	-0.35 (-0.66,0)	**0.034**	-
	TSIcatHot	-0.19 (-0.55,0.16)	0.253	-
	poly(age, 2)1	2.56 (-2.08,6.98)	0.279	-
	poly(age, 2)2	-5.11 (-7.53,-2.38)	**0.001**	-
Random effect var.	TSIcatBenign	0.304 (0.171,0.645)	-	name
	TSIcatCold	0.396 (0.172,0.811)	-	name
	TSIcatHot	0.417 (0.224,1.064)	-	name
	year	0.074 (0.015,0.246)	-	year
	TSIcatBenign	0.827 (0.763,0.901)	-	units
	TSIcatCold	0.892 (0.778,1.096)	-	units
	TSIcatHot	1.232 (1.09,1.471)	-	units
Correlations	TSIcatCold:TSIcatBenign.name	0.587 (0.127,0.824)	**0.026**	-
	TSIcatHot:TSIcatBenign.name	0.563 (0.061,0.786)	**0.046**	-

For fixed effects the posterior mean is reported

1.4.3 Supplementary Table 14: Egg mass (character-state)

Testing the effect of thermal stress on egg mass using a character-state model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	1.44 (1.42,1.45)	**0.001**	-
	TSIcatCold	-0.01 (-0.02,0)	**0.034**	-
	TSIcatHot	0 (-0.01,0.01)	0.646	-
	dam_subpopZB	0.07 (0.04,0.1)	**0.001**	-
	dam_subpopCross	0.04 (0.01,0.07)	**0.006**	-
	sire_subpopZB	0.01 (0.01,0.02)	**0.001**	-
	sire_subpopCross	0 (0,0.01)	0.384	-
	poly(days.since.prev.eggLOG_z, 2)1	-5.17 (-5.32,-5.02)	**0.001**	-
	poly(days.since.prev.eggLOG_z, 2)2	-1.85 (-1.99,-1.68)	**0.001**	-
	dam_age_z	0 (-0.01,0)	**0.001**	-
	TSIcatCold:dam_subpopZB	0.01 (-0.02,0.03)	0.642	-
	TSIcatHot:dam_subpopZB	0 (-0.03,0.02)	0.749	-
	TSIcatCold:dam_subpopCross	-0.01 (-0.03,0.01)	0.57	-
	TSIcatHot:dam_subpopCross	0 (-0.02,0.02)	0.8	-
Random effect var.	TSIcatBenign	0.017 (0.015,0.019)	-	damid
	TSIcatCold	0.017 (0.015,0.019)	-	damid
	TSIcatHot	0.019 (0.016,0.021)	-	damid
	enclosure	0.002 (0.002,0.002)	-	enclosure
	year	0 (0,0.001)	-	year
	TSIcatBenign	0.005 (0.005,0.005)	-	units
	TSIcatCold	0.006 (0.006,0.006)	-	units
	TSIcatHot	0.005 (0.005,0.006)	-	units
Correlations	TSIcatCold:TSIcatBenign.damid	0.761 (0.733,0.795)	**0.001**	-
	TSIcatHot:TSIcatBenign.damid	0.767 (0.738,0.803)	**0.001**	-

For fixed effects the posterior mean is reported

1.4.4 Supplementary Table 15: Sperm viability (character-state)

Testing the effect of thermal stress on sperm viability using a character-state model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	-1.93 (-2.19,-1.6)	**0.001**	-
	TSIcatCold	-0.11 (-0.41,0.17)	0.455	-
	TSIcatHot	0.03 (-0.23,0.3)	0.842	-
	poly(age, 2)1	-2.3 (-4.48,0.16)	0.061	-
	poly(age, 2)2	1.29 (0.03,2.67)	0.067	-
Random effect var.	TSIcatBenign	0.167 (0.092,0.379)	-	name
	TSIcatCold	0.203 (0.106,0.484)	-	name
	TSIcatHot	0.143 (0.074,0.341)	-	name
	year	0.052 (0.014,0.224)	-	year
	TSIcatBenign	0.035 (0.031,0.041)	-	units
	TSIcatCold	0.089 (0.073,0.12)	-	units
	TSIcatHot	0.051 (0.043,0.067)	-	units
Correlations	TSIcatCold:TSIcatBenign.name	0.426 (-0.1,0.721)	0.176	-
	TSIcatHot:TSIcatBenign.name	0.215 (-0.265,0.631)	0.418	-

For fixed effects the posterior mean is reported

1.4.5 Supplementary Table 16: Hatching success (character-state)

Testing the effect of thermal stress on hatching success using a character-state model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	-0.11 (-0.31,0.09)	0.226	-
	TSIcatCold	-0.09 (-0.18,0.02)	0.101	-
	TSIcatHot	-0.05 (-0.13,0.05)	0.325	-
	dam_subpopZB	0.05 (-0.24,0.4)	0.743	-
	dam_subpopCross	-0.02 (-0.28,0.24)	0.907	-
	sire_subpopZB	0.22 (0.05,0.41)	**0.016**	-
	sire_subpopCross	0.49 (0.21,0.82)	**0.002**	-
	dam_age_z	-0.09 (-0.14,-0.03)	**0.001**	-
	TSIcatCold:dam_subpopZB	0.02 (-0.31,0.31)	0.867	-
	TSIcatHot:dam_subpopZB	-0.01 (-0.27,0.3)	0.917	-
	TSIcatCold:dam_subpopCross	-0.13 (-0.39,0.1)	0.317	-
	TSIcatHot:dam_subpopCross	-0.02 (-0.27,0.2)	0.927	-
Random effect var.	TSIcatBenign	0.906 (0.761,1.078)	-	damid
	TSIcatCold	0.961 (0.798,1.254)	-	damid
	TSIcatHot	1.027 (0.813,1.231)	-	damid
	enclosure	0.192 (0.135,0.275)	-	enclosure
	year	0.091 (0.06,0.237)	-	year
	TSIcatBenign	0.628 (0.57,0.703)	-	units
	TSIcatCold	0.652 (0.527,0.835)	-	units
	TSIcatHot	0.613 (0.49,0.723)	-	units

For fixed effects the posterior mean is reported

1.4.6 Supplementary Table 17: Number of offspring (character-state)

Testing the effect of thermal stress on number of offspring using a character-state model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	Intercept	-1.41 (-1.59,-1.23)	**0.001**	-
	TSIcatCold	-0.26 (-0.36,-0.16)	**0.001**	-
	TSIcatHot	-0.66 (-0.77,-0.57)	**0.001**	-
	dam_subpopZB	-0.26 (-0.54,0.02)	0.071	-
	dam_subpopCross	-0.12 (-0.35,0.11)	0.341	-
	sire_subpopZB	0.05 (-0.12,0.22)	0.547	-
	sire_subpopCross	0.26 (-0.03,0.52)	0.077	-
	dam_age_z	-0.07 (-0.11,-0.02)	**0.006**	-
	TSIcatCold:dam_subpopZB	-0.01 (-0.28,0.25)	0.956	-
	TSIcatHot:dam_subpopZB	-0.14 (-0.41,0.12)	0.307	-
	TSIcatCold:dam_subpopCross	-0.28 (-0.5,-0.05)	**0.014**	-
	TSIcatHot:dam_subpopCross	0.09 (-0.14,0.33)	0.442	-
Random effect var.	TSIcatBenign	0.891 (0.744,1.023)	-	damid
	TSIcatCold	0.995 (0.839,1.29)	-	damid
	TSIcatHot	1.184 (0.983,1.468)	-	damid
	enclosure	0.221 (0.164,0.3)	-	enclosure
	year	0.088 (0.05,0.209)	-	year
	TSIcatBenign	0.643 (0.591,0.699)	-	units
	TSIcatCold	1.021 (0.846,1.166)	-	units
	TSIcatHot	1.039 (0.925,1.198)	-	units

For fixed effects the posterior mean is reported

1.5 Results from two-trait models (Supplementary Tables 18-22)

1.5.1 Supplementary Table 18: Phenotypic correlations from character state two-trait models

Testing for trade-offs between fertility traits in cold and hot environments. Phenotypic correlations among and within individuals were estimated using two-trait character-state models in MCMCglmm. Credible intervals not overlapping with zero are written in bold. See supplementary tables 19-20 below for model details.

Trait compairon	Level	Cold	Benign	Hot
Eggs laying vs eggmass	Among individuals	0.068 (-0.098,0.186)	0.105 (-0.015,0.193)	0.147 (-0.011,0.251)
	Within individuals	**0.186 (0.105,0.257)**	**0.172 (0.106,0.226)**	**0.117 (0.049,0.189)**
Number of sperm vs sperm viability	Among individuals	-0.195 (-0.645,0.348)	-0.237 (-0.614,0.339)	0.078 (-0.416,0.601)
	Within individuals	**0.166 (0.017,0.3)**	**0.19 (0.098,0.263)**	0.149 (-0.029,0.247)

1.5.2 Supplementary Table 19: Character state model of number of sperm vs sperm viability

Testing for correlations between sperm viability and number of sperm in the effect of thermal stress using a bivariate character state model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	traitsperm_num5	10.35 (9.8,11.01)	**0.001**	-
	traitsperm_live	-1.92 (-2.36,-1.53)	**0.001**	-
	poly(age, 2)1	8.03 (2.14,14.34)	**0.014**	-
	poly(age, 2)2	-7.27 (-12.09,-2.23)	**0.001**	-
	traitsperm_num5:TSIcatCold	-0.26 (-0.7,0.2)	0.238	-
	traitsperm_live:TSIcatCold	-0.1 (-0.37,0.17)	0.499	-
	traitsperm_num5:TSIcatHot	-0.01 (-0.44,0.46)	0.958	-
	traitsperm_live:TSIcatHot	0.03 (-0.2,0.28)	0.822	-
	traitsperm_live:poly(age, 2)1	-10.81 (-17.88,-4.14)	**0.002**	-
	traitsperm_live:poly(age, 2)2	9.2 (4.3,14.62)	**0.001**	-
Random effect var.	traitsperm_num5:at.level(TSIcat, Cold)	0.277 (0.14,0.749)	-	name
	traitsperm_live:at.level(TSIcat, Cold)	0.155 (0.073,0.345)	-	name
	traitsperm_num5:at.level(TSIcat, Benign)	0.311 (0.123,0.648)	-	name
	traitsperm_live:at.level(TSIcat, Benign)	0.114 (0.06,0.245)	-	name
	traitsperm_num5:at.level(TSIcat, Hot)	0.321 (0.163,0.898)	-	name
	traitsperm_live:at.level(TSIcat, Hot)	0.087 (0.046,0.213)	-	name
	traitsperm_num5	0.192 (0.086,0.983)	-	year
	traitsperm_live	0.202 (0.071,0.694)	-	year
	traitsperm_num5:at.level(TSIcat, Cold)	0.618 (0.508,0.733)	-	units
	traitsperm_live:at.level(TSIcat, Cold)	0.088 (0.065,0.107)	-	units
	traitsperm_num5:at.level(TSIcat, Benign)	0.788 (0.698,0.855)	-	units
	traitsperm_live:at.level(TSIcat, Benign)	0.032 (0.028,0.038)	-	units
	traitsperm_num5:at.level(TSIcat, Hot)	0.765 (0.631,0.875)	-	units
	traitsperm_live:at.level(TSIcat, Hot)	0.048 (0.037,0.06)	-	units
Correlations	traitsperm_live:at.level(TSIcat, Cold):traitsperm_num5:at.level(TSIcat, Cold).name	-0.195 (-0.645,0.348)	0.523	-
	traitsperm_live:at.level(TSIcat, Benign):traitsperm_num5:at.level(TSIcat, Benign).name	-0.237 (-0.614,0.339)	0.622	-
	traitsperm_live:at.level(TSIcat, Hot):traitsperm_num5:at.level(TSIcat, Hot).name	0.078 (-0.416,0.601)	0.681	-
	traitsperm_live:at.level(TSIcat, Cold):traitsperm_num5:at.level(TSIcat, Cold).units	0.166 (0.017,0.3)	**0.024**	-
	traitsperm_live:at.level(TSIcat, Benign):traitsperm_num5:at.level(TSIcat, Benign).units	0.19 (0.098,0.263)	**0.001**	-
	traitsperm_live:at.level(TSIcat, Hot):traitsperm_num5:at.level(TSIcat, Hot).units	0.149 (-0.029,0.247)	0.109	-

For fixed effects the posterior mean is reported

1.5.3 Supplementary Table 20: Character state model of egg-laing vs egg mass

Testing for correlations between egg mass and egg laying in the effect of thermal stress using a bivariate character state model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	traitmass	1.44 (1.34,1.53)	**0**	-
	traitNeggs	0.17 (-0.01,0.38)	0.094	-
	dam_subpopZB	0.08 (0.05,0.11)	**0**	-
	dam_subpopCross	0.05 (0.02,0.08)	**0**	-
	TSIcatCold	-0.01 (-0.03,0)	0.109	-
	TSIcatHot	0 (-0.02,0.02)	0.929	-
	sire_subpopZB	0 (-0.01,0.01)	0.73	-
	sire_subpopCross	-0.01 (-0.03,0.01)	0.558	-
	dam_age_z	0 (-0.01,0)	0.176	-
	dam_subpopZB:TSIcatCold	0.01 (-0.04,0.06)	0.58	-
	dam_subpopCross:TSIcatCold	-0.01 (-0.05,0.03)	0.651	-
	dam_subpopZB:TSIcatHot	0 (-0.05,0.04)	0.843	-
	dam_subpopCross:TSIcatHot	0 (-0.03,0.04)	0.874	-
	traitNeggs:dam_subpopZB	-0.57 (-0.78,-0.38)	**0**	-
	traitNeggs:dam_subpopCross	-0.24 (-0.39,-0.07)	**0.007**	-
	traitNeggs:TSIcatCold	0.1 (0,0.21)	0.067	-
	traitNeggs:TSIcatHot	-0.47 (-0.56,-0.36)	**0**	-
	traitNeggs:sire_subpopZB	-0.18 (-0.3,-0.06)	**0.004**	-
	traitNeggs:sire_subpopCross	-0.21 (-0.41,-0.02)	**0.034**	-
	traitNeggs:dam_age_z	0.02 (-0.01,0.06)	0.221	-
	traitNeggs:dam_subpopZB:TSIcatCold	0.01 (-0.3,0.34)	0.944	-
	traitNeggs:dam_subpopCross:TSIcatCold	-0.29 (-0.54,-0.03)	**0.024**	-
	traitNeggs:dam_subpopZB:TSIcatHot	0.1 (-0.18,0.41)	0.491	-
	traitNeggs:dam_subpopCross:TSIcatHot	0.35 (0.1,0.59)	**0.007**	-

(continued)

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Random effect var.	traitmass:at.level(TSIcat, Cold)	0.013 (0.011,0.015)	-	damid
	traitNeggs:at.level(TSIcat, Cold)	0.238 (0.156,0.351)	-	damid
	traitmass:at.level(TSIcat, Benign)	0.013 (0.011,0.015)	-	damid
	traitNeggs:at.level(TSIcat, Benign)	0.284 (0.227,0.356)	-	damid
	traitmass:at.level(TSIcat, Hot)	0.014 (0.013,0.017)	-	damid
	traitNeggs:at.level(TSIcat, Hot)	0.292 (0.213,0.39)	-	damid
	traitmass	0.007 (0.006,0.008)	-	enclosure
	traitNeggs	0.126 (0.097,0.171)	-	enclosure
	traitmass	0.045 (0.026,0.087)	-	year
	traitNeggs	0.151 (0.078,0.282)	-	year
	traitmass:at.level(TSIcat, Cold)	0.006 (0.006,0.007)	-	units
	traitNeggs:at.level(TSIcat, Cold)	0.832 (0.718,0.966)	-	units
	traitmass:at.level(TSIcat, Benign)	0.003 (0.003,0.003)	-	units
	traitNeggs:at.level(TSIcat, Benign)	0.511 (0.46,0.562)	-	units
	traitmass:at.level(TSIcat, Hot)	0.004 (0.004,0.005)	-	units
	traitNeggs:at.level(TSIcat, Hot)	0.72 (0.643,0.827)	-	units
Correlations	traitNeggs:at.level(TSIcat, Cold):traitmass:at.level(TSIcat, Cold).damid	0.068 (-0.098,0.186)	0.589	-
	traitNeggs:at.level(TSIcat, Benign):traitmass:at.level(TSIcat, Benign).damid	0.105 (-0.015,0.193)	0.089	-
	traitNeggs:at.level(TSIcat, Hot):traitmass:at.level(TSIcat, Hot).damid	0.147 (-0.011,0.251)	0.074	-
	traitNeggs:at.level(TSIcat, Cold):traitmass:at.level(TSIcat, Cold).units	0.186 (0.105,0.257)	**0**	-
	traitNeggs:at.level(TSIcat, Benign):traitmass:at.level(TSIcat, Benign).units	0.172 (0.106,0.226)	**0**	-
	traitNeggs:at.level(TSIcat, Hot):traitmass:at.level(TSIcat, Hot).units	0.117 (0.049,0.189)	**0**	-

