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Matrilineal inheritance of a key mediator of prenatal maternal effects

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Sex-linkage is predicted to evolve in response to sex-specific or sexually antagonistic selection. In line with this prediction, most sex-linked genes are associated with reproduction in the respective sex. In addition to traits directly involved in fertility and fecundity, mediators of maternal effects may be predisposed to evolve sex-linkage, because they indirectly affect female fitness through their effect on offspring phenotype. Here, we test for sex-linked inheritance of a key mediator of prenatal maternal effects in oviparous species, the transfer of maternally derived testosterone to the eggs. Consistent with maternal inheritance, we found that in Japanese quail (*Coturnix japonica*) granddaughters resemble their maternal (but not their paternal) grandmother in yolk testosterone deposition. This pattern of resemblance was not due to non-genetic priming effects of testosterone exposure during prenatal development, as an experimental manipulation of yolk testosterone levels did not affect the females' testosterone transfer to their own eggs later in life. Instead, W chromosome and/or mitochondrial variation may underlie the observed matrilineal inheritance pattern. Ultimately, the inheritance of mediators of maternal effects along the maternal line will allow for a fast and direct response to female-specific selection, thereby affecting the dynamics of evolutionary processes mediated by maternal effects.

1. Introduction

Sexual antagonism is common in nature and has important consequences for the genomic arrangement of loci under sex-specific selection, as well as their inheritance [1–3]. Indeed, because daughters are more likely to obtain high female fitness alleles from their mother than from their father, and vice versa, sex-specific (or sexually antagonistic) selection will favour sex-linkage of traits differentially linked to male and female fitness [4,5]. A classic example for the evolution of sex-linkage in response to sexually antagonistic selection is coloration in guppies (*Poecilia reticulata*), which is associated with attractiveness in males [6], but makes males and females more vulnerable to predation [7]. In response to these conflicting selection pressures, a large proportion of the genetic variation in coloration has become linked to the male-specific Y chromosome [8].

Even when selection is not acting in a sexually *antagonistic* way, sex-linkage may be adaptive, because it allows for a faster and more direct response to sex-specific selection. Furthermore, if a trait is expressed in a sex-limited way, sex-linkage prevents deleterious alleles from being sheltered from selection in the non-expressing sex [4], again accelerating adaptive responses to selection. In line with these ideas, male-specific fitness traits, such as sperm motility [9] or spermatogenesis [10], are linked to the male-specific Y chromosome in species where the male is the heterogametic sex (XY). And similarly, in species where

the female is the heterogametic sex (ZW), female fecundity and fertility traits are associated with the female-specific W chromosome [11,12].

We propose that in addition to traits directly involved in fecundity and fertility, mediators of maternal effects (i.e. maternally expressed traits that affect offspring phenotype) may be predisposed to evolve sex-linkage because they indirectly affect female fitness through their effect on offspring phenotype [13]. Furthermore, we argue that the potential for such sex-linkage of maternal effect mediators is particularly high in taxa where the female is the heterogametic sex (such as birds).

Here we used a three-generation breeding design (electronic supplementary material, S1) in a captive Japanese quail (*Coturnix japonica*) population to test for sex-linkage of a key mediator of prenatal maternal effects in birds: the transfer of maternally derived testosterone (T) to the eggs (yolk T transfer) [14–16]. Maternally transferred T affects a wide range of morphological, physiological, behavioural and life-history traits in the offspring (i.e. it acts as a mediator of maternal effects [14–16]), and the costs and benefits of T exposure during prenatal development appear to depend on the social and environmental conditions encountered by the offspring [17–19]. Yolk T transfer is known to be heritable [20–22], but the design of previous studies did not allow detection of potential sex-linkage. We predict that if yolk T transfer is inherited along the maternal line, females will resemble their maternal, but not their paternal grandmother in their transfer of T to the eggs.

2. Material and methods

(a) Study population

The study was conducted in a population of Japanese quail kept at the University of Zurich, Switzerland. Males and females were housed in separate outdoor aviaries (7 × 5.5 m each). For breeding, male–female pairs were transferred to cages (122 × 50 × 50 cm) within our facility. Cages contained *ad libitum* food, water, grit, a source of calcium, a shelter and a sand bath. The bottom of the cages was lined with sawdust. The breeding facility was kept on a 16 L : 8 D cycle at 20 ± 3°C (see [23] for a detailed description of animal husbandry).

