Matrilineal inheritance of a key mediator of prenatal maternal effects

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Running title: Inheritance of maternal effectors

Abstract

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.

Keywords: maternal effects; yolk androgens; sex-specific selection; *Coturnix japonica;* hormones

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 effects mediators is particularly high in taxa where the female is the heterogametic sex (such as birds). Here we used a three-generation breeding design (ESM 1) 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 to detect 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.

Material and methods

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 x 5.5 m each). For breeding, male-female pairs were transferred to cages (122 x 50 x 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 h : 8 h light : dark cycle at 20 ± 3°C (see [23] for a detailed description of animal husbandry).

94

95 *Egg collection, incubation and offspring rearing*

96 Eggs were collected daily, labelled with a non-toxic marker, and weighed. To
97 standardise incubation and rearing conditions, we artificially incubated the eggs
98 (mean ± SD: 9.5 ± 0.84 eggs per female) (Favorit, HEKA Brutgeräte, Germany;
99 37.8°C, 55% humidity). For hatching, eggs were placed in individual containers to be
100 able to determine which chick hatched from which egg. After hatching, chicks were
101 raised in heated cages in mixed family groups (109 x 57 x 25 cm, Kükenaufzuchtbox
102 4002/C, HEKA Brutgeräte, Germany). Variation in the number of eggs laid while in
103 the breeding cages was small and there was no mother-daughter resemblance in the
104 number of eggs laid (generalised linear mixed model: $\chi^2 = 0.264$, $P = 0.607$).
105 For the yolk T analysis, yolk and albumen of one egg per female (the 5th) were
106 separated, weighed, homogenised, and frozen at –20°C. Previous work has shown that
107 within-clutch variation in yolk T concentration is small in Japanese quail (within-
108 female repeatability across different stages of the reproductive cycle > 0.7 [24]) and
109 the 5th egg is thus representative of a female's yolk T deposition to her eggs. Yolks
110 were collected across three generations (hereafter referred to as maternal and paternal
111 grandmothers, mothers, and (grand-) daughters) to assess the inheritance pattern (see
112 ESM 1). Within a generation, all females had the same age and had experienced the
113 same period of reproductive activity when eggs were collected.

114

115 *Yolk testosterone analysis*

116 Yolk T extraction and radioimmunoassay were performed following previously
117 published protocols [22]. In short, 100-110 mg of yolk were spiked with
118 approximately 2500 dpm of [^{3}H]-testosterone (PerkinElmer, USA) and extracted

119 twice with a mixture of diethyl and petroleum ether (7 : 3). Yolk T concentrations (pg
120 / mg yolk) were quantified in 10 µl aliquots using [1,2,6,7-^{3}H]-testosterone
121 (PerkinElmer, USA, specific activity 63.47 Ci/mmoL) and a specific antibody
122 generated in rabbits against testosterone-3-(carboxy-methyl) oxime bovine serum
123 albumin conjugate [25]. The sensitivity of the assay was 1.62 ± 0.17 pg per tube. The
124 mean recovery rate ± SD was 79.3 ± 6.4%. The samples were analysed in two assays.
125 The intra- and inter-assay coefficients of variation were 4.7% and 6.5%, respectively.
126 To test for (matrilineal) inheritance of yolk T transfer, we analysed the yolk T
127 concentration in the eggs of 22 maternal grandmothers, 24 paternal grandmothers, 29
128 mothers and 40 (grand-) daughters (ESM 1). Yolk T concentrations were log
129 transformed and standardised within generation before analysis to ensure normality of
130 the residuals and equal variances across generations.
131
132
133 *Yolk testosterone manipulation*
134 To explore whether the resemblance in yolk T transfer along the maternal line (see
135 Results) is due to non-genetic priming effects, we experimentally manipulated yolk T
136 levels in eggs and tested 1) if T levels experienced during a female's prenatal
137 development affect the transfer of T into her own eggs later in life, and 2) if the
138 manipulation affects the transfer of T into the eggs of the daughters of these females
139 (i.e. if the manipulation has a transgenerational effect). To this end, we experimentally
140 increased yolk T concentrations in the eggs of half of the females of the second
141 generation before incubation. This manipulation simulates an environmental effect on
142 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 SD of the yolk T content in the study population (mean ± SD: 48.4 ± 16.9 ng / yolk; range: 18.5 – 83.9 ng / yolk). 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 (ESM 2). 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.