For fixed effects the posterior mean is reported

1.5.4 Supplementary Table 21: Random regression model of number of sperm vs sperm viability

Testing for correlations between sperm viability and number of sperm in the effect of thermal stress using a bivariate random slope model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	traitsperm_num5	10.38 (9.62,11.11)	**0.001**	-
	traitsperm_live	-1.93 (-2.61,-1.26)	**0.001**	-
	poly(age, 2)1	2.58 (-13.84,16.75)	0.76	-
	poly(age, 2)2	-6.42 (-20.73,9.61)	0.408	-
	traitsperm_live:poly(age, 2)1	-7.35 (-28.03,12.28)	0.473	-
	traitsperm_live:poly(age, 2)2	8.35 (-12.87,27.52)	0.39	-
	traitsperm_num5:TSI:StressCold	-0.5 (-1.1,0.06)	0.109	-
	traitsperm_live:TSI:StressCold	-0.2 (-0.53,0.19)	0.319	-
	traitsperm_num5:TSI:StressHeat	-0.24 (-0.85,0.41)	0.436	-
	traitsperm_live:TSI:StressHeat	0.16 (-0.22,0.55)	0.406	-
Random effect var.	traitsperm_num5:TSI:at.level(Stress, Heat)	0.522 (0.186,1.541)	-	name
	traitsperm_live:TSI:at.level(Stress, Heat)	0.385 (0.117,0.81)	-	name
	traitsperm_num5:TSI:at.level(Stress, Cold)	0.431 (0.155,0.894)	-	year_name
	traitsperm_live:TSI:at.level(Stress, Cold)	0.213 (0.116,0.446)	-	year_name
	traitsperm_num5:TSI:at.level(Stress, Heat)	0.73 (0.253,1.73)	-	year_name
	traitsperm_live:TSI:at.level(Stress, Heat)	0.267 (0.128,0.442)	-	year_name
	traitsperm_num5:TSI:at.level(Stress, Cold)	0.584 (0.209,1.569)	-	name
	traitsperm_live:TSI:at.level(Stress, Cold)	0.33 (0.155,0.8)	-	name
	traitsperm_num5	0.222 (0.071,1.004)	-	year
	traitsperm_live	0.152 (0.063,0.793)	-	year
	traitsperm_num5	0.691 (0.641,0.751)	-	units
	traitsperm_live	0.037 (0.032,0.041)	-	units
Correlations	traitsperm_live:TSI:at.level(Stress, Heat):traitsperm_num5:TSI:at.level(Stress, Heat).name	-0.071 (-0.633,0.497)	0.766	-
	traitsperm_live:TSI:at.level(Stress, Cold):traitsperm_num5:TSI:at.level(Stress, Cold).name	-0.052 (-0.603,0.526)	0.871	-
	traitsperm_live:traitsperm_num5.units	0.175 (0.095,0.224)	**0.001**	-
	traitsperm_live:TSI:at.level(Stress, Cold):traitsperm_num5:TSI:at.level(Stress, Cold).year_name	-0.04 (-0.425,0.447)	0.952	-
	traitsperm_live:TSI:at.level(Stress, Heat):traitsperm_num5:TSI:at.level(Stress, Heat).year_name	-0.08 (-0.398,0.494)	0.952	-

For fixed effects the posterior mean is reported

1.5.5 Supplementary Table 22: Random regression model of egg laying vs egg mass

Testing for correlations between egg mass and egg laying in the effect of thermal stress using a bivariate random slope model in the R-package MCMCglmm.

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Fixed effects	traitmass	1.47 (1.35,1.59)	**0**	-
	traitNeggs	0.1 (-0.23,0.43)	0.53	-
	dam_subpopSAB	-0.04 (-0.09,0)	0.081	-
	dam_subpopZB	0.03 (-0.03,0.1)	0.32	-
	sire_subpopSAB	0.01 (-0.05,0.06)	0.798	-
	sire_subpopZB	0.02 (-0.04,0.08)	0.611	-
	dam_age_z	0 (-0.01,0.01)	0.587	-
	traitNeggs:sire_subpopSAB	0.24 (-0.04,0.52)	0.092	-
	traitNeggs:sire_subpopZB	0.01 (-0.3,0.34)	0.962	-
	traitNeggs:dam_age_z	0 (-0.05,0.05)	0.888	-
	traitmass:TSIcon:StressCold	-0.11 (-0.16,-0.05)	**0**	-
	traitNeggs:TSIcon:StressCold	-4.18 (-5.04,-3.37)	**0**	-
	traitmass:TSIcon:StressHeat	0.02 (-0.01,0.05)	0.149	-
	traitNeggs:TSIcon:StressHeat	-1.55 (-2.03,-1.13)	**0**	-
	traitmass:dam_subpopSAB:TSIcon:StressCold	0.01 (-0.05,0.07)	0.782	-
	traitNeggs:dam_subpopSAB:TSIcon:StressCold	1.49 (0.6,2.44)	**0.001**	-
	traitmass:dam_subpopZB:TSIcon:StressCold	0.04 (-0.05,0.13)	0.454	-
	traitNeggs:dam_subpopZB:TSIcon:StressCold	0.5 (-0.9,1.77)	0.463	-
	traitmass:dam_subpopSAB:TSIcon:StressHeat	-0.02 (-0.05,0.01)	0.266	-
	traitNeggs:dam_subpopSAB:TSIcon:StressHeat	-0.06 (-0.54,0.46)	0.794	-
	traitmass:dam_subpopZB:TSIcon:StressHeat	-0.06 (-0.11,-0.01)	**0.02**	-
	traitNeggs:dam_subpopZB:TSIcon:StressHeat	-1.1 (-1.82,-0.4)	**0.001**	-
Random effect var.	traitmass:TSIcon:at.level(Stress, Heat)	0.015 (0.014,0.018)	-	damid
	traitNeggs:TSIcon:at.level(Stress, Heat)	1.879 (1.231,2.491)	-	damid
	traitmass:TSIcon:at.level(Stress, Cold)	0.034 (0.027,0.041)	-	damid
	traitNeggs:TSIcon:at.level(Stress, Cold)	0.657 (0.237,2.356)	-	damid
	traitmass:TSIcon:at.level(Stress, Heat)	0.014 (0.013,0.015)	-	year_damid
	traitNeggs:TSIcon:at.level(Stress, Heat)	6.547 (5.83,7.352)	-	year_damid
	traitmass:TSIcon:at.level(Stress, Cold)	0.049 (0.043,0.059)	-	year_damid
	traitNeggs:TSIcon:at.level(Stress, Cold)	14.405 (11.016,18.023)	-	year_damid
	traitmass	0.009 (0.007,0.01)	-	enclosure
	traitNeggs	0.109 (0.073,0.157)	-	enclosure
	traitmass	0.045 (0.024,0.087)	-	year
	traitNeggs	0.12 (0.064,0.259)	-	year
	traitmass	0.002 (0.002,0.002)	-	units
	traitNeggs	0.211 (0.186,0.23)	-	units

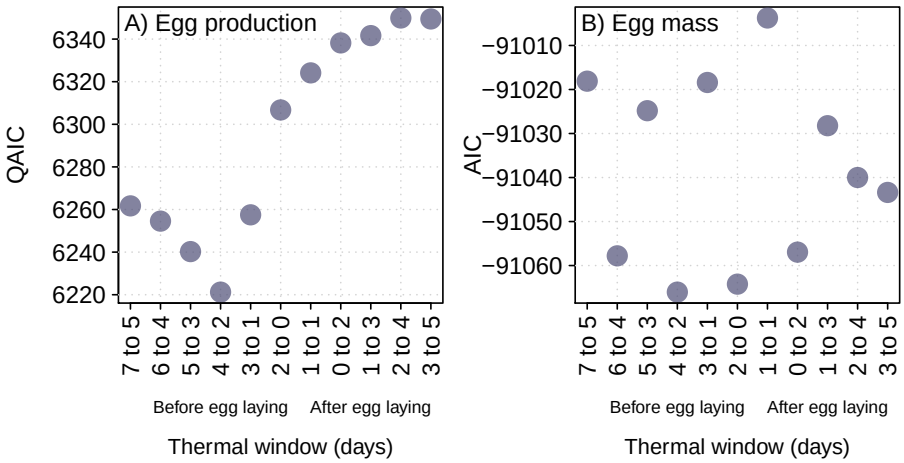
(continued)

Type	Parameter	Posterior mode (CI)	pMCMC	Level
Correlations	traitNeggs:TSIcon:at.level(Stress, Heat):traitmass:TSIcon:at.level(Stress, Heat).damid	0.135 (0.031,0.269)	**0.015**	-
	traitNeggs:TSIcon:at.level(Stress, Cold):traitmass:TSIcon:at.level(Stress, Cold).damid	0.087 (-0.129,0.27)	0.515	-
	traitNeggs:traitmass.units	0.067 (0.025,0.104)	**0.003**	-
	traitNeggs:TSIcon:at.level(Stress, Heat):traitmass:TSIcon:at.level(Stress, Heat).year_damid	0.218 (0.128,0.288)	**0**	-
	traitNeggs:TSIcon:at.level(Stress, Cold):traitmass:TSIcon:at.level(Stress, Cold).year_damid	0.25 (0.105,0.368)	**0.001**	-

For fixed effects the posterior mean is reported

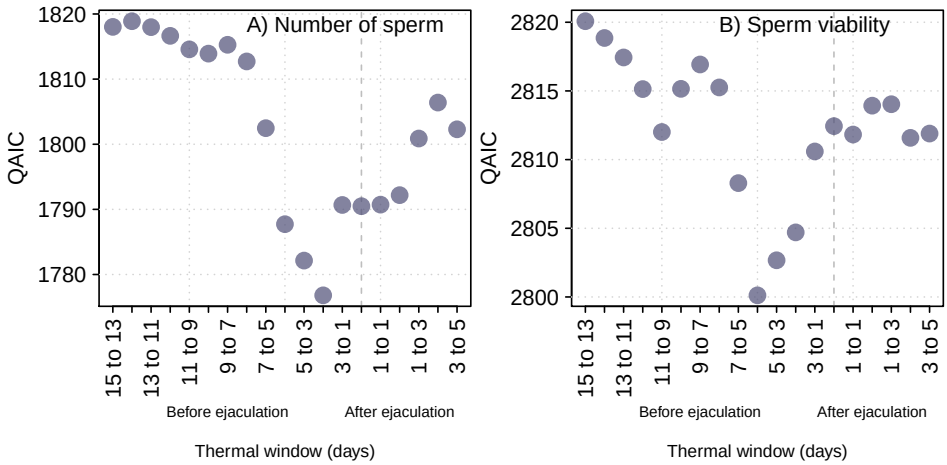
2 Supplementary Figures

2.1 Supplementary Fig. 1: Identification of critical thermal window for egg traits



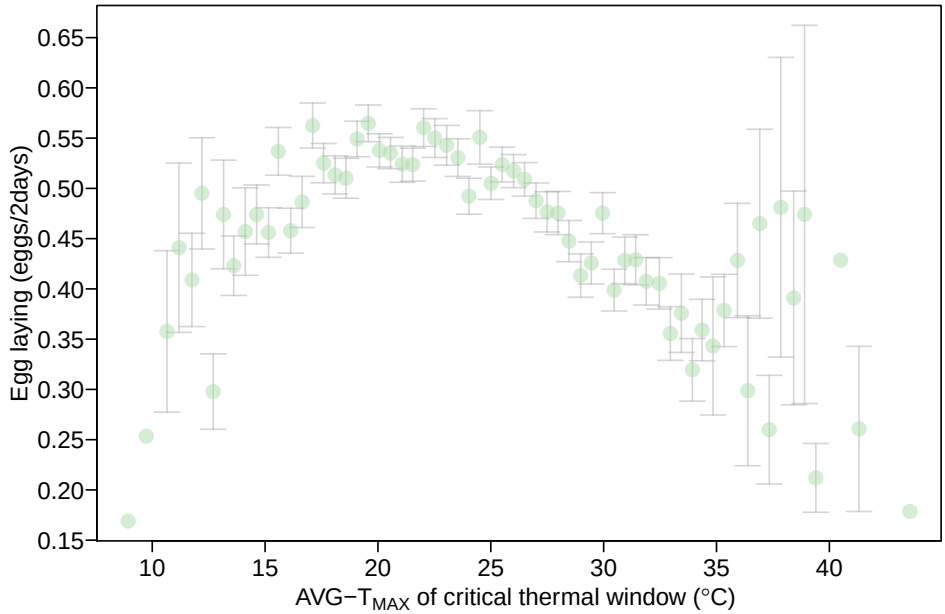
The sensitivity of female and male fertility traits to local temperature fluctuations may not only be a product of the immediate environment, but also time-lag effects. When comparing population-level GLMs using different windows to predict the sensitivity of egg production to fluctuating temperatures, we found improved predictive power of the days preceding egg-laying compared to the days around egg-laying **(A)**. AVG-T_{MAX} of the window spanning 4 to 2 days before egg-laying was the best predictor of egg-laying (i.e. the critical thermal window). This is in accordance with the duration of egg formation in the ostrich being approximately 2 days. For egg mass **(B)** the ranking of AIC was highly sensitive to outliers and we therefore removed eggs laid at the 0.5% hottest and 0.5% coldest days. The critical thermal windows of 2 to 0 days before egg-laying was choosen the best predictor, but several windows show similar performance.

2.2 Supplementary Fig. 2: Identification of critical thermal window for sperm traits



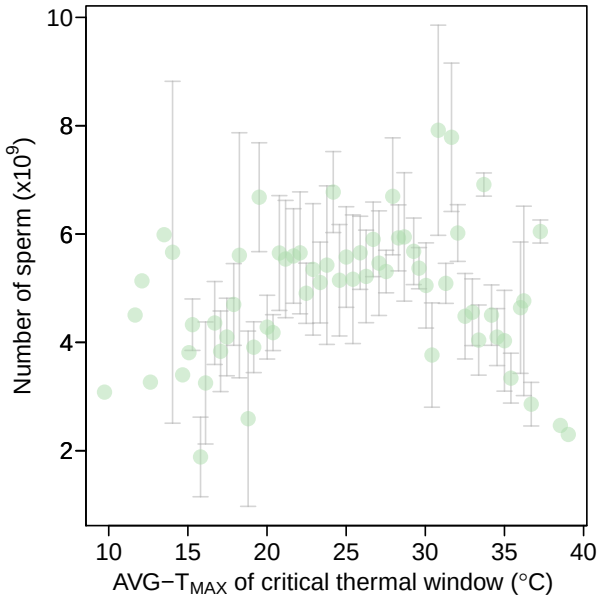
For the number of sperm **(A)** produced by males, we also found improved model fit in the days before ejaculation, with AVG-T_{MAX} of the window spanning 4 to 2 days before delivery shown to be the critical thermal window. Sperm viability **(B)** showed a similar pattern but with the best model fit using AVG-T_{MAX} being the window spanning 6 to 4 days before ejaculation. The vertical lines indicate the time of egg-laying or time of ejaculation.

2.3 Supplementary Fig. 3: The relationship between ambient temperature and egg-laying.



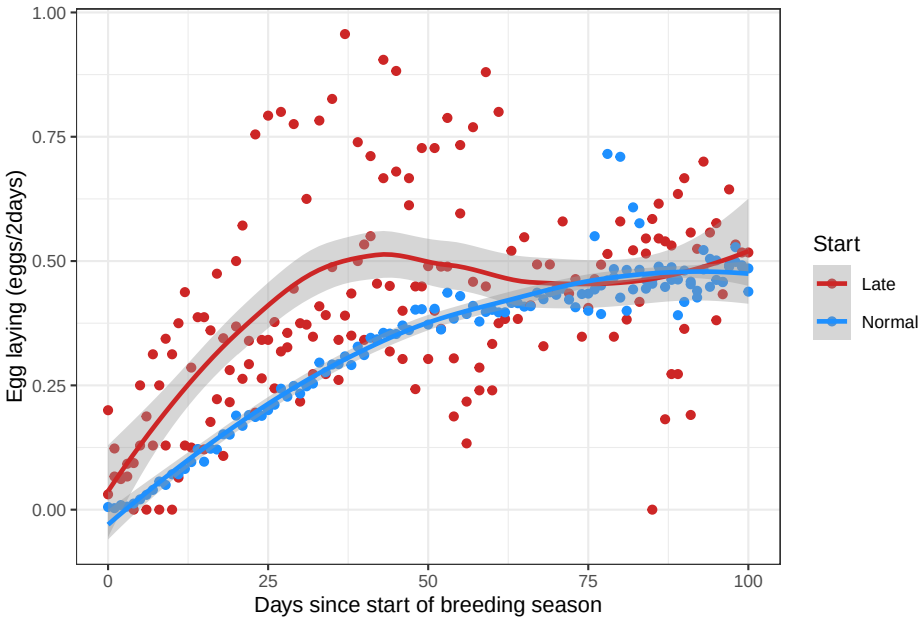
Points show observed probabilities of egg laying (n = 652 females) for the binned temperature variable. Data are presented as mean values +/- SEM.