(b) Egg collection, incubation and offspring rearing

Eggs were collected daily, labelled with a non-toxic marker and weighed. To standardize incubation and rearing conditions, we artificially incubated the eggs (mean ± s.d.: 9.5 ± 0.84 eggs per female; Favorit, HEKA Brutgeräte, Germany; 37.8°C, 55% humidity). For hatching, eggs were placed in individual containers to be able to determine which chick hatched from which egg. After hatching, chicks were raised in heated cages in mixed family groups (109 × 57 × 25 cm, Kükenaufzuchtbox 4002/C, HEKA Brutgeräte, Germany). Variation in the number of eggs laid while in the breeding cages was small, and there was no mother–daughter resemblance in the number of eggs laid (generalized linear mixed model: $\chi^2 = 0.264$, $p = 0.607$).

For the yolk T analysis, yolk and albumen of one egg per female (the fifth) were separated, weighed, homogenized and frozen at −20°C. Previous work has shown that within-clutch variation in yolk T concentration is small in Japanese quail (within-female repeatability across different stages of the reproductive cycle greater than 0.7 [24]) and the fifth egg is thus representative of a female's yolk T deposition to her eggs. Yolks were collected across three generations (hereafter referred to as maternal and paternal grandmothers, mothers and (grand-) daughters) to assess the inheritance pattern (see the electronic supplementary material, S1). Within a generation, all females had the same age and had experienced the same period of reproductive activity when eggs were collected.

(c) Yolk testosterone analysis

Yolk T extraction and radioimmunoassay were performed following previously published protocols [22]. In short, 100–110 mg of yolk were spiked with approximately 2500 dpm of [^{3}H]-testosterone (PerkinElmer, USA) and extracted twice with a mixture of diethyl and petroleum ether (7 : 3). Yolk T concentrations (pg/mg yolk) were quantified in 10 µl aliquots using [1,2,6,7-^{3}H]-testosterone (PerkinElmer, USA, specific activity 63.47 Ci mmol^{-1}) and a specific antibody generated in rabbits against testosterone-3-(carboxy-methyl) oxime bovine serum albumin conjugate [25]. The sensitivity of the assay was 1.62 ± 0.17 pg per tube. The mean recovery rate ± s.d. was 79.3 ± 6.4%. The samples were analysed in two assays. The intra- and inter-assay coefficients of variation were 4.7% and 6.5%, respectively.

To test for (matrilineal) inheritance of yolk T transfer, we analysed the yolk T concentration in the eggs of 22 maternal grandmothers, 24 paternal grandmothers, 29 mothers and 40 (grand-) daughters (electronic supplementary material, S1). Yolk T concentrations were log transformed and standardized within generation before analysis to ensure normality of the residuals and equal variances across generations.

(d) Yolk testosterone manipulation

To explore whether the resemblance in yolk T transfer along the maternal line (see Results) is due to non-genetic priming effects, we experimentally manipulated yolk T levels in eggs and tested (i) if T levels experienced during a female's prenatal development affect the transfer of T into her own eggs later in life and (ii) if the manipulation affects the transfer of T into the eggs of the daughters of these females (i.e. if the manipulation has a transgenerational effect). To this end, we experimentally increased yolk T concentrations in the eggs of half of the females of the second generation before incubation. This manipulation simulates an environmental effect on maternal yolk T transfer (i.e. an environmental maternal effect), as for example observed in response to breeding density [26,27], food availability [28,29] or parasite abundance [19].

We injected eggs with 15 ng testosterone (Sigma-Aldrich, Switzerland) dissolved in 20 µl safflower oil (Sigma-Aldrich, Switzerland) (T-treatment) or with 20 µl safflower oil as a control (C-treatment). Clutches ($n = 29$) were assigned randomly to one of the two treatment groups. The injected dose is equivalent to approximately 1 s.d. of the yolk T content in the study population (mean ± s.d.: 48.4 ± 16.9 ng yolk^{-1}; range: 18.5–83.9 ng yolk^{-1}). Injections were performed at the pointed end of the egg, using an insulin syringe (Terumo, Belgium). The hole in the shell was closed with an adhesive film (Opsite, Smith & Nephew, Switzerland). There was no statistically significant difference in hatching success between T-injected and control eggs [30]. Furthermore, the yolk T manipulation did not significantly affect brood sex ratio (electronic supplementary material, S2). When females originating from T-manipulated and control eggs reached adulthood, we measured the T concentration they transferred to their own eggs (see above). Moreover, we measured the yolk T concentration in the eggs of 26 daughters of these females (as described above) to test for a transgenerational effect of the yolk T manipulation on yolk T transfer.

(e) Statistical analysis

First, we used a linear mixed model to quantify the relationship between the yolk T concentration in the eggs of mothers (explanatory variable) and daughters (response variable). Family ID was included as a random effect to control for the non-independence of siblings.