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

168 relationship between the yolk T concentration in the eggs of both grandmothers and
169 their granddaughters. To confirm the results of these linear mixed models, we
170 conducted a model selection procedure using AICc criteria to determine if a model
171 that contains maternal and / or paternal grandmother yolk T best explains yolk T
172 transfer of granddaughters. Candidate models contained combinations of the maternal
173 grandmother's and paternal grandmother's yolk T concentrations. All candidate
174 models contained family ID as a random effect. Model selection was performed using
175 the 'MuMIn' package [31] in R [32].
176 Third, we tested for an effect of the experimental yolk T manipulation on the transfer
177 of yolk T later in life in 1) females that developed in the manipulated eggs (i.e.
178 directly experienced manipulated T concentrations during their embryonic
179 development), and 2) in the daughters of these females (to test for transgenerational
180 effects of the manipulation) using linear mixed models that included T treatment, the
181 yolk T concentration in the eggs of the mother and their interaction as fixed effects,
182 and family ID as a random effect. For all linear mixed models, analyses were
183 performed using the package 'lme4' [33] in R [32]. *P* values were obtained by
184 comparing two nested models, with and without the variable of interest, using
185 likelihood ratio tests.
186
187 **Results**
188 There was a significant positive relationship between the yolk T concentration in the
189 eggs of mothers and daughters ($b \pm$ SE: 0.437 ± 0.142; $\chi^2 = 8.185$, $P = 0.004$; Fig. 1A).
190 Similarly, a significant positive relationship between the yolk T concentrations in the
191 eggs of maternal grandmothers and granddaughters was found ($b \pm$ SE: 0.366 ± 0.147;
192 $\chi^2 = 5.415$, $P = 0.020$; Fig. 1B). In contrast, yolk T concentrations in the eggs of

193 paternal grandmothers and granddaughters were unrelated (b ± SE: -0.027 ± 0.159; χ^2
194 = 0.001, P = 0.973; Fig. 1C). In comparison, the resemblance in yolk mass between
195 granddaughters and their maternal (b ± SE: 0.266 ± 0.158) or paternal grandmother (b
196 ± SE: 0.250 ± 0.184) was very similar. As a consequence, analysing total yolk T
197 content instead of yolk T concentration gave comparable results in all analyses.
198 The finding that yolk T deposition is inherited along the maternal line was confirmed
199 by a model selection procedure based on AICc, which revealed that a model
200 containing only the maternal grandmother's yolk T concentration explained the
201 granddaughters' yolk T transfer best. Models that contained additionally the paternal
202 grandmother's yolk T concentration or only the paternal grandmother's yolk T
203 concentration all had ΔAICc > 4.5.
204 There was no indication that an experimental increase of yolk T levels experienced
205 during prenatal development influences a female's own transfer of yolk T later in life
206 (χ^2 = 0.243, P = 0.622; Fig. 2). Furthermore, the manipulation had no significant
207 transgenerational effect on the yolk T transfer of the daughters of females that
208 developed in the manipulated eggs (χ^2 = 0.035, P = 0.851).
209
210 **Discussion**
211 Using a three-generation breeding design, we provide evidence for a significant
212 within-family resemblance in the transfer of yolk T, an important mediator of prenatal
213 maternal effects in oviparous species [15, 16]. However, in contrast to what is
214 expected under autosomal inheritance, the resemblance in yolk T transfer between
215 mothers and daughters, and between maternal grandmothers and granddaughters was
216 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.

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 few genes [34, 35], it plays a key role in regulating female fertility and fecundity [11, 12], likely through epistatic interactions between the W 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 (RMR) 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

241 allow for an in-depth investigation of the molecular mechanisms underlying the
242 maternal inheritance pattern observed in our study.
243 Besides sex-limited genetic variation, non-genetic mechanisms [43-45] may
244 contribute to the resemblance in yolk T transfer along the maternal line. For example,
245 prenatal exposure to yolk T may prime ('program') a female's yolk T transfer to her
246 own eggs at adulthood. Indeed, experimental manipulations have shown that variation
247 in prenatal T exposure has long-term effects on both circulating T levels as well as T
248 sensitivity later in life [46, 47]. We directly tested this hypothesis, but found no
249 evidence that females originating from an egg with experimentally increased T
250 concentration differed in their yolk T transfer from control females. Moreover, we
251 found no evidence for a transgenerational effect of the yolk T manipulation on the
252 deposition of yolk T in the next generation (i.e. in the daughters of females that
253 developed in the manipulated eggs).
254 The former finding is in line with previous studies in pheasants (*Phasianus*
255 *colchicus*)[48] and canaries (*Serinus canaria*)[49] that found no effect of
256 experimentally increased prenatal T exposure on T transfer to the eggs. We can
257 exclude that the lack of an effect was due to an unsuccessful manipulation, because
258 the yolk T treatment affected a range of other behavioural and physiological traits in
259 our study [30] as well as in [48] and [49]. Rather, it suggests that whereas prenatal
260 exposure to T has long-term effects on both circulating T levels and T sensitivity [46,
261 47], it does not affect the transfer of T to the eggs.
262 Whereas we found no evidence that the T manipulation affected the (overall) yolk T
263 transfer in the next two generations, the manipulation may differentially affect the
264 deposition of yolk T to male and female eggs. However, this scenario appears
265 unlikely given that evidence for differential allocation of T to male and female eggs is