2.4 Supplementary Fig. 4: The relationship between ambient temperature and number of sperm.



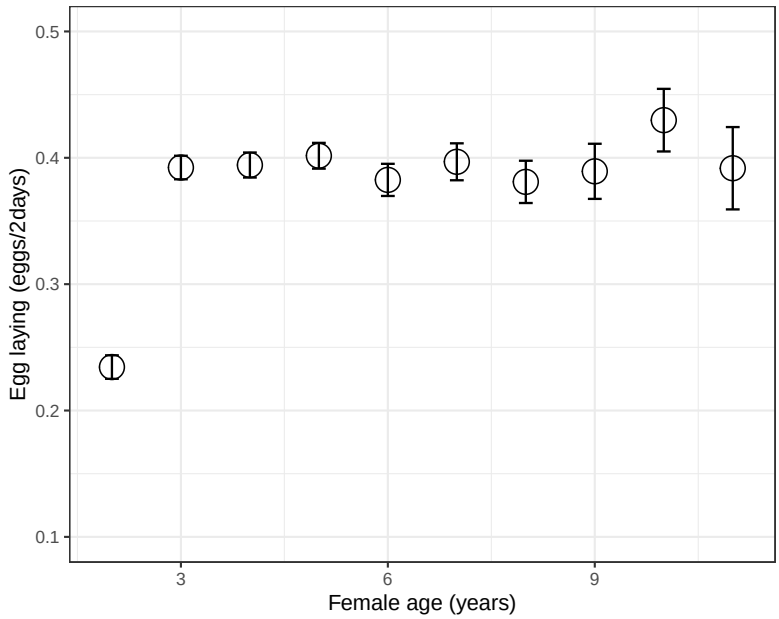
Points show observed number of sperm (n = 22 males) for the binned temperature variable. Data are presented as mean values +/- SEM.

2.5 Supplementary Fig. 5: Acclimation duration in the start of a new breeding season.



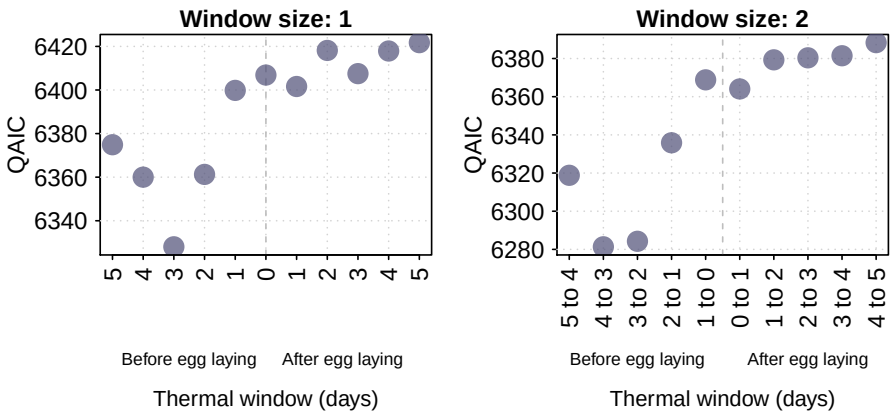
When ostrich pairs are established in the enclosures in May/June (normal start: 746 females) there is a steep increase in rate of egg-laying for the first 75 days. This increase likely reflects two effects, one being the increase in temperature at this time of year and the other being the acclimation to the enclosure and assigned partner. To determine the duration of acclimation to enclosure and partner, we investigated the reaction norm of ostrich pairs that, for various reasons, started their breeding season between July and October (Late start: 93 females) (note that these pairs are not included in the analyses presented in the main document). These late starters also show an increase in laying probability at the start and seem to be fully acclimated around 45 days after they were assigned to an enclosure. Estimated probability and 95% confidence band were obtained from a cubic spline model. Days after 100 days since start of breeding season are not shown.

2.6 Supplementary Fig. 6: Female age influences egg-laying.



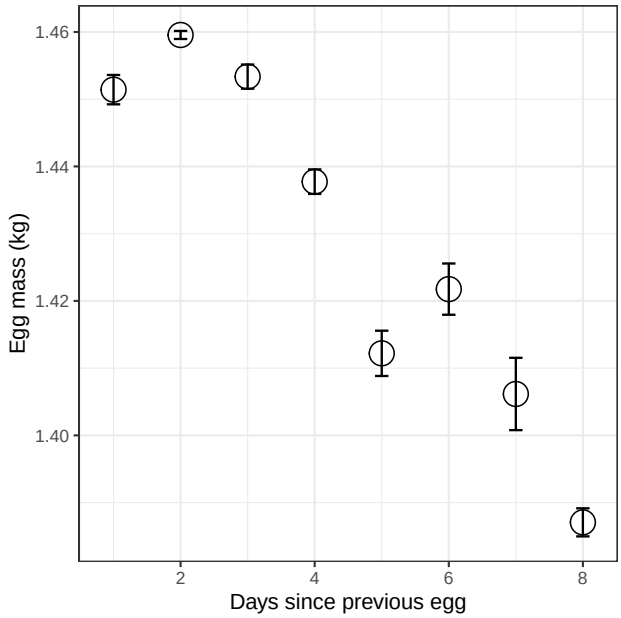
Data are presented as mean values +/- SEM across females (n = 756 females). Only 7 females had an age > 11 and are not shown.

2.7 Supplementary Fig. 7: Inspecting ranking of one-day and two-day thermal windows in egg-laying



The temperature at several days before are important for egg-laying, showing that a window of three days is an acceptable way to capture the most important immediate thermal fluctuations. Best thermal windows (lowest QAIC values) are consistent with the critical three-day thermal window identified in **Supplementary Fig. 2**.

2.8 Supplementary Fig. 8: Change in egg mass with number of days since previous egg.



When more than three days pass since the previous egg was laid, the egg mass of the next egg starts to decline. A decrease of 70 g is substantial, since the average standard deviation of egg mass for an ostrich pair amounts to 73 g. If more than eight days passed since the previous egg, we assigned eight days since previous egg for illustration purposes. Data are presented as mean values +/- SEM across females (n = 652).

Paper IV

Genetic adaptations to cope with heat predict the emergence of cooperative behaviour

Julian Melgar[1,+], Mads F. Schou[1], Maud Bonato[2], Zanell Brand[3], Anel Engelbrecht[3], Schalk Cloete[4,3], Pfunzo T. Muvhali[4], Bengt Hansson[1] & Charlie K. Cornwallis[1,+]

[1] Department of Biology, Lund University, Lund, Sweden [2] Kynsna Elephant Park, Kynsna, South Africa [3] Directorate Animal Sciences, Western Cape Department of Agriculture, Elsenburg, South Africa [4] Department of Animal Sciences, University of Stellenbosch, Matieland, South Africa

+Corresponding authors.

Email: julian.melgar@biol.lu.se, charlie.cornwallis@biol.lu.se

Abstract

Cooperative breeding has enabled animals to inhabit areas where extreme temperatures make reproduction difficult. The predominant explanation for this is that raising offspring under harsh conditions can only be achieved by sharing the burden of parental care. However, in the vast majority of cooperative breeding species some individuals manage to breed independently, even under extreme conditions, challenging our current understanding of cooperative breeding. Here we test the hypothesis that genetic variation in the ability to cope with extreme temperatures determines the need to cooperate over reproduction using a novel study system, the ostrich, *Struthio camelus*. Using experimental manipulations of the number of males and females in cooperative groups, we show that cooperation over incubation lightens the load of parental care under challenging thermal conditions. Furthermore, the genetic ability of individuals to cope with high temperatures predicted cooperative behaviour within groups. However, high heat tolerance only resulted in lower levels of cooperation among males. In females, higher heat tolerance was associated with greater incubation effort, irrespective of social context, resulting in higher, not lower, levels of cooperation. Our results show that genetic variation in the ability to cope with environmental challenges has important, yet unexpected effects, on the emergence of cooperation.

Introduction

Cooperation has been shown to facilitate survival and reproduction in environments where independent living is hard (AlRashidi et al., 2011; Cornwallis et al., 2017; Emlen, 1982; Firman et al., 2020; Jetz & Rubenstein, 2011; Koenig & Dickinson, 2016; Lukas & Clutton-Brock, 2017; Rubenstein & Lovette, 2007; Sun et al., 2014). Many studies have shown that the occurrence of cooperation is greater in habitats with extraordinary environmental conditions (Cornwallis et al., 2017; Davies, 2003; Firman et al., 2020; Jetz & Rubenstein, 2011; Lukas & Clutton-Brock, 2017). Yet, even in challenging environments, individuals vary dramatically in how cooperative they are. For example, some individuals breed independently foregoing cooperation, and those that do cooperate, can vary in how much they contribute to the common cause (Hatchwell, 2009). How can animals of the same species, in the same environment, vary so much in their cooperative behaviour?

One potential answer to this question is that individuals vary in their ability to overcome environmental challenges and hence the need to cooperate. Individuals that are more tolerant to environmental conditions might be capable of reproducing independently under stressful conditions, reducing the benefits of cooperating. In contrast, individuals that are more sensitive to environmental conditions, might need

to be part of a cooperative group to survive and reproduce. Such environmental buffering effects of cooperation have been proposed to explain variation in cooperation across species (Cornwallis et al., 2017; Firman et al., 2020; Jetz & Rubenstein, 2011; Lukas & Clutton-Brock, 2017). However, whether variation in tolerance to environmental conditions also explains levels of cooperation within species remains unclear.

Testing how individual tolerance to environmental conditions influences cooperative behaviour is challenging. First, key environmental factors that influence cooperation have to be identified. Second, individual tolerance to such environmental factors have to be quantified. Third, the behaviour of individuals has to be monitored while manipulating opportunities for cooperation, which is difficult to do successfully in cooperative breeding animals (Cockburn et al., 2008; Dickinson & Hatchwell, 2004; Downing et al., 2020).

One of the key environmental variables influencing animals is temperature (Chown et al., 2004; Parmesan, 2006; Sunday et al., 2012), and in birds it is the predominant driver of cooperative breeding (Cornwallis et al., 2017; Jetz & Rubenstein, 2011). With this as a backdrop, we examine how tolerance to temperature influences the probability that individuals engage in cooperation, and how individuals that do cooperate vary in their investment. To overcome the difficulties in testing how individual tolerance to temperature influences cooperation, we have developed a unique study system using ostriches, *Struthio camelus*. Ostriches breed both independently and in groups where multiple females and multiple males cooperate over the incubation of eggs, which represents a substantial proportion of the total parental care provided. Ostriches also inhabit some of the most extreme thermal environments: during the study period temperatures ranged from 5 to 45°C, just at the field site. Such fluctuations significantly reduce the reproductive success of males and females, but individuals do vary markedly in how sensitive they are to changes in temperature (Schou et al., 2021).

To quantify individual tolerance to temperature change, we used an eight-generation pedigree and 21 years of data on the reproductive success of breeding pairs exposed to natural fluctuations in temperature (756 females and 701 males). Using these data, genetic breeding values of heat tolerance were estimated as the ability of individuals to maintain reproduction when hot and without opportunity for cooperation. We focused on tolerance to high temperatures because incubation limits behavioural thermoregulation in ostriches, making it difficult for individuals to reduce their body temperatures under hot conditions, whereas elevating their body temperatures when it is cold appears to be less challenging during incubation (Svensson et al., unpublished data; Fuller et al., 2003; Maloney, 2008). Using these data, genetic breeding values of heat tolerance were estimated as the ability of individuals to maintain reproduction when hot and without opportunity for cooperation. Maintenance of reproduction was measured as the change in egg laying rate when temperatures increased from the optimum temperature for reproduction (20°C, see

Schou et al., 2021 for details). In males, individual heat tolerance reflects genetic correlation to a female trait, but ultimately we assume this measure to reflect inherent physiological heat tolerance. Individual heat tolerance was estimated for the whole population, of which 162 females and 143 males were used in the experiments presented here. In these experiments, the opportunities for cooperation over incubation were manipulated by varying the number of males and females in groups. Groups were also established so that the frequency of heat tolerant individuals varied across groups to test if cooperation was more likely to emerge when all individuals in a group were sensitive to high temperatures. The incubation behaviour of individuals in experimental groups was monitored and related to hourly temperature measurements recorded by an onsite weather station.

Results

Extreme temperatures increase the need for incubation eliciting a division of labour between males and females

It is widely recognised that for embryos to successfully develop in birds eggs need to be protected against low temperatures (Berntsen & Bech, 2016; DuRant et al., 2013; Hepp & Kennamer, 2012; Nord & Nilsson, 2016; Olson et al., 2008). Our results show that ostrich eggs also require protection from the heat, as they frequently experience temperatures that are lethal to embryos (Figure 1. See also Bertram, 1992; Deeming & Ayres, 1993). Eggs incubated by adults were protected from the heat (Figure 1B), being kept at temperatures close to the optimum for embryo development (Deeming & Ayres, 1993). This required adults to increase the protection of eggs at extreme temperatures (Figure2. Temperature^2: posterior mode (PM) and credible interval (CI) = 0.88 (0.65 , 1.09), pMCMC = 0.001. Table S1), which was facilitated by a division of labour between males and females (Figure 2C & 2D). During cold periods, males performed the majority of incubation, whereas females incubated more at high temperatures (Figure 2. Male investment: Temperature PM (CI) = -1.11 (-1.46 , -0.81), pMCMC = 0.001. Female investment: Temperature PM (CI) = 1.45 (1.09 , 1.61), pMCMC = 0.001. Temperature^2 PM (CI) = -0.78 (-1.02 , -0.53), pMCMC = 0.001.Table S2).

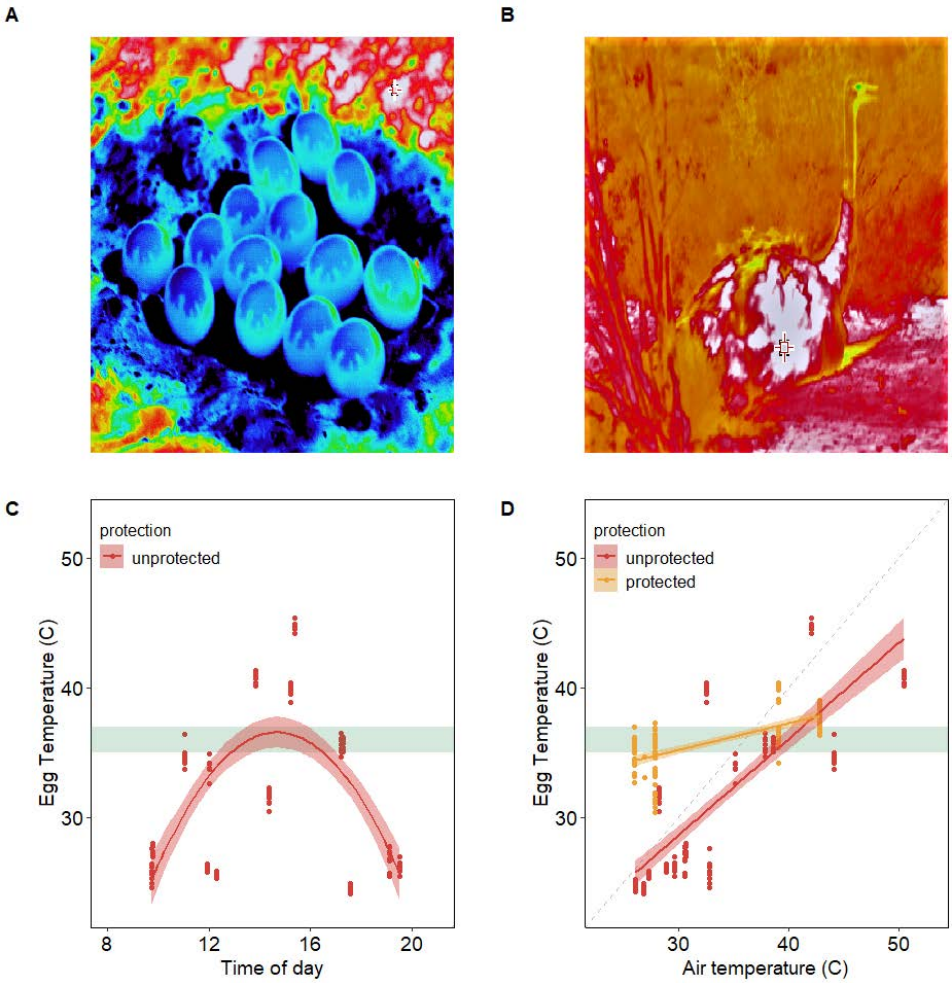


Figure 1: Temperature fluctuations and the need for incubation. (A) Thermal image of a nest with 14 eggs. A female was incubating the nest just seconds before the picture was taken. Note how eggs are maintained much cooler than the surrounding ground. (B) Thermal image of a panting incubating female. (C) Hot as well as cold temperatures increase the need for incubation. (D) Eggs that were incubated maintained temperatures that were close to those optimal for embryo development (grey area). Brighter colours in A and B indicate higher temperatures. Regression lines with 95% confidence intervals are presented (C = quadratic fit, D = linear fit).