Second, a similar model, this time with the T concentration in the eggs of the maternal and paternal grandmother as explanatory variables, was used in order to estimate the relationship between the yolk T concentration in the eggs of both grandmothers and their granddaughters. To confirm the results of these linear mixed models, we conducted a model selection procedure using AICc criteria to determine if a model that contains maternal and/or paternal grandmother yolk T best explains yolk T transfer of granddaughters. Candidate models contained combinations of the maternal grandmother's and paternal grandmother's yolk T concentrations. All candidate models contained family ID as a random effect. Model selection was performed using the 'MuMIn' package [31] in R [32].

Third, we tested for an effect of the experimental yolk T manipulation on the transfer of yolk T later in life in (i) females that developed in the manipulated eggs (i.e. directly experienced manipulated T concentrations during their embryonic development) and (ii) in the daughters of these females (to test for transgenerational effects of the manipulation) using linear mixed models that included T treatment, the yolk T concentration in the eggs of the mother and their interaction as fixed effects, and family ID as a random effect. For all linear mixed models, analyses were performed using the package 'lme4' [33] in R [32]. *p*-values were obtained by comparing two nested models, with and without the variable of interest, using likelihood ratio tests.

3. Results

There was a significant positive relationship between the yolk T concentration in the eggs of mothers and daughters ($b \pm$ s.e.: 0.437 ± 0.142; $\chi^2 = 8.185$, $p = 0.004$; figure 1*a*). Similarly, a significant positive relationship between the yolk T concentrations in the eggs of maternal grandmothers and granddaughters was found ($b \pm$ s.e.: 0.366 ± 0.147; $\chi^2 = 5.415$, $p = 0.020$; figure 1*b*). By contrast, yolk T concentrations in the eggs of paternal grandmothers and granddaughters were unrelated ($b \pm$ s.e.: -0.027 ± 0.159; $\chi^2 = 0.001$, $p = 0.973$; figure 1*c*). In comparison, the resemblance in yolk mass between granddaughters and their maternal ($b \pm$ s.e.: 0.266 ± 0.158) or paternal grandmother ($b \pm$ s.e.: 0.250 ± 0.184) was very similar. As a consequence, analysing total yolk T content instead of yolk T concentration gave comparable results in all analyses.

The finding that yolk T deposition is inherited along the maternal line was confirmed by a model selection procedure based on AICc, which revealed that a model containing only the maternal grandmother's yolk T concentration explained the granddaughters' yolk T transfer best. Models that contained additionally the paternal grandmother's yolk T concentration or only the paternal grandmother's yolk T concentration all had ΔAICc > 4.5.

There was no indication that an experimental increase of yolk T levels experienced during prenatal development influences a female's own transfer of yolk T later in life ($\chi^2 = 0.243$, $p = 0.622$; figure 2). Furthermore, the manipulation had no significant transgenerational effect on the yolk T transfer of the daughters of females that developed in the manipulated eggs ($\chi^2 = 0.035$, $p = 0.851$).

4. Discussion

Using a three-generation breeding design, we provide evidence for a significant within-family resemblance in the transfer of yolk T, an important mediator of prenatal maternal effects in oviparous species [15,16]. However, in contrast to what is expected under autosomal inheritance, the resemblances in yolk T transfer between mothers and daughters, and between maternal grandmothers and granddaughters, were very similar, whereas yolk T concentrations in eggs of paternal grandmothers and granddaughters were unrelated. This pattern of resemblance is consistent with female-linked inheritance.

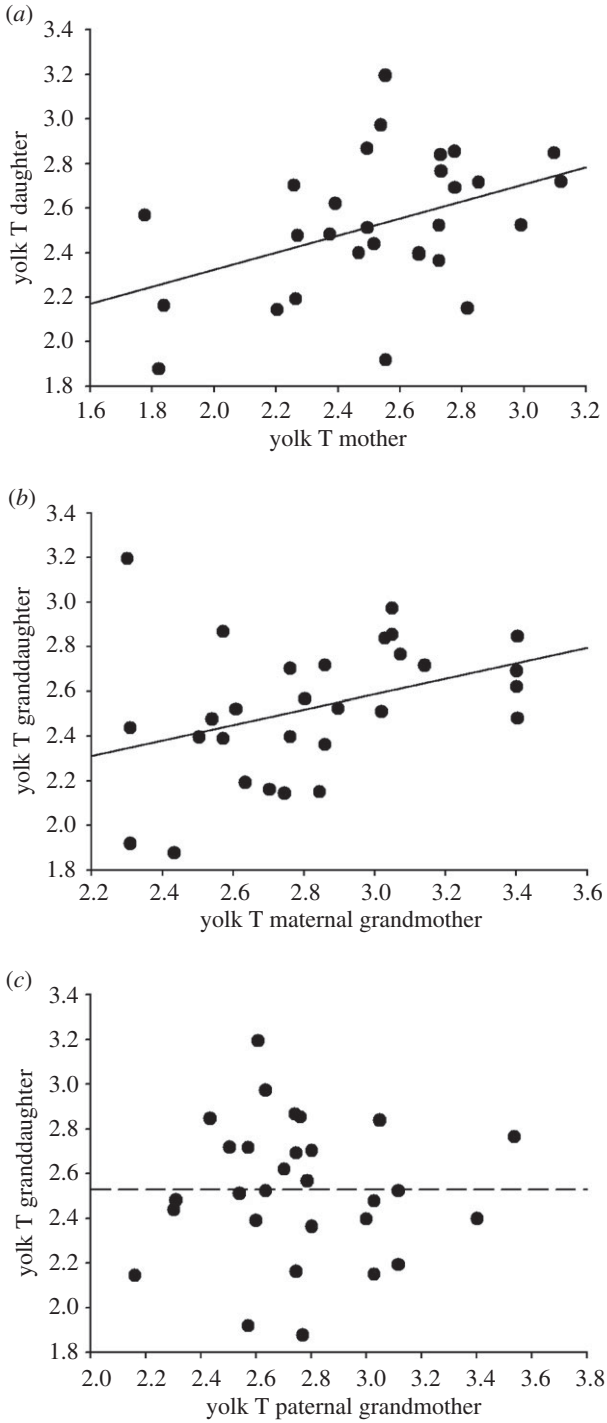

Figure 1. Resemblance in yolk testosterone deposition (log yolk T; pg/mg yolk) among family members. (*a*) Relationship between mothers and daughters; (*b*) relationship between maternal grandmothers and granddaughters; (*c*) relationship between paternal grandmothers and granddaughters.

Sex-linked inheritance can be caused by several non-mutually exclusive mechanisms. First, information on the avian female-specific W chromosome, which is passed on from mothers to daughters, may influence yolk T transfer. Although the W chromosome contains only a few genes [34,35], it plays a key role in regulating female fertility and fecundity [11,12], probably through epistatic interactions between the W

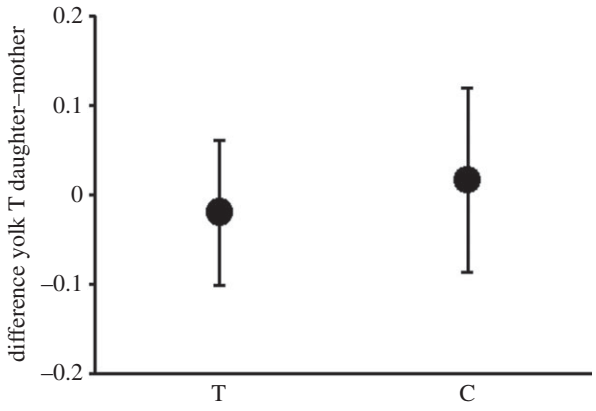

Figure 2. Effect of prenatal testosterone manipulation on the transfer of yolk testosterone to the eggs. Shown is the difference between the yolk testosterone concentration (log yolk T pg/mg yolk) in the eggs of females that have experienced an experimentally increased yolk testosterone level during their prenatal development (T) and females that developed in a control egg (C), and their mother. Means ± s.e. are shown.

chromosome and other parts of the genome [36]. Moreover, the expression of W-chromosome-linked genes has been found to rapidly respond to artificial selection on female reproductive performance [12], again highlighting the important role of W-linked variation in mediating female fitness.

Second, mitochondrial effects may underlie the observed maternal resemblance in yolk T transfer. Mitochondria are, like W chromosomes, inherited along the maternal line and there is accumulating evidence that mitochondrial genetic variation is non-neutral [37,38]. If mitochondrial variation affects yolk T transfer, for example, by influencing a female's metabolic rate [39], this could explain the female-linked inheritance pattern. Indeed, there is a strong positive relationship between a female's resting metabolic rate and the amount of T she transfers to her eggs [40], making this a plausible scenario. Interestingly, positive selection has shaped *ATP5A1W*, a gene on the avian W chromosome that encodes a mitochondrial ATP synthase subunit [41], suggesting that W and mtDNA variation may epistatically interact in shaping female-specific fitness traits [36]. Testing for associations between sequence or structural [42] variation on the W chromosome and/or the mitochondria and variation in yolk T transfer will thus be a fruitful next step, and will allow for an in-depth investigation of the molecular mechanisms underlying the maternal inheritance pattern observed in our study.