Social group complexity increases the amount of time eggs are incubated, but reduces individual work loads

Groups with more males and females were able to protect their eggs for a greater amount of time than smaller groups (Figure2. N_{males}: PM (CI) = 0.39 (0.04 , 0.9), pMCMC = 0.04. $N_{females}$: PM (CI) = 0.8 (0.43 , 1.3), pMCMC = 0.001. Table S1). However, the amount of time individual males and females invested in incubation decreased with the number of individuals in groups at all temperatures (Figure 2.

Male investment: N_{males} PM (CI) = -1.06 (-1.65 , -0.58), pMCMC = 0.001. Female investment: $N_{females}$ PM (CI) = -1.62 (-1.95 , -1.06), pMCMC = 0.001. Table S2). Such reductions in incubation effort are likely to be important, at least for females, as there was evidence that long periods of incubation at higher temperatures were difficult: The rate at which females ended incubation was significantly higher under hotter temperatures (Figure S2. Temperature PM (CI) = 0.68 (0.48 , 0.85), pMCMC = 0.001. Table S3). These results are consistent with previous findings (see paper 1), further showing that the costs of parental care are reduced in larger groups across highly variable temperature conditions.

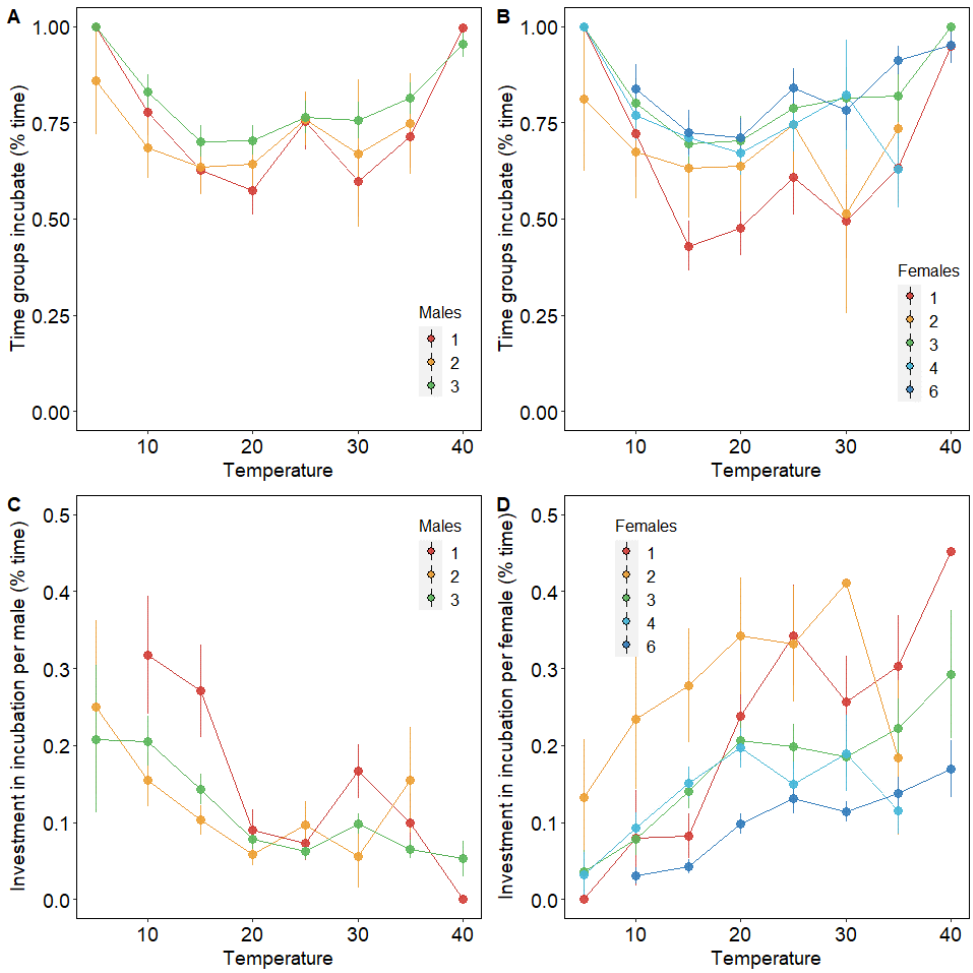


Figure 2: Social group complexity and the incubation of eggs at extreme temperatures. Groups with more males (A) and more females (B) increase the time that their nest is incubated compared to smaller groups. However, this was not because males (C) or females (D) in the larger groups invested more in incubation. Means ± SE are plotted.

Genetic heat tolerance explains variation in incubation behaviour

Individual contributions to cooperative incubation were highly variable, even after taking into account the effects of the number of males and females in groups. Both the probability that individuals contributed to incubation, as well as their incubation effort, varied markedly within groups (proportion incubating: males = 0.3 to 1, females = 0.2 to 1. Proportion of time invested in incubation: males = 0 to 0.61, females = 0.01 to 0.76). We examined whether such variation was predicted by the genetic heat tolerance of individuals when experiencing relatively benign (<25°C) and hot temperatures (>=25°C: see Methods for justification of cut-off).

Females predicted to be more heat tolerant were much more likely to engage in incubation than less heat tolerant females, both at benign and hot temperatures (Figure 3A & S3. Benign: PM (CI) = 0.28 (0.12 , 0.55), pMCMC = 0.001. Hot: PM (CI) = 0.22 (-0.03 , 0.44), pMCMC = 0.05. Table S4. See also figure S2.). Heat tolerant females also invested more time in incubation than less heat tolerant females (Figure 3C. Benign: PM (CI) = 0.89 (0.29 , 1.51), pMCMC = 0.004. Hot: PM (CI) = 0.63 (0.02 , 1.32), pMCMC = 0.05. Table S5). This was most pronounced in larger groups where females with low heat tolerance reduced their incubation effort, whereas heat tolerant females largely maintained their incubation effort, even when it was hot (Figure 3C. Tables S5). Furthermore, females with higher heat tolerance were less likely to end incubation when it was hot compared to females with lower heat tolerance, which was not the case under benign temperatures (Benign: PM (CI) = -0.08 (-0.24 , 0.12), pMCMC = 0.64. Hot: PM (CI) = -0.18 (-0.36 , 0.02), pMCMC = 0.048. Figure S3. Table S6).

In contrast to females, male genetic heat tolerance did not influence the probability that they incubated or the amount of time they invested in incubation, at either high or benign temperatures (Figures 3B & 3D. Tables S4 & S5). In fact, the only evidence that male heat tolerance influenced incubation behaviour was a significant increase in the rate that they ended incubation under hot conditions as the number of males in their group increased (Hot: PM (CI) = 0.46 (0.08 , 0.83), pMCMC = 0.008. Table S6). Such an effect was not apparent at benign temperatures, suggesting that, if anything, more heat tolerant males were less inclined to incubate in groups under hotter conditions rather than the opposite (Benign: PM (CI) = 0.04 (-0.21 , 0.26), pMCMC = 0.92. Figure S3. Table S6).

Female heat tolerance enhances cooperation whereas male heat tolerance reduces it

We tested whether differences in the incubation behaviour of low and high heat tolerant individuals influenced the emergence of cooperation at the group level. This was done by examining if the frequency of heat tolerant females and males (genetic breeding value > median) in groups predicted the number of individuals that

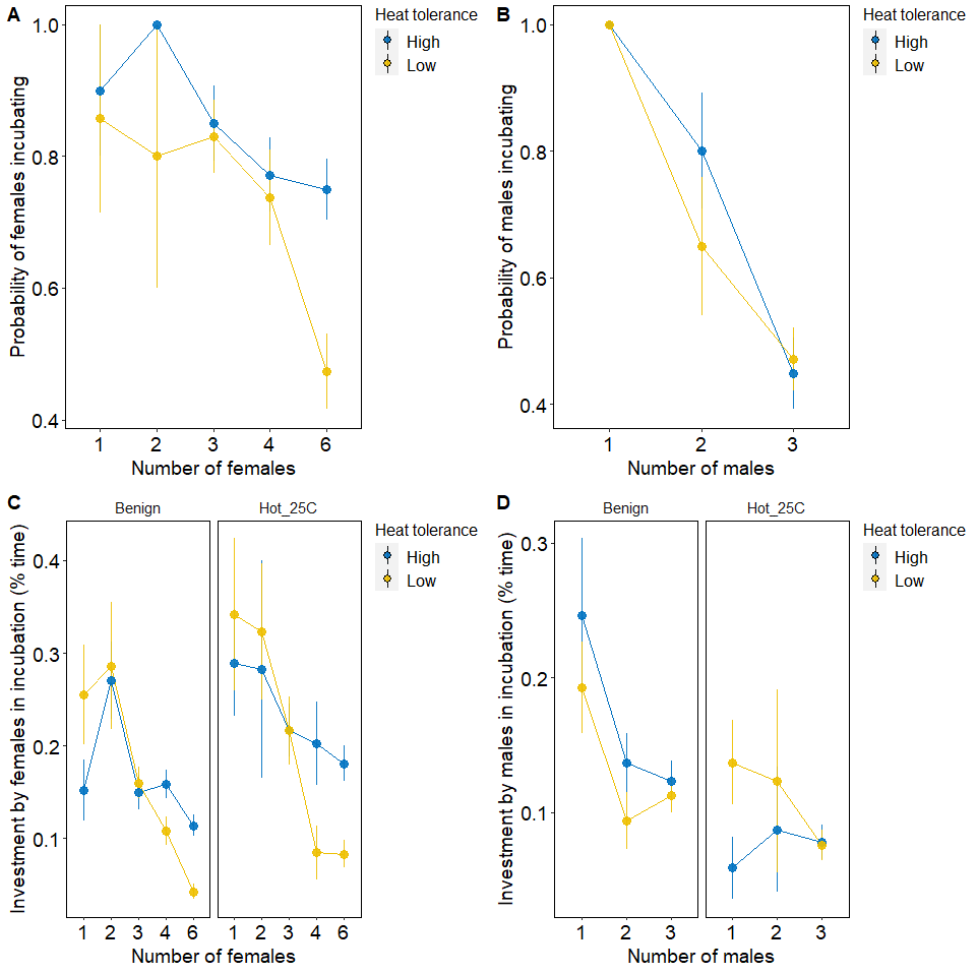


Figure 3: Genetic heat tolerance influences incubation behaviour. The probability that females (A) and males (B) with high (>median) and low (<=median) heat tolerance contributed to incubation. The proportion of time females (C) and males (D) with high and low heat tolerance invested in incubation under benign (<25°C) and hot temperatures (>25°C). Heat tolerance was categorised for illustration purposes only, models analyse continuous values. Means ± SE are plotted.

cooperated over incubation. We found that, as the opportunities for cooperation (number of females in groups) increased, groups with the highest frequency of heat tolerant females had the most cooperators (Figure 4A). This effect was only apparent under hot conditions, suggesting that higher frequencies of heat tolerant females results in greater cooperation over incubation during periods of heat stress (Benign: PM (CI) = 0.39 (-0.16 , 0.72), pMCMC = 0.22. Hot: PM (CI) = 0.62 (0.05 , 0.99), pMCMC = 0.036. Table S7). This was not because more heat tolerant females had a greater propensity to cooperate *per se*: Out of the females that contributed to incubation, heat tolerant females were no more or less likely to share

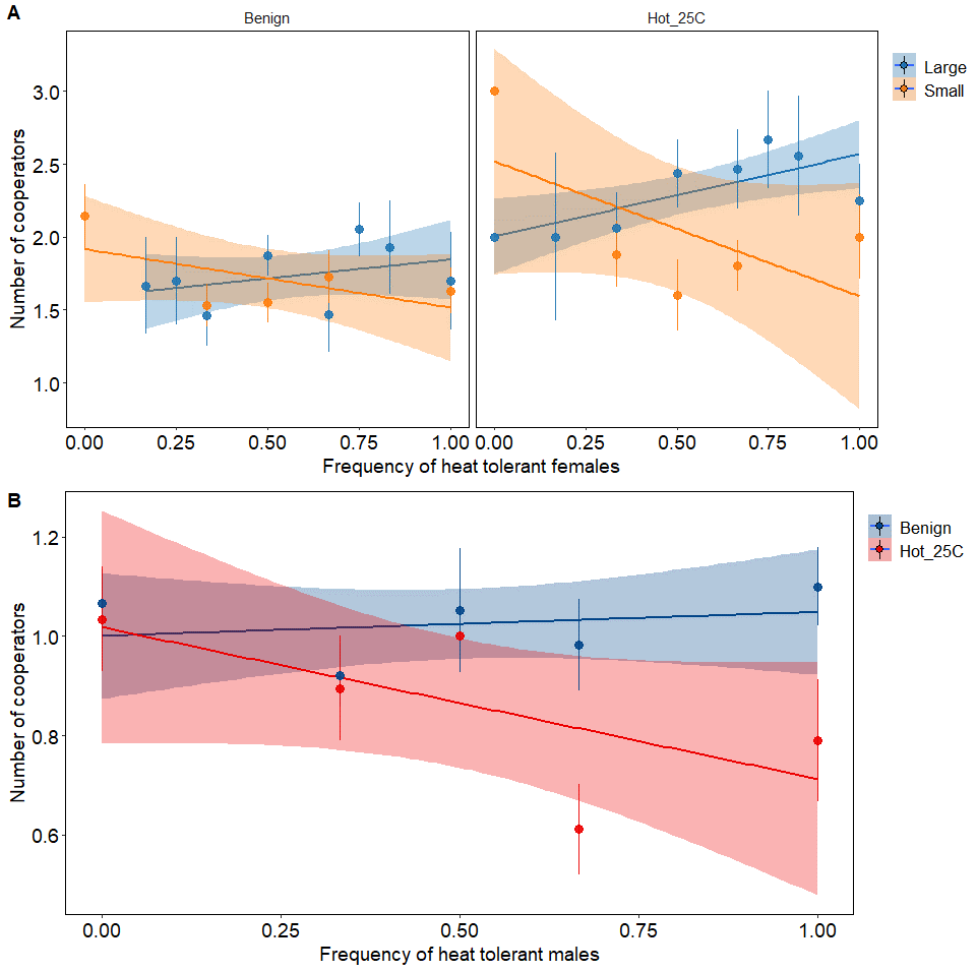


Figure 4: The emergence of cooperation in groups with different frequencies of heat tolerant individuals. (A) The number of females that cooperated over incubation in relation to the frequency of heat tolerant females in small (<=3 females) and large groups (>3 females) under benign (<25°C) and hot temperature (>25°C) conditions. Female group size was categorised for graphical purposes only. (B) The number of males that cooperated over incubation in relation to the frequency of heat tolerant males under benign (<25°C) and hot temperature (>25°C) conditions. Regression lines with 95% confidence intervals are plotted. Points plotted are means ± SE.

incubation during benign or hot periods than less heat tolerant females (Benign: PM (CI) = 0 (-0.05 , 0.06), pMCMC = 0.824. Hot: PM (CI) = -0.03 (-0.08 , 0.05), pMCMC = 0.638. Figure S4. Table S8). Instead, higher levels of cooperation in groups appeared to emerge in groups with more heat tolerant females due to their inherent higher probability of engaging in incubation (Figures 3A & S3. Table S4.). These results are contrary to the idea that increased heat tolerance negates the need for cooperation, and show that higher heat tolerance in females may in fact drive cooperation.

The effect of heat tolerance in males was the opposite to that of females. As the frequency of high heat tolerant males increased, the number of males that cooperated over incubation decreased (Figure 4B. Benign: PM (CI) = -0.14 (-0.33 , 0.07), pMCMC = 0.3. Hot: PM (CI) = -0.31 (-0.58 , -0.04), pMCMC = 0.03. Figure S4. Table S9). Reductions in the number of cooperating males with increasing frequencies of heat tolerant males were particularly evident during hot periods (Figure 4B). While in females heat tolerance was associated with more individuals cooperating over incubation, the general trend in males appears to be that higher heat tolerance reduced cooperation (Figures S3B & S4E).

Discussion

Why individuals of the same species vary in how cooperative they are, even under the same environmental conditions, has been challenging to explain. Here we show that individual heat tolerance influences variation in cooperation, although not in the way initially expected. In female ostriches, high tolerance leads to an increase in cooperation over incubation, rather than the predicted decrease (Figure 3A, 3C an 4A). Moreover, females with low heat tolerance were able to make substantial investments in incubation when alone or in small groups (Figures 3A and 3C). These findings are inconsistent with the idea that individuals that are less tolerant to environmental stress are more dependent on cooperation to successfully reproduce. However, in male ostriches, heat tolerance was associated with a reduction in cooperative behaviour under hot conditions (figure 4B), but our results did not support that this was because they were more capable of incubating when temperatures were high. In fact, heat tolerant males were neither more likely to incubate, nor invested more in incubation, than heat sensitive males (Figures 3B & 3D). Together, our findings suggest that the inherent properties of individuals within groups, in our case their degree of heat tolerance, is an important factor in predicting variation in the size of cooperative groups: both heat tolerant and heat sensitive males and females can breed on their own, explaining the occurrence of small groups, but females that are vulnerable to heat stress appear to benefit more from being in larger groups.

Only females with low heat tolerance significantly decreased their incubation effort when opportunities for cooperation increased (Figures 3C and S3). This suggests that only females that are vulnerable to heat stress capitalize on the rewards of cooperation over incubation. Why do females with high heat tolerance not reap the benefits of cooperation? One potential answer is that heat tolerance reduces the cost of incubation (Amat & Masero, 2004; Grant, 1982; Vincze et al., 2013; Ward, 1990), and increases the number of eggs in nests (Schou et al., 2021). Therefore, failure to incubate may come at a higher cost for heat tolerant females, selecting for greater investment in incubation. Females with low heat tolerance, on the other

hand, reduced their investment in incubation when groups became larger (Figure 3C). This suggests that cooperation buffers the costs of incubation for heat sensitive females. The presence of heat tolerant individuals is thus likely to allow individuals with low heat tolerance to breed under hot conditions while decreasing their reproductive investment. One potential implication of this result is that variation in heat sensitivity may mediate the maintenance of cooperative behaviour and vice versa.

In contrast to females, male heat tolerance had a negative effect on cooperation (Figure 4B). In this context, it is important to point out that our estimates of heat tolerance were based on egg laying rates in relation to increasing temperatures. Egg laying is a trait tightly linked to female, not male, reproductive physiology. Our initial assumption was that genetic variation underlying heat tolerance would be captured by egg laying rates, and not be sex or trait specific. While our estimates of heat tolerance do not appear to be trait specific, given they predicted incubation behaviour, whether it reflects heat tolerance in males is unclear. Genetic conflict between sexes can lead to opposing correlations between a given trait and fitness in males and females (Arnqvist, 2004; Chippindale et al., 2001; Fedorka & Mousseau, 2004; Foerster et al., 2007; Gibson et al., 2002; Meagher, 1992; Rice, 1984, 1992; Rice & Chippindale, 2001). It is therefore possible that, while our breeding value estimates correspond to increased heat tolerance in females, they reflect decreased tolerance in males as seen by the elevated rate of ending incubation at high temperatures (Table S6). Alternatively, our estimates may capture the heat tolerance of males, but given they specialize in incubation at lower temperatures (Figure 1C) (Bertram, 1992; Kennou Sebei & Bergaoui, 2009; Sauer & Sauer, 1966; Siegfried, 1974), higher heat tolerance may reduce incubation effort.

The benefits of cooperation are predicted to increase when a division of labour is possible, which is more likely to occur if different individuals in a group are specialized in different tasks (Cooper & West, 2018). This is generally thought to be unlikely in groups of unrelated individuals because, when group relatedness is low individuals are expected to pursue reproduction (direct fitness) rather than invest in other activities (Queller, 2000; Queller & Strassmann, 1998; Szathmáry & Smith, 1995; Wilson, 2000). However, where individuals in groups gain mutual direct fitness benefits from specializing in specific tasks then a division of labour may still be possible. Our results raise the possibility that high heat tolerance in females may be complemented by high *cold* tolerance in males, allowing a division of labour over incubating under different temperature conditions. While previous studies of ostriches have showed males and females incubate at different times (Bertram, 1992; Kennou Sebei & Bergaoui, 2009; Sauer & Sauer, 1966; Siegfried, 1974), our results indicate that this difference may in fact be a response to the climatic condition (Figure S1). Interestingly the same climatic conditions believed to influence group size. The fact that male and female ostriches seem to have different temperature optima for incubation may also help explain variation in group

size: groups with many females but few males may have high hatching success under hot conditions, but this may be compromised if hot days are followed by cold nights. Fluctuating temperatures may therefore select for groups with more equal sex ratios and offset the costs of sexual competition for males (see paper 1).

Although our results suggest that division of labour in ostriches is associated with intersexual differences in heat tolerance, our experiments were not designed to disentangle the effects of temperature from those of time of day. However, when we restricted our data to observations around noon, we found that temperature still had a negative effect on male incubation effort (Figure S1). This gives further support to the idea that division of labour in ostriches is driven by temperature effects rather than other factors associated with time of day, such as predator avoidance, as previuosly suggested (Sauer & Sauer, 1966). Moreover, the fact that male and female ostriches seem to have different temperature optima for incubation can help explain variation in group size: groups with many females but few males may have high hatching success under hot conditions, but this may be compromised if hot days are followed by cold nights. Fluctuating temperatures may therefore select for groups with more equal sex ratios and offset the costs of sexual competition for males (see paper 1).

Together our results show that individual variation in tolerance to environmental conditions has important implications for cooperative behaviour, and vice versa. It is possible that genetic diversity in heat tolerance is maintained by the benefits that cooperation confers to individuals with low heat tolerance. The benefits of cooperation are in turn likely to be influenced by differences in heat sensitivity, both within and between the sexes.

Methods

Study population

The research was conducted on a captive population of ostriches kept in fenced areas (range: 2400 and 70600 m2, median = 4700 m2) of Karoo habitat at Oudtshoorn Research Farm, South Africa (33° 38’ 21.5“S, 22° 15’ 17.4”E). The experiments involved 118 breeding groups monitored from 2012 to 2018 involving 147 males and 170 females. All individuals were individually identifiable by coloured neck tags.

Egg temperatures in protected and unprotected nests

The effect of temperature on ostrich eggs was examined across five days in early November 2014.

To examine the effects of temperature on unattended ostrich eggs, we surveyed how the temperature of ten fresh, unfertilized eggs oscillated with natural temperature variation when left unprotected on bare soil. Measurements of the temperature of the surface of each egg, as well as ambient temperature were taken with an infrared camera (InfReC R500). These measurements were taken in 22 different occasions across five days at different times of the day and night, covering ambient temperatures from 19.4 to 50.5˚C.

The thermal camera was also used to examine the thermal conditions of eggs that were naturally incubated. 124 surface temperature measurements were taken on at least 63 different newly incubated eggs in nine nests in the experimental groups. These measurements were taken in two consecutive days at ambient temperatures ranging from 26 to 42.8˚C.

The thermal images were analyzed using specialized software (InfReC analyzer NS9500 Lite).

Experimental design

We manipulated the complexity of ostrich groups experimentally across a seven-year period (16-18 groups per year). The number of males in groups ranged from 1 to 3 and the number of females ranged from 1 to 6. Due to limitations in the number of birds accessible for our experiments, and other experiments being conducted on the same population, not all combinations of male and female group sizes were possible.

The final sample size of our study population was reduced by a number of reasons: individual ostriches were removed from the study due to casualties, injuries and aggressive behaviour. Entire groups were excluded when the removal of any of their members was likely to cause a disruption in their breeding behaviour. Groups were also excluded from the study if no incubation or copulation behaviour was observed. This resulted in a final sample size of 143 males and 162 females, many of which were included in the study in more than one year, across 107 groups.

The breeding season was typically from May to December. During the first ~5 months of the season, eggs were collected twice a day and incubated artificially. During the last ~2 months, when the data for this study was collected, eggs were left in nests and incubation behaviour was monitored. During the last ~2 months of the breeding season, the incubation behaviour of individuals in the breeding groups was monitored by conducting ~3 hour observations at least three times a week using binoculars (10 x 40) and a telescope (12-36 x 50). The observer sat camouflaged in

a 10-meter-tall observation tower in the middle of the field site. Each group was observed for between 47 and 91 hours. The identity of each incubating individual, as well as the incubation's start and end time, was recorded. Nests were checked daily and new eggs were marked with the date and an egg identification number.

During the whole breeding season, ostriches received a balanced ostrich breeder diet (90 to 120 g protein, 7.5 to 10.5 MJ metabolizable energy, 26 g calcium and 6 g phosphorus per kg feed) and ad-libitum water.

Temperature

Temperature measurements were retrieved from an onsite weather station during the whole observation period, except for 11/12/2014, when hourly temperature data were not available from this weather station and had to be retrieved from a different weather station, situated 53 km from the study site (33°32'05.4"S, 22°49'02.2"E). Temperature measures were taken every hour on the hour (XX:00). For the analyses, we divided temperatures into two categories: "Heat stress" for temperatures $>$ 25°C and "No heat stress" for temperatures $\leq$ 25°C. These categories were based on Schou et al 2021, and Maloney 2008.

Estimating heat tolerance using long-term data of reproductive success in pairs

In the majority of the enclosures on Oudtshoorn Experimental Farm (n = 197) ostriches are restricted to breeding in pairs. We used the daily records of egg production of the ostriches in these enclosures between 1998 and 2018 as a measure of reproductive success at increasingly hot temperatures. Data was filtered following (Schou et al., 2021), but without removing pairs with low reproductive output, resulting in data from 678 females. The average maximum daily temperature 2 to 4 days before egg laying is the best predictor of egg-laying rate, and shows a quadratic relationship with egg-laying with the highest egg-laying rate at 20ºC (Schou et al., 2021). Using an available pedigree for the pair breeders, we constructed a random regression animal model of the individual change in egg-laying rate with increasing or decreasing temperatures from the optimum. The animal model was run in R v.3.6.0 (Team, 2020) using the Bayesian framework implemented in the R-package MCMCglmm v.2.29 (Hadfield, 2010).

The model setup was similar to the primary model of egg-laying in (Schou et al., 2021). Briefly, egg-laying is a binomial trait and was modelled as a multinomial2 trait. We used the weakly informative inverse-Gamma distribution (scale = 0.001, shape = 0.001, i.e. V = 1, nu = 0.002) as priors for the variance components, which included unstructured variance-covariance matrices for individual id (permanent environment variance) and an identical individual id linked to the pedigree (genetic

variance). With each of these matrices the model estimated random intercept and random slope variance with increasing or decreasing temperatures as well as their covariances. The model performed 8100000 iterations of which the initial 100000 were discarded and only one in 8000 runs was used for estimating posterior probabilities. When estimating the breeding values of the individuals' rate of egg-laying at increasingly hot temperatures we also accounted for the impact of the subpopulation origin (>85% Southern Africa Black or <85% Southern Africa Black) of each individual used in the group manipulation. The breeding value was estimated as the mode of a composite posterior consisting of the individual random slope posterior and the posterior of the fixed effect slope of subpopulation. The extracted breeding values are on the logit scale. Throughout the text and in figures were refer to breeding values as 'heat tolerance'.

Incubation behaviour

To be able to match behavioural observations to the hourly temperature measurements, observation bouts were split into hour breaks. Each hour break started 31 minutes past a given hour, and ended 30 minutes past the next hour. In this way, the temperature measurement, taken on the hour, was taken in the middle of each hour break.

Probability of incubation

Individuals that were observed incubating in at least one occasion were given an overall probability of incubation of 1 and were considered "cooperators". In contrast, individuals that were never seen incubating were given an overall probability of incubation of 0. The probability of incubation in a given temperature category was computed for every individual in the same way as the overall probability of incubation.

Incubation effort

To compute incubation effort, the data was subset to only include hour breaks when a given individual was observed incubating. Incubation effort was then defined as the daily proportion of the total observation time that an individual was observed incubating.

Probability of ending incubation bout

To compute the rate of ending incubation, the data was subset to only include hour breaks when a given individual was observed incubating. The probability of ending incubation was then defined by the daily proportion of observation hours, in a given temperature category, in which an individual was observed incubating, and in which it also ended at least one incubation bout.

Quantification and Statistical Analyses

General approach

Data were analysed in R using Bayesian Linear Mixed Models (BLMM) with Markov chain Monte Carlo (MCMC) estimation in the package MCMCglmm. The non-independence of data arising from multiple data points per group, per enclosure, per year and per individual was modelled using random effects. Default fixed effect priors were used (independent normal priors with zero mean and large variance (10^{10})) and for random effects inverse gamma priors were used. Apart from in binary threshold models. For binary models fixed effect priors were specified as mu = 0, V = 1 + π2 / 3 (relatively flat on the logit scale), residual variance is not identifiable and was fixed at 1, and for random effects parameter expanded priors were used (V = 1, nu = 0.002, alpha.mu = 0, alpha.V = 1000) (Hadfield, 2021). Each analysis was run for 1100000 iterations with a burn-in of 100000 and a thinning interval of 1000. Convergence was checked by running models three times and examining the overlap of traces, levels of autocorrelation, and testing with Gelman and Rubin's convergence diagnostic (potential scale reduction factors < 1.1).

Fixed effects were considered significant when 95% credible intervals (CIs) did not overlap with 0 and pMCMC were less than 0.05 (pMCMC = proportion of iterations above or below a test value correcting for the finite sample size of posterior samples). To estimate the magnitude of random effects we calculated the percentage of variation explained by each random term (I2%) after accounting for variation attributable to fixed effects. By default MCMCglmm reports parameter estimates for fixed factors as differences from the global intercept. This does not allow absolute estimates and 95% CIs for all factor levels to be estimated or custom hypothesis tests of differences between factor levels. Consequently, we removed the global intercept from all models and present absolute estimates for factor levels. Differences between factor levels were estimated by subtracting the posterior samples from one level from the second level and calculating the posterior mode, 95% CI and pMCMC. Parameter estimates for fixed effects are reported from models that included all terms of the same order and lower. For example, all main effect estimates are from models where all other main effects are included, all estimates of two-way interactions are from models that included all two-way interactions and main effects, and so forth. Random effect estimates are from models that included the highest order fixed effect terms. All continuous explanatory variables were z transformed using the scale() function in R. Curvilinear effects of continuous explanatory variables were modelled using the quadratics of the z transformed values computed before running the models. As we were interested in the effects of the number of individuals of the same sex on incubation behaviour we restricted the effect of the number of males to male investment in incubation, and the effect of number of females to female investment in incubation using the 'at.level' term in MCMCglmm (e.g. at.level(sex,"M") : number_males).

Specific analyses

1. Testing how the numbers of males and females in groups influenced the time eggs were incubated at different temperatures.

The effect that temperature (fixed effect: continuous variable of 5°C temperature windows ranging from 5 to 45°C), the number of males (fixed effect: continuous variable) and the number of females (fixed effect: continuous variable) had on the proportion of time eggs were protected was modelled using a BLMM with a binomial (multinomial2) error distribution. The response variable was the total amount of time a nest was protected versus the total time it was not protected summarized for 5°C temperature windows. Year, enclosure and group were included as random effects (see R code: M1 for details).

2. Testing how individual investment in incubation changed with the numbers of males and females in groups at different temperatures.

The effect that temperature (fixed effect: continuous variable of 5°C temperature windows), sex (fixed effect: two level factor), number of males (fixed effect: continuous variable) and number of females (fixed effect: continuous variable) had on individual investment in incubation was modelled using a BLMM with a binomial (multinomial2) error distribution. The response variable was the total time an individual was observed incubating versus the time it was not incubating. Year, enclosure, group and individual were included as random effects (see R code: M2 for details).

3. Testing how the rate at which individuals ended incubation was influenced by the numbers of males and females in groups at different temperatures.

The effect that temperature (fixed effect: continuous variable of 5°C temperature windows), sex (fixed effect: two level factor), number of males (fixed effect: continuous variable) and number of females (fixed effect: continuous variable) had on rates of ending an incubation was modelled using a BLMM with a poisson error distribution. The response variable was the number of times an individual stopped incubating at a given temperature. Since response variables in Poisson models in MCMCglmm need to be whole numbers, rates were multiplied by 24 to covert rates to per day and rounded to the nearest whole number. Year, enclosure, group and individual were included as random effects (see R code: M3 for details).

4. Testing how individual probability of incubation was influenced by breeding value at different temperatures.

The effect that temperature (fixed effect: 2-level factor hot vs benign), breeding value (fixed effect: continuous variable), sex (fixed effect: two level factor), number of males (fixed effect: continuous variable) and number of females (fixed effect:

continuous variable) had on the probability that individuals incubated was modelled using a binary (threshold) BLMM with a probit link function. The response variable was the probability that a given individual was ever seen contributing to incubation (1 if an individual was observed incubating for more than 10 minutes in a given temperature condition across the entire breeding season, otherwise 0). Year, enclosure, group and individual were included as random effects (see R code: M4 for details).

5. Testing how individual investment in incubation and the rate individuals ended incubation was influenced by breeding value at different temperatures.