Besides sex-limited genetic variation, non-genetic mechanisms [43–45] may contribute to the resemblance in yolk T transfer along the maternal line. For example, prenatal exposure to yolk T may prime (program) a female's yolk T transfer to her own eggs at adulthood. Indeed, experimental manipulations have shown that variation in prenatal T exposure has long-term effects on both circulating T levels as well as T sensitivity later in life [46,47]. We directly tested this hypothesis, but found no evidence that females originating from an egg with experimentally increased T concentration differed in their yolk T transfer from control females. Moreover, we found no evidence for a transgenerational effect of the yolk T manipulation on the deposition of yolk T in the next generation (i.e. in the daughters of females that developed in the manipulated eggs).

The former finding is in line with previous studies in pheasants (*Phasianus colchicus*) [48] and canaries (*Serinus canaria*) [49] that found no effect of experimentally increased prenatal T exposure on T transfer to the eggs. We can exclude that the lack of an effect was due to an unsuccessful manipulation, because the yolk T treatment affected a range of other behavioural and physiological traits in our study [30] as well as in other studies [48,49]. Rather, it suggests that whereas prenatal exposure to T has long-term effects on both circulating T levels and T sensitivity [46,47], it does not affect the transfer of T to the eggs.

Whereas we found no evidence that the T manipulation affected the (overall) yolk T transfer in the next two generations, the manipulation may differentially affect the deposition of yolk T to male and female eggs. However, this scenario appears unlikely given that evidence for differential allocation of T to male and female eggs is weak across species [50], and absent in Japanese quail [51] (see also the electronic supplementary material, S2). Furthermore, although the T manipulation was performed within the natural range, it is possible that the lack of a difference might be due to dose–response effects [52].

Given the highly controlled egg handling, incubation and chick rearing conditions in our study, we can exclude that common postnatal environmental effects contribute to the observed within-family resemblance. However, as a third potential source of matrilineal resemblance, other non-genetic effects such as the transmission of epigenetic states across generations [45], other egg components (e.g. nutrients) that indirectly prime yolk T transfer or genomic imprinting may play a role. Although we can currently not exclude such mechanisms, they are unlikely to explain our results because to date neither the transgenerational transmission of epigenetic marks [53] nor genomic imprinting [54,55] have been documented in birds.

Ultimately, sex-linkage of yolk T transfer may have evolved in response to female-specific selection and/or in order to resolve sexual conflict [2,3]. Although yolk T transfer is a trait that is expressed only in females, any underlying autosomal genes might have pleiotropic effects on traits expressed in males as well [56]. For example, yolk T transfer may not be independent of T levels in the circulation, on which strong sexually antagonistic selection is acting on [57]. Interestingly, the relationship between yolk T and plasma T levels differs across species [58], which may reflect different stages in the resolution of this conflict. Under this scenario, we would predict pronounced sex-linkage of yolk T transfer in species where yolk T and circulating T levels are not correlated (anymore) (e.g. our study species [22]), but no or limited sex-linkage in species where the two traits are (still) correlated (e.g. canary *Serinus canaria* [59]).

In conclusion, we show that yolk T transfer, an important mediator of prenatal maternal effects in oviparous species, is inherited along the maternal line in Japanese quail. We can exclude the possibility that this maternal resemblance is due to common postnatal environmental effects or non-genetic priming effects of prenatal exposure to T on yolk T transfer later in life. Instead, our findings suggest that W-linked and/or mitochondrial variation might underlie the observed inheritance pattern. Female-linked inheritance of maternal effect mediators allows for a fast and direct response to female-specific selection and will thereby affect the dynamics of evolutionary processes mediated by maternal effects, such as the adaptation of populations to changing environments [60] or mother–offspring coadaptation [61].

Ethics. All procedures conform to the relevant regulatory standards and were conducted under licences provided by the Veterinary Office of the Canton of Zurich, Zurich, Switzerland (195/2010; 14/2014; 156).

Data accessibility. Data are available from Dryad http://dx.doi.org/10.5061/dryad.j76q1 [62].

Authors' contributions. B.T. conceived and coordinated the project, conducted the statistical analysis and wrote the manuscript. A.-K.Z., J.L.P. and M.G. collected data and performed the egg manipulation, A.-K.Z., M.O. and M.Z. performed the hormone assays. All authors commented on the manuscript.

Competing interests. We have no competing interests.

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