Investment in incubation was modelled in the same way as 2, but heat tolerance was included as a fixed effect (continuous) (see R code: M5 for details). The rate that individuals ended incubation was modelled in the same way as 3, but heat tolerance was included as a fixed effect (continuous) (see R code: M6 for details).

6. Testing how the frequency of high and low heat tolerant individuals influenced the number of cooperators in groups.

The effect that the frequency of high tolerance individuals (fixed effect: continuous), temperature (fixed effect: 2-level factor hot vs benign), number of males (fixed effect: continuous variable) and number of females (fixed effect: continuous variable) had on the numbers of individuals that cooperated over incubation was modelled using a BLMM with a gaussian error distribution. The response variable of the model was the average number of cooperators in a group under hot and benign conditions. Individuals that had a breeding value equal to or above the median of the whole population were considered to have high heat tolerance. The frequency of high tolerant individuals was then calculated as the number of high tolerant individuals / number of same sex individuals in the group. Separate analyses were performed for females and males (see R code: M7 for details of females and M8 for males).

7. Testing if the heat tolerance was associated with a greater propensity to cooperate over incubation.

To examine if heat tolerance influenced the number of individuals that shared incubation during hot and benign periods, we restricted data to only include those individuals that incubated. With this data we ran a BLMM with Poisson error distribution. The response variable was number of cooperators in the group. Sex (fixed effect: 2-level factor), temperature (fixed effect: 2- level factor benign vs hot), breeding value (fixed effect: continuous) and number of males and females (fixed effect: continuous) were entered as fixed effects. Year, enclosure, group and individual were included as random effects. (see R code: M9 for details).

References

AlRashidi, M., Kosztolányi, A., Shobrak, M., Küpper, C., & Székely, T. (2011). Parental cooperation in an extreme hot environment: Natural behaviour and experimental evidence. *Animal Behaviour*, *82*(2), 235–243. https://doi.org/10.1016/j.anbehav.2011.04.019

Amat, J. A., & Masero, J. A. (2004). How kentish plovers, charadrius alexandrinus , cope with heat stress during incubation. *Behavioral Ecology and Sociobiology*, *56*(1), 26–33. https://doi.org/10.1007/s00265-004-0758-9

Arnqvist, G. (2004). Sexual conflict and sexual selection: Lost in the chase. *Evolution*, *58*(6), 1383–1388. https://doi.org/https://doi.org/10.1111/j.0014-3820.2004.tb01716.x

Berntsen, H. H., & Bech, C. (2016). Incubation temperature influences survival in a small passerine bird. *Journal of Avian Biology*, *47*, 141–145. https://doi.org/doi:10.1111/jav.00688

Bertram, B. C. R. (1992). *The ostrich communal nesting system:* Princeton University Press. https://doi.org/10.2307/j.ctt7ztm99

Chippindale, A. K., Gibson, J. R., & Rice, W. R. (2001). Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in drosophila. *Proceedings of the National Academy of Sciences*, *98*(4), 1671–1675. https://doi.org/10.1073/pnas.98.4.1671

Chown, S. L., Sinclair, B. J., Leinaas, H. P., & Gaston, K. J. (2004). Hemispheric asymmetries in biodiversity— a serious matter for ecology. *PLoS Biology*, *2*(11), 7.

Cockburn, A., Sims, R. A., Osmond, H. L., Green, D. J., Double, M. C., & Mulder, R. A. (2008). Can we measure the benefits of help in cooperatively breeding birds: The case of superb fairy-wrens malurus cyaneus? *Journal of Animal Ecology*, *77*(3), 430–438. https://doi.org/https://doi.org/10.1111/j.1365-2656.2007.01351.x

Cooper, G. A., & West, S. A. (2018). Division of labour and the evolution of extreme specialization. *Nature Ecology & Evolution*, *2*(7), 1161–1167. https://doi.org/10.1038/s41559-018-0564-9

Cornwallis, C. K., Botero, C. A., Rubenstein, D. R., Downing, P. A., West, S. A., & Griffin, A. S. (2017). Cooperation facilitates the colonization of harsh environments. *Nature Ecology & Evolution*, *1*(3), 0057. https://doi.org/10.1038/s41559-016-0057

Davies, D. (2003). Understanding biofilm resistance to antibacterial agents. *Nature Reviews Drug Discovery*, *2*(2), 114–122. https://doi.org/10.1038/nrd1008

Deeming, D. C., & Ayres, F. J. (1993). Observations on the commercial production of ostrich (struthio camelus) in the united kingdom: Incubation. *The Veterinary Record*, *24*(132), 602–607. https://europepmc.org/article/med/8337808

Dickinson, J. L., & Hatchwell, B. J. (2004). Fitness consequences of helping. In J. L. Dickinson & W. D. Koenig (Eds.), *Ecology and evolution of cooperative breeding in birds* (pp. 48–66). Cambridge University Press. https://doi.org/10.1017/CBO9780511606816.004

Downing, P. A., Griffin, A. S., & Cornwallis, C. K. (2020). The benefits of help in cooperative birds: Nonexistent or difficult to detect? *The American Naturalist*, *195*(6), 1085–1091. https://doi.org/10.1086/708515

DuRant, S. E., Hopkins, W. A., Hepp, G. R., & Walters, J. R. (2013). Ecological, evolutionary, and conservation implications of incubation temperature-dependent phenotypes in birds. *Biological Reviews*, *88*, 499–509. https://doi.org/doi:10.1111/brv.12015

Emlen, S. T. (1982). The evolution of helping. I. An ecological constraints model. *The American Naturalist*, *119*(1), 29–39. https://www.jstor.org/stable/2460654

Fedorka, K. M., & Mousseau, T. A. (2004). Female mating bias results in conflicting sex-specific offspring fitness. *Nature*, *429*(6987), 65–67. https://doi.org/10.1038/nature02492

Firman, R. C., Rubenstein, D. R., Moran, J. M., Rowe, K. C., & Buzatto, B. A. (2020). Extreme and variable climatic conditions drive the evolution of sociality in australian rodents. *Current Biology*, *30*(4), 691–697.e3. https://doi.org/10.1016/j.cub.2019.12.012

Foerster, K., Coulson, T., Sheldon, B. C., Pemberton, J. M., Clutton-Brock, T. H., & Kruuk, L. E. B. (2007). Sexually antagonistic genetic variation for fitness in red deer. *Nature*, *447*(7148), 1107–1110. https://doi.org/10.1038/nature05912

Fuller, A., Kamerman, P. R., Maloney, S. K., Mitchell, G., & Mitchell, D. (2003). Variability in brain and arterial blood temperatures in free-ranging ostriches in their natural habitat. *Journal of Experimental Biology*, *206*(7), 1171–1181. https://doi.org/10.1242/jeb.00230

Gibson, J. R., Chippindale, A. K., & Rice, W. R. (2002). The x chromosome is a hot spot for sexually antagonistic fitness variation. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1490), 499–505. https://doi.org/10.1098/rspb.2001.1863

Grant, G. S. (1982). Avian incubation: Egg temperature, nest humidity, and behavioral thermoregulation in a hot environment. *Ornithological Monographs*, *30*, iii–75. https://doi.org/10.2307/40166669

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: The **mcmcglmm** *r* package. *Journal of Statistical Software*, *33*(2). https://doi.org/10.18637/jss.v033.i02

Hadfield, J. D. (2021). *MCMCglmm course notes*. https://cran.r-project.org/web/packages/MCMCglmm/vignettes/CourseNotes.pdf

Hatchwell, B. J. (2009). The evolution of cooperative breeding in birds: Kinship, dispersal and life history. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1533), 3217–3227. https://doi.org/10.1098/rstb.2009.0109

Hepp, G. R., & Kennamer, R. A. (2012). Warm is better: Incubation temperature influences apparent survival and recruitment of wood ducks (aix sponsa). *PLOS ONE*, *7*(10), e47777. https://doi.org/10.1371/journal.pone.0047777

Jetz, W., & Rubenstein, D. R. (2011). Environmental uncertainty and the global biogeography of cooperative breeding in birds. *Current Biology*, *21*(1), 72–78. https://doi.org/10.1016/j.cub.2010.11.075

Kennou Sebei, S., & Bergaoui, R. (2009). Ostriches' reproduction behaviour and mastery of natural incubation under farming conditions. *Tropical Animal Health and Production*, *41*(3), 353–361. https://doi.org/10.1007/s11250-008-9196-4

Koenig, W. D., & Dickinson, J. L. (2016). *Cooperative breeding in vertebrates: Studies of ecology, evolution, and behavior*. Cambridge University Press.

Lukas, D., & Clutton-Brock, T. (2017). Climate and the distribution of cooperative breeding in mammals. *Royal Society Open Science*, *4*(1), 160897. https://doi.org/10.1098/rsos.160897

Maloney, S. K. (2008). Thermoregulation in ratites: A review. *Aust. J. Exp. Agric.*, *48*(10), 1293. https://doi.org/10.1071/EA08142

Meagher, T. R. (1992). The quantitative genetics of sexual dimorphism in silene latifolia (caryophyllaceae). I. Genetic variation. *Evolution*, *46*(2), 445–457. https://doi.org/https://doi.org/10.1111/j.1558-5646.1992.tb02050.x

Nord, A., & Nilsson, J.-Å. (2016). Long-term consequences of high incubation temperature in a wild bird population. *Biology Letters*, *12*(4), 20160087. https://doi.org/10.1098/rsbl.2016.0087

Olson, C. R., Vleck, C. M., & Adams, D. C. (2008). Decoupling morphological development from growth in periodically cooled zebra finch embryos. *Journal of Morphology*, *269*, 875–883. https://doi.org/doi:10.1002/jmor.10635

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, *37*(1), 637–669. https://doi.org/10.1146/annurev.ecolsys.37.091305.110100

Queller, D. C. (2000). Relatedness and the fraternal major transitions. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*. https://doi.org/10.1098/rstb.2000.0727

Queller, D. C., & Strassmann, J. E. (1998). Kin selection and social insects. *BioScience*, *48*(3), 165–175. https://doi.org/10.2307/1313262

Rice, W. R. (1984). Sex chromosomes and the evolution of sexual dimorphism. *Evolution*, *38*(4), 735–742. https://doi.org/10.2307/2408385

Rice, W. R. (1992). Sexually antagonistic genes: Experimental evidence. *Science*, *256*(5062), 1436–1439. https://doi.org/10.1126/science.1604317

Rice, W. R., & Chippindale, A. K. (2001). Intersexual ontogenetic conflict. *Journal of Evolutionary Biology*, *14*(5), 685–693. https://doi.org/https://doi.org/10.1046/j.1420-9101.2001.00319.x

Rubenstein, D. R., & Lovette, I. J. (2007). Temporal environmental variability drives the evolution of cooperative breeding in birds. *Current Biology*, *17*(16), 1414–1419. https://doi.org/10.1016/j.cub.2007.07.032

Sauer, E. G. F., & Sauer, E. M. (1966). The behaviour and ecology of the south african ostrich. *Living Bird*, *5*, 45–75.

Schou, M. F., Bonato, M., Engelbrecht, A., Brand, Z., Svensson, E. I., 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*(1), 666. https://doi.org/10.1038/s41467-021-20937-7

Siegfried, W. R. \. F. P. G. H. (1974). Egg temperature and incubation behaviour of the ostrich. *Madoqua*, *1974*(8), 63–66. https://doi.org/10.10520/AJA10115498_78

Sun, S.-J., Rubenstein, D. R., Chen, B.-F., Chan, S.-F., Liu, J.-N., Liu, M., Hwang, W., Yang, P.-S., & Shen, S.-F. (2014). Climate-mediated cooperation promotes niche expansion in burying beetles. *eLife*, *3*, e02440. https://doi.org/10.7554/eLife.02440

Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2012). Thermal tolerance and the global redistribution of animals. *Nature Climate Change*, *2*(9), 686–690. https://doi.org/10.1038/nclimate1539

Szathmáry, E., & Smith, J. M. (1995). The major evolutionary transitions. *Nature*, *374*(6519), 227–232. https://doi.org/10.1038/374227a0

Team, R. C. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Vincze, O., Székely, T., Küpper, C., AlRashidi, M., Amat, J. A., Ticó, A. A., Burgas, D., Burke, T., Cavitt, J., Figuerola, J., Shobrak, M., Montalvo, T., & Kosztolányi, A. (2013). Local environment but not genetic differentiation influences biparental care in ten plover populations. *PLOS ONE*, *8*(4), e60998. https://doi.org/10.1371/journal.pone.0060998

Ward, D. (1990). Incubation temperatures and behavior of crowned, black-winged, and lesser black-winged plovers. *The Auk*, *107*(1), 10–17. https://doi.org/10.1093/auk/107.1.10

Wilson, E. O. (2000). *Sociobiology: The new synthesis, twenty-fifth anniversary edition*. Harvard University Press. https://www.jstor.org/stable/j.ctvjnrttd

Supplementary Information: Genetic adaptations to cope with heat predict the emergence of cooperative behaviour

Julian Melgar[1,+], Mads F. Schou[1], Maud Bonato[2], Zanell Brand[3], Anel Engelbrecht[3], Schalk Cloete[4,3], Pfunzo T. Muvhali[4], Bengt Hansson[1] & Charlie K. Cornwallis[1,+]

[1] Department of Biology, Lund University, Lund, Sweden [2] Kynsna Elephant Park, Kynsna, South Africa [3] Directorate Animal Sciences, Western Cape Department of Agriculture, Elsenburg, South Africa [4] Department of Animal Sciences, University of Stellenbosch, Matieland, South Africa

[+]Corresponding authors: Email: julian.melgar@biol.lu.se (mailto:julian.melgar@biol.lu.se), charlie.cornwallis@biol.lu.se (mailto:charlie.cornwallis@biol.lu.se)

This file includes: **Figures S1-S6** and **Tables S1-S9**.

Supplementary Figures

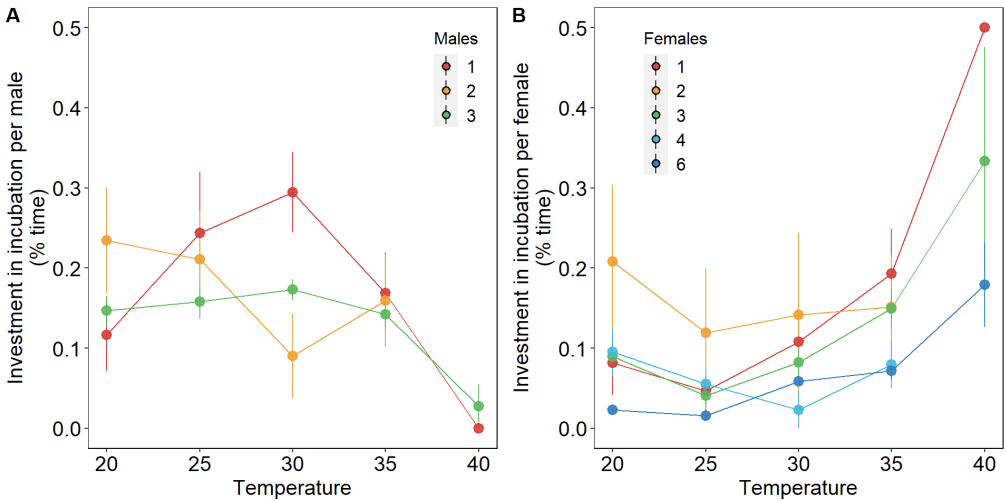


Figure S1: The division of incubation between males and females excluding morning observations

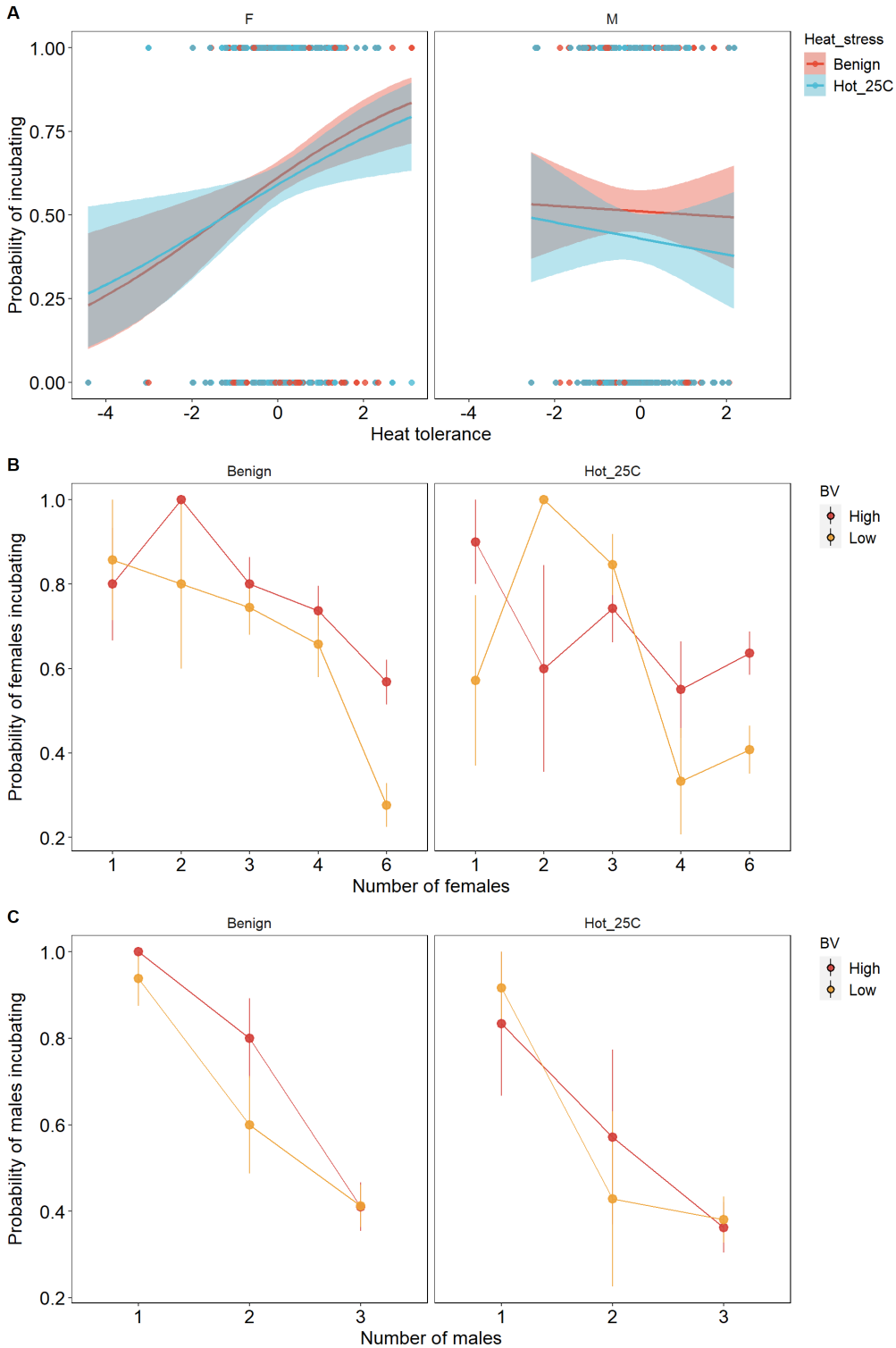


Figure S2. Probability of incubating at high and low temperatures in relation to genetic heat tolerance

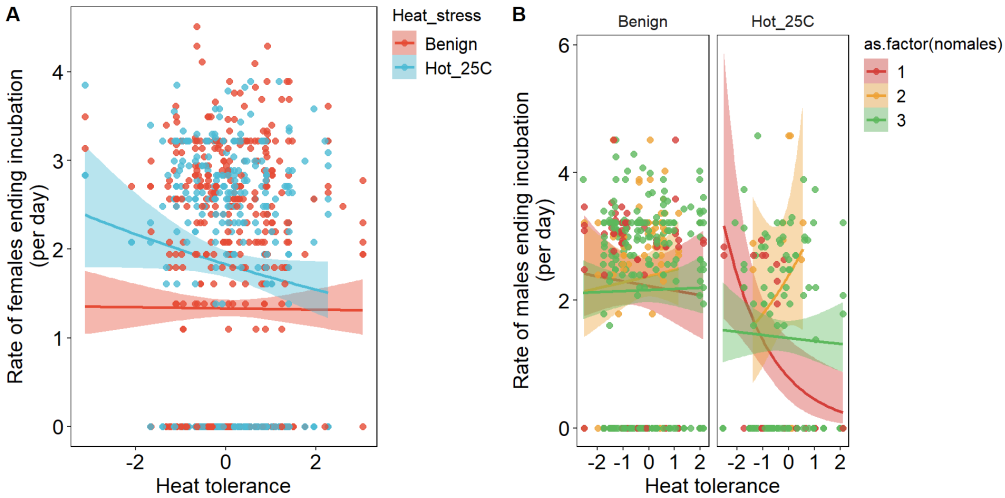


Figure S3: The rate that females (A) and males (B) ended incubation at benign and hot temperatures in relation to their genetic heat tolerance

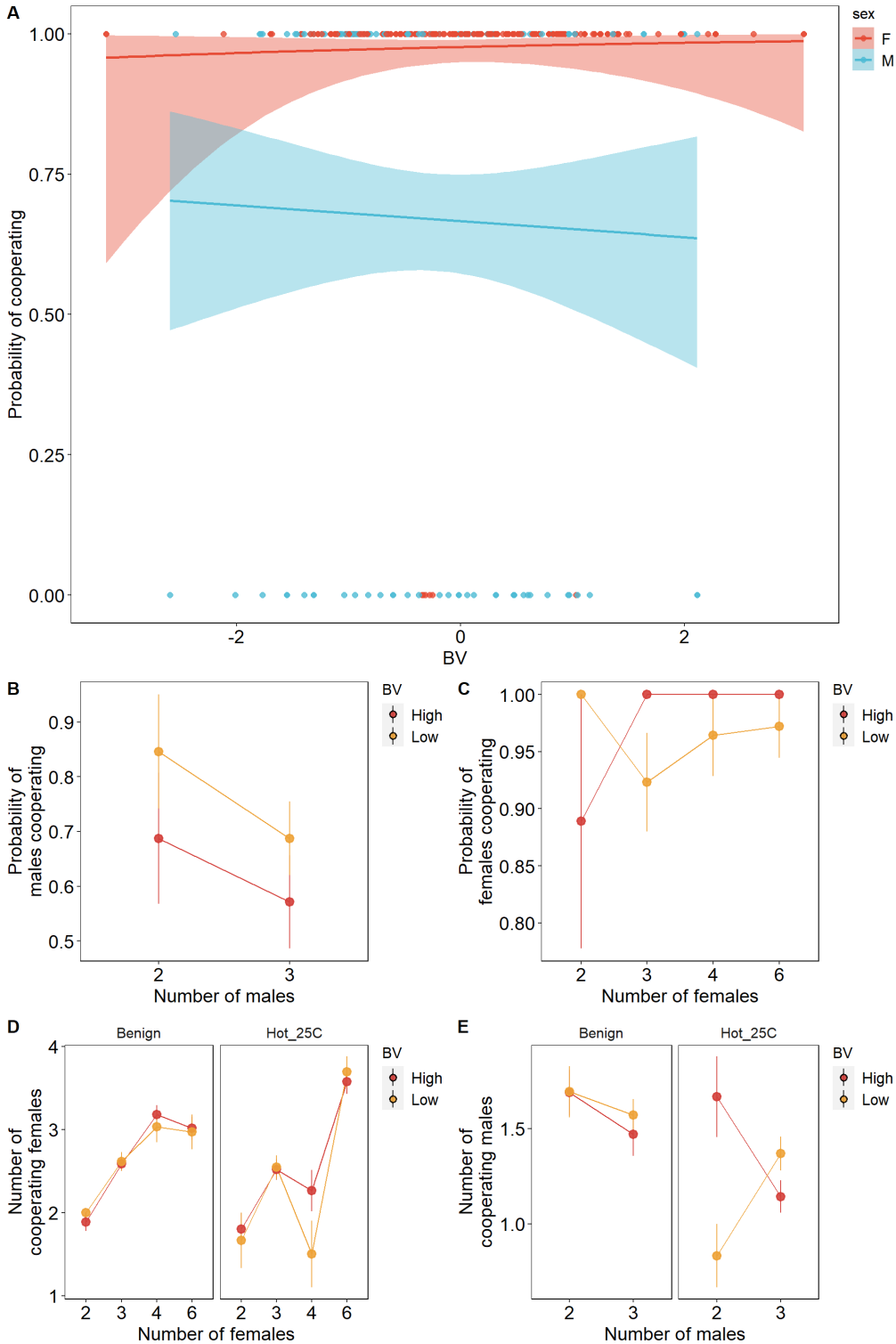


Figure S4. Genetic heat tolerance of the number of cooperating individuals

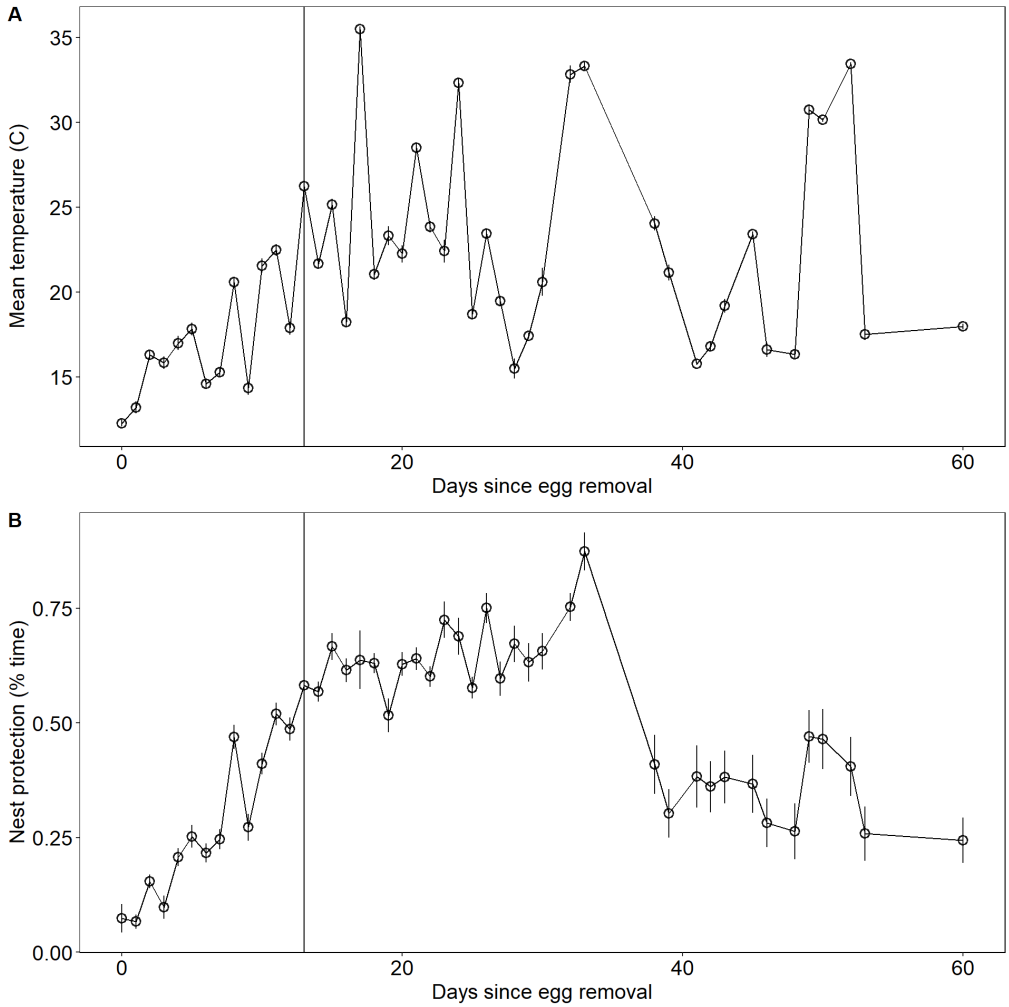


Figure S5. Does the data need filtering to remove trends over time due to birds building nests and starting to incubate as well as seasonal increases in temperature increases?

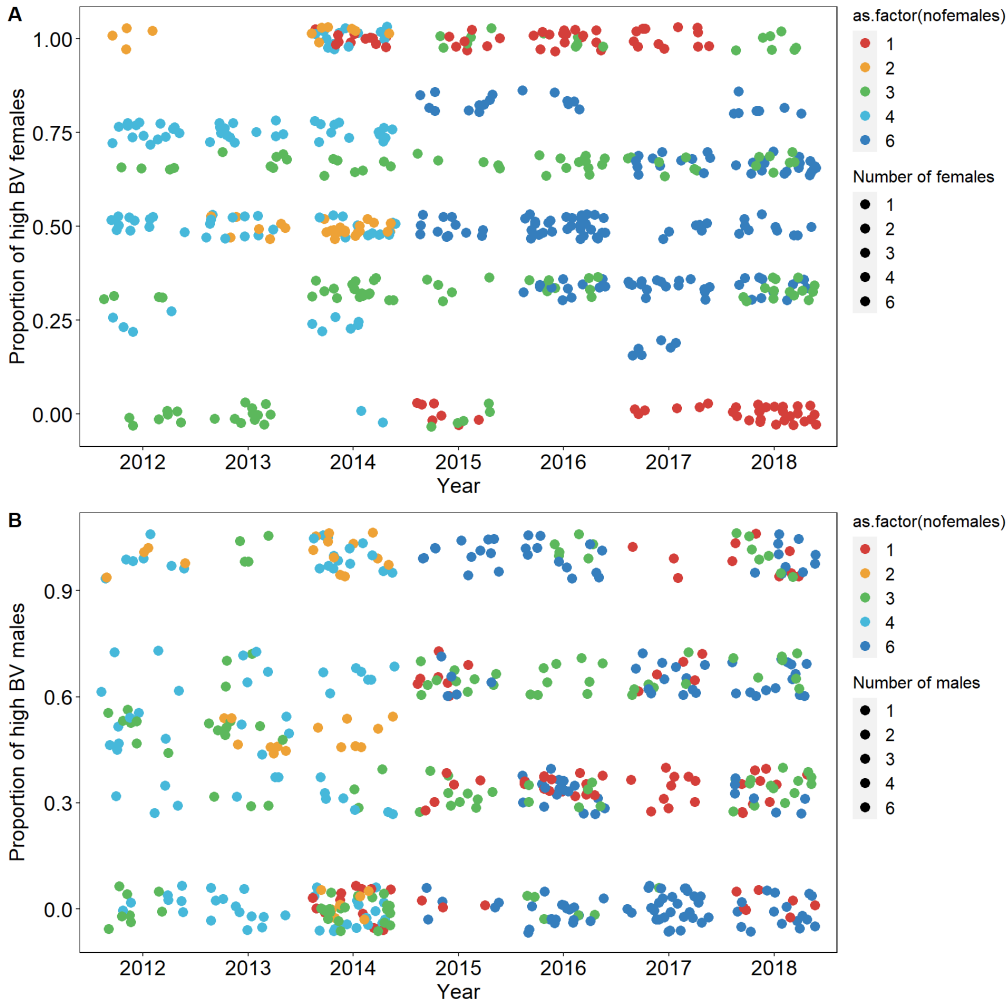


Figure S6. Relative frequencies of high and low BV individuals across years

Supplementary Tables

Table S1: The % time nests were protected by groups with different numbers of males and females at different temperatures

Fixed Effects	Posterior Mode (CI)	pMCMC
Intercept	1.21 (0.53 , 1.89)	**0.008**
Nmales	0.39 (0.04 , 0.9)	**0.04**
Nfemales	0.8 (0.43 , 1.3)	**0.001**
Temperature	0.17 (-0.07 , 0.36)	0.178
Temperature2	0.88 (0.65 , 1.09)	**0.001**
Nmales*Temperature	0.15 (-0.02 , 0.39)	0.082
Nfemales*Temperature	0.12 (-0.07 , 0.33)	0.184

Fixed Effects	Posterior Mode (CI)	pMCMC
Nmales*Temperature2	-0.04 (-0.26 , 0.17)	0.782
Nfemales*Temperature2	0.08 (-0.12 , 0.31)	0.536
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.014 (0 , 1.627)	5.321 (0.003 , 18.889)
Camp	0.001 (0 , 0.292)	0.884 (0.003 , 3.973)
Group	3.082 (2.147 , 4.584)	42.194 (29.232 , 54.577)
Residual	4.085 (3.197 , 4.532)	51.602 (41.012 , 63.049)

Table S2: Individual investment in incubation in groups with different numbers of males and females at different temperatures

Fixed Effects	Posterior Mode (CI)	pMCMC
Female	-5.99 (-6.68 , -5)	**0.001**
Male	-8.51 (-9.55 , -7.46)	**0.001**
Male: Nmales	-1.06 (-1.65 , -0.58)	**0.001**
Female: Nfemales	-1.62 (-1.95 , -1.06)	**0.001**
Female: Temperature	1.45 (1.09 , 1.61)	**0.001**
Male: Temperature	-1.11 (-1.46 , -0.81)	**0.001**
Female: Temperature2	-0.78 (-1.02 , -0.53)	**0.001**
Male: Temperature2	0.17 (-0.22 , 0.41)	0.51
Male: Nmales*Temperature	0.31 (-0.16 , 0.55)	0.246
Female: Nfemales*Temperature	0.17 (-0.09 , 0.44)	0.2
Male: Nmales*Temperature2	0.09 (-0.33 , 0.41)	0.846
Female: Nfemales*Temperature2	-0.12 (-0.32 , 0.23)	0.692
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.002 (0 , 0.248)	0.138 (0.001 , 0.595)
Camp	0.003 (0 , 0.435)	0.219 (0.001 , 1.021)
Group	3.687 (2.461 , 5.87)	9.551 (6.397 , 13.541)
Individual	19.321 (15.229 , 24.258)	46.516 (40.577 , 52.157)
Residual	17.87 (16.364 , 19.63)	43.576 (38.76 , 50.095)

Table S3: The rate individuals ended incubation in groups with different numbers of males and females at different temperatures

Fixed Effects	Posterior Mode (CI)	pMCMC
Female	0.98 (0.63 , 1.47)	**0.001**
Male	1.84 (1.36 , 2.31)	**0.001**
Male: Nmales	-0.18 (-0.39 , 0.08)	0.206
Female: Nfemales	0.07 (-0.16 , 0.24)	0.68

Fixed Effects	Posterior Mode (CI)	pMCMC
Female: Temperature	0.68 (0.48 , 0.85)	**0.001**
Male: Temperature	-0.58 (-0.85 , -0.43)	**0.001**
Female: Temperature2	-0.34 (-0.55 , -0.24)	**0.001**
Male: Temperature2	-0.36 (-0.51 , -0.1)	**0.001**
Male: Nmales*Temperature	0.09 (-0.1 , 0.34)	0.314
Female: Nfemales*Temperature	0.03 (-0.09 , 0.24)	0.29
Male: Nmales*Temperature2	-0.21 (-0.37 , 0.03)	0.084
Female: Nfemales*Temperature2	-0.04 (-0.21 , 0.1)	0.54
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.048 (0.001 , 0.416)	3.077 (0.013 , 8.322)
Camp	0.09 (0.002 , 0.453)	4.241 (0.053 , 9.556)
Group	0.142 (0.001 , 0.379)	3.787 (0.008 , 8.123)
Individual	0.002 (0 , 0.102)	0.593 (0.007 , 2.286)
Residual	4.076 (3.584 , 4.664)	88.302 (80.275 , 95.151)

Table S4: The probability that individuals incubated in relation to their heat tolerance

Fixed Effects	Posterior Mode (CI)	pMCMC
Female Benign	0.37 (0.17 , 0.77)	**0.008**
Female Hot_25C	0.55 (0.21 , 0.89)	**0.001**
Male Benign	0.26 (-0.14 , 0.57)	0.246
Male Hot_25C	0.14 (-0.29 , 0.48)	0.674
Male Benign: Nmales	-0.88 (-1.25 , -0.65)	**0.001**
Male Hot_25C: Nmales	-0.56 (-0.78 , -0.38)	**0.001**
Female Benign: Nfemales	0.28 (0.07 , 0.48)	**0.008**
Female Hot_25C: Nfemales	-0.04 (-0.25 , 0.23)	0.892
Female Benign: Heat tolerance	0.28 (0.12 , 0.55)	**0.001**
Female Hot_25C: Heat tolerance	0.22 (-0.03 , 0.44)	0.05
Male Benign: Heat tolerance	-0.01 (-0.28 , 0.29)	0.958
Male Hot_25C: Heat tolerance	-0.04 (-0.38 , 0.27)	0.816
Female Benign: Heat tolerance*Nfemales	-0.02 (-0.15 , 0.12)	0.734
Female Hot_25C: Heat tolerance*Nfemales	0.03 (-0.12 , 0.17)	0.72
Male Benign: Heat tolerance*Nmales	-0.01 (-0.46 , 0.48)	0.918
Male Hot_25C: Heat tolerance*Nmales	-0.05 (-0.51 , 0.58)	0.918
Female Benign vs Female Hot_25C	-0.09 (-0.35 , 0.12)	0.326
Male Benign vs Male Hot_25C	0.15 (-0.16 , 0.45)	0.38
Male Benign: Nmales vs Male Hot_25C: Nmales	-0.3 (-0.7 , -0.01)	**0.046**

Fixed Effects	Posterior Mode (CI)	pMCMC
Female Benign: Nfemales vs Female Hot_25C: Nfemales	0.29 (-0.04 , 0.6)	0.086
Female Benign: Heat tolerance vs Female Hot_25C: Heat tolerance	0.1 (-0.15 , 0.32)	0.396
Male Benign: Heat tolerance vs Male Hot_25C: Heat tolerance	0.04 (-0.24 , 0.38)	0.748
Female Benign: Heat tolerance*Nfemales vs Female Hot_25C: Heat tolerance*Nfemales	-0.05 (-0.21 , 0.12)	0.572
Male Benign: Heat tolerance*Nmales vs Male Hot_25C: Heat tolerance*Nmales	0.12 (-0.72 , 0.62)	0.986
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.001 (0 , 0.16)	3.326 (0 , 11.713)
Camp	0.001 (0 , 0.146)	3.674 (0 , 11.607)
Group	0.001 (0 , 0.224)	7.296 (0 , 17.224)
Residual	1 (1 , 1)	85.704 (71.375 , 96.412)

Table S5: Investment in incubation by individuals in relation to temperature and heat tolerance

Fixed Effects	Posterior Mode (CI)	pMCMC
Female Benign	-4.94 (-5.39 , -4.06)	**0.001**
Female Hot_25C	-4.35 (-4.86 , -3.45)	**0.001**
Male Benign	-5.98 (-6.6 , -5)	**0.001**
Male Hot_25C	-6.28 (-7.18 , -5.51)	**0.001**
Male Benign: Nmales	-1.68 (-2.21 , -1.1)	**0.001**
Male Hot_25C: Nmales	-1.26 (-1.7 , -0.91)	**0.001**
Female Benign: Nfemales	0.89 (0.29 , 1.37)	**0.002**
Female Hot_25C: Nfemales	-0.08 (-0.77 , 0.54)	0.692
Female Benign: Heat tolerance	0.89 (0.29 , 1.51)	**0.004**
Female Hot_25C: Heat tolerance	0.63 (0.02 , 1.32)	0.05
Male Benign: Heat tolerance	-0.07 (-0.72 , 0.68)	0.938
Male Hot_25C: Heat tolerance	-0.21 (-1.08 , 0.51)	0.514
Female Benign: Heat tolerance*Nfemales	0.14 (-0.19 , 0.4)	0.446
Female Hot_25C: Heat tolerance*Nfemales	0.12 (-0.13 , 0.49)	0.26
Male Benign: Heat tolerance*Nmales	0.38 (-0.54 , 0.99)	0.534
Male Hot_25C: Heat tolerance*Nmales	0.23 (-0.79 , 0.92)	0.76
Female Benign vs Female Hot_25C	-0.62 (-1.07 , 0.09)	0.072
Male Benign vs Male Hot_25C	0.42 (-0.31 , 1.18)	0.264
Male Benign: Nmales vs Male Hot_25C: Nmales	-0.44 (-1.05 , 0.34)	0.28
Female Benign: Nfemales vs Female Hot_25C: Nfemales	0.99 (0.1 , 1.73)	**0.026**
Female Benign: Heat tolerance vs Female Hot_25C: Heat tolerance	0.29 (-0.38 , 0.8)	0.456

Fixed Effects	Posterior Mode (CI)	pMCMC
Male Benign: Heat tolerance vs Male Hot_25C: Heat tolerance	0.23 (-0.5 , 1.05)	0.554
Female Benign: Heat tolerance*Nfemales vs Female Hot_25C: Heat tolerance*Nfemales	-0.03 (-0.45 , 0.28)	0.754
Male Benign: Heat tolerance*Nmales vs Male Hot_25C: Heat tolerance*Nmales	0.01 (-0.95 , 1.17)	0.826
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.004 (0 , 0.31)	0.35 (0.001 , 1.514)
Camp	0.002 (0 , 0.376)	0.448 (0.001 , 1.804)
Group	0.817 (0.001 , 1.584)	3.72 (0.003 , 7.428)
Individual	8.402 (6.55 , 11.448)	43.097 (35.195 , 50.548)
Residual	10.588 (8.98 , 12.357)	52.385 (43.513 , 60.465)

Table S6: The rate that individuals with different heat tolerance ended incubation in groups with different numbers of males and females at different temperatures

Fixed Effects	Posterior Mode (CI)	pMCMC
Female Benign	1.65 (1.35 , 1.92)	**0.001**
Female Hot_25C	2.01 (1.66 , 2.23)	**0.001**
Male Benign	2.62 (2.26 , 2.88)	**0.001**
Male Hot_25C	1.45 (1.06 , 1.8)	**0.001**
Male Benign: Nmales	-0.09 (-0.25 , 0.12)	0.48
Male Hot_25C: Nmales	0.09 (-0.07 , 0.23)	0.438
Female Benign: Nfemales	-0.1 (-0.25 , 0)	0.084
Female Hot_25C: Nfemales	-0.06 (-0.22 , 0.1)	0.4
Female Benign: Heat tolerance	-0.08 (-0.24 , 0.12)	0.64
Female Hot_25C: Heat tolerance	-0.18 (-0.36 , 0.02)	**0.048**
Male Benign: Heat tolerance	-0.05 (-0.23 , 0.14)	0.782
Male Hot_25C: Heat tolerance	-0.2 (-0.44 , 0.09)	0.252
Female Benign: Heat tolerance*Nfemales	-0.1 (-0.2 , 0.04)	0.184
Female Hot_25C: Heat tolerance*Nfemales	0.02 (-0.13 , 0.1)	0.9
Male Benign: Heat tolerance*Nmales	0.04 (-0.21 , 0.26)	0.92
Male Hot_25C: Heat tolerance*Nmales	0.46 (0.08 , 0.83)	**0.008**
Female Benign vs Female Hot_25C	-0.29 (-0.55 , -0.07)	**0.004**
Male Benign vs Male Hot_25C	1.23 (0.83 , 1.5)	**0.001**
Male Benign: Nmales vs Male Hot_25C: Nmales	-0.16 (-0.35 , 0.12)	0.258
Female Benign: Nfemales vs Female Hot_25C: Nfemales	-0.05 (-0.25 , 0.17)	0.67

Fixed Effects	Posterior Mode (CI)	pMCMC
Female Benign: Heat tolerance vs Female Hot_25C: Heat tolerance	0.15 (-0.12 , 0.39)	0.222
Male Benign: Heat tolerance vs Male Hot_25C: Heat tolerance	0.09 (-0.18 , 0.47)	0.4
Female Benign: Heat tolerance*Nfemales vs Female Hot_25C: Heat tolerance*Nfemales	-0.06 (-0.23 , 0.09)	0.4
Male Benign: Heat tolerance*Nmales vs Male Hot_25C: Heat tolerance*Nmales	-0.48 (-0.84 , 0.01)	**0.046**
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.004 (0 , 0.16)	3.346 (0.011 , 10.215)
Camp	0.091 (0.001 , 0.227)	6.412 (0.035 , 14.576)
Group	0.003 (0 , 0.188)	5.662 (0.022 , 13.157)
Individual	0.002 (0 , 0.08)	1.41 (0.014 , 5.484)
Residual	1.22 (0.996 , 1.439)	83.171 (70.324 , 94.15)

Table S7: The number of female cooperators in groups with different frequencies of heat tolerant females at different temperatures

Fixed Effects	Posterior Mode (CI)	pMCMC
Benign	1.57 (1.1 , 1.96)	**0.001**
Hot_25C	1.9 (1.46 , 2.32)	**0.001**
Nmales	0.08 (-0.02 , 0.23)	0.114
Nfemales	0.44 (0.32 , 0.59)	**0.001**
Benign: Freq tolerant females	0.02 (-0.29 , 0.44)	0.656
Hot_25C: Freq tolerant females	0.3 (-0.31 , 0.84)	0.316
Benign: Freq tolerant females*Nfemales	0.39 (-0.16 , 0.72)	0.22
Hot_25C: Freq tolerant females*Nfemales	0.62 (0.05 , 0.99)	**0.036**
Benign vs Hot_25C	-0.38 (-0.56 , -0.13)	**0.001**
Benign: Freq tolerant females vs Hot_25C: Freq tolerant females	-0.19 (-0.88 , 0.44)	0.518
Benign: Freq tolerant females*Nfemales vs Hot_25C: Freq tolerant females*Nfemales	-0.14 (-0.81 , 0.45)	0.486
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.061 (0.001 , 0.421)	21.259 (2.226 , 48.523)
Camp	0.002 (0 , 0.074)	3.662 (0.032 , 11.935)
Group	0.003 (0.001 , 0.221)	16.621 (0.05 , 34.897)
Residual	0.375 (0.237 , 0.485)	58.459 (29.557 , 84.182)

Table S8: Variation in the number of cooperators individuals that incubated at different temperatures in relation to their heat tolerance

Fixed Effects	Posterior Mode (CI)	pMCMC
Female Benign	1.01 (0.88 , 1.14)	**0.001**
Female Hot_25C	1.06 (0.91 , 1.19)	**0.001**
Male Benign	0.52 (0.26 , 0.68)	**0.001**
Male Hot_25C	0.28 (0.03 , 0.54)	**0.022**
Male Benign: Nmales	-0.1 (-0.3 , 0.18)	0.646
Male Hot_25C: Nmales	0.14 (0.06 , 0.21)	**0.001**
Female Benign: Nfemales	0 (-0.05 , 0.06)	0.976
Female Hot_25C: Nfemales	0 (-0.12 , 0.11)	0.926
Female Benign: Heat tolerance	0.02 (-0.05 , 0.1)	0.67
Female Hot_25C: Heat tolerance	-0.03 (-0.1 , 0.06)	0.654
Male Benign: Heat tolerance	-0.07 (-0.14 , 0.13)	0.994
Male Hot_25C: Heat tolerance	-0.06 (-0.2 , 0.17)	0.892
Female Benign: Heat tolerance*Nfemales	0 (-0.05 , 0.06)	0.824
Female Hot_25C: Heat tolerance*Nfemales	-0.03 (-0.08 , 0.05)	0.638
Male Benign: Heat tolerance*Nmales	0 (-0.37 , 0.35)	0.874
Male Hot_25C: Heat tolerance*Nmales	-0.25 (-1.47 , 0.39)	0.226
Female Benign vs Female Hot_25C	-0.04 (-0.16 , 0.06)	0.376
Male Benign vs Male Hot_25C	0.13 (-0.06 , 0.46)	0.168
Male Benign: Nmales vs Male Hot_25C: Nmales	-0.12 (-0.46 , 0.03)	0.072
Female Benign: Nfemales vs Female Hot_25C: Nfemales	0 (-0.12 , 0.13)	0.92
Female Benign: Heat tolerance vs Female Hot_25C: Heat tolerance	0.06 (-0.07 , 0.14)	0.538
Male Benign: Heat tolerance vs Male Hot_25C: Heat tolerance	-0.01 (-0.23 , 0.24)	0.96
Female Benign: Heat tolerance*Nfemales vs Female Hot_25C: Heat tolerance*Nfemales	0.02 (-0.07 , 0.1)	0.608
Male Benign: Heat tolerance*Nmales vs Male Hot_25C: Heat tolerance*Nmales	0.49 (-0.38 , 1.59)	0.218
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.002 (0 , 0.041)	42.539 (3.77 , 84.419)
Camp	0.001 (0 , 0.006)	10.581 (0.199 , 28.585)
Group	0.002 (0 , 0.019)	29.359 (0.533 , 66.208)
Individual	0.001 (0 , 0.005)	9.017 (0.381 , 25.589)
Residual	0.001 (0 , 0.004)	8.504 (0.152 , 23.562)

Table S9: The number of male cooperators in groups with different frequencies of heat tolerant males at different temperatures

Fixed Effects	**Posterior Mode (CI)**	**pMCMC**
Benign	0.97 (0.79 , 1.16)	**0.001**
Hot_25C	0.85 (0.71 , 1.09)	**0.001**
Nmales	0.1 (0.02 , 0.19)	**0.02**
Nfemales	0.03 (-0.07 , 0.11)	0.638
Benign: Freq tolerant males	-0.14 (-0.33 , 0.07)	0.3
Hot_25C: Freq tolerant males	-0.31 (-0.58 , -0.04)	**0.03**
Benign: Freq tolerant males*Nmales	-0.15 (-0.33 , 0.06)	0.162
Hot_25C: Freq tolerant males*Nmales	-0.17 (-0.36 , 0.07)	0.128
Benign vs Hot_25C	0.1 (-0.01 , 0.17)	0.08
Benign: Freq tolerant males vs Hot_25C: Freq tolerant males	0.19 (-0.14 , 0.52)	0.27
Benign: Freq tolerant males*Nmales vs Hot_25C: Freq tolerant males*Nmales	0.07 (-0.26 , 0.31)	0.822
Random Effects	**Posterior Mode (CI)**	**I2 % (CI)**
Year	0.004 (0 , 0.069)	10.476 (0.195 , 28.732)
Camp	0.002 (0 , 0.052)	8.154 (0.081 , 22.623)
Group	0.074 (0.034 , 0.138)	41.376 (15.506 , 62.021)
Residual	0.072 (0.054 , 0.115)	39.994 (23.762 , 58.11)