266 weak across species [50], and absent in Japanese quail [51] (see also ESM 2).
267 Furthermore, although the T manipulation was performed within the natural range, it
268 is possible that the lack of a difference might be due to dose-response effects [52].
269 Given the highly controlled egg handling, incubation and chick rearing conditions in
270 our study, we can exclude that common postnatal environmental effects contribute to
271 the observed within-family resemblance. However as a third potential source of
272 matrilineal resemblance, other non-genetic effects such as the transmission of
273 epigenetic states across generations [45], other egg components (e.g. nutrients) that
274 indirectly prime yolk T transfer, or genomic imprinting may play a role. Although we
275 can currently not exclude such mechanisms, they are unlikely to explain our results
276 because to date neither the transgenerational transmission of epigenetic marks [53],
277 nor genomic imprinting [54, 55] have been documented in birds.
278 Ultimately, sex-linkage of yolk T transfer may have evolved in response to female-
279 specific selection and / or in order to resolve sexual conflict [3, 56]. Although yolk T
280 transfer is a trait that is expressed only in females, any underlying autosomal genes
281 might have pleiotropic effects on traits expressed in males as well [57]. For example,
282 yolk T transfer may not be independent of T levels in the circulation, on which strong
283 sexually antagonistic selection is acting on [58]. Interestingly, the relationship
284 between yolk T and plasma T levels differs across species [59], which may reflect
285 different stages in the resolution of this conflict. Under this scenario, we would
286 predict pronounced sex-linkage of yolk T transfer in species where yolk T and
287 circulating T levels are not correlated (anymore) (e.g. our study species [22]), but no
288 or limited sex-linkage in species where the two traits are (still) correlated (e.g. canary
289 *Serinus canaria* [60]).

290 In conclusion, we show that yolk T transfer, an important mediator of prenatal
291 maternal effects in oviparous species, is inherited along the maternal line in Japanese
292 quail. We can exclude the possibility that this maternal resemblance is due to common
293 postnatal environmental effects or non-genetic priming effects of prenatal exposure to
294 T on yolk T transfer later in life. Instead, our findings suggest that W-linked and / or
295 mitochondrial variation might underlie the observed inheritance pattern. Female-
296 linked inheritance of maternal effect mediators allows for a fast and direct response to
297 female-specific selection and will thereby affect the dynamics of evolutionary
298 processes mediated by maternal effects, such as the adaptation of populations to
299 changing environments [61] or mother-offspring coadaptation [62].
300

301 **Ethics**

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

305

306 **Data accessibility**

307 Data are available from Dryad (doi:10.5061/dryad.j76q1).

308

309 **Competing interests**

310 We have no competing interests.

311

Authors' contributions

BT conceived and coordinated the project, conducted the statistical analysis and wrote the manuscript. AKZ, JLP and MG collected data and performed the egg manipulation, AKZ, MO and MZ performed the hormone assays. All authors commented on the manuscript.

Acknowledgements

We thank Alison Pick, Barbara Schnüriger, Pascale Hutter, Christina Ebneter and Elif Hanic for help with animal husbandry and data collection, and Erik Postma and two anonymous reviewers for comments on the manuscript.

Funding

The study was financially supported by Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung (PP00P3_128386 and PP00P3_157455) and Fonds zur Förderung des akademischen Nachwuchses (FAN).

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519 **Figure legends**

520

521 **Fig. 1. Resemblance in yolk testosterone deposition (log yolk T; pg / mg yolk)**

522 **among family members.** A) relationship between mothers and daughters; B)

523 relationship between maternal grandmothers and granddaughters; C) relationship

524 between paternal grandmothers and granddaughters.

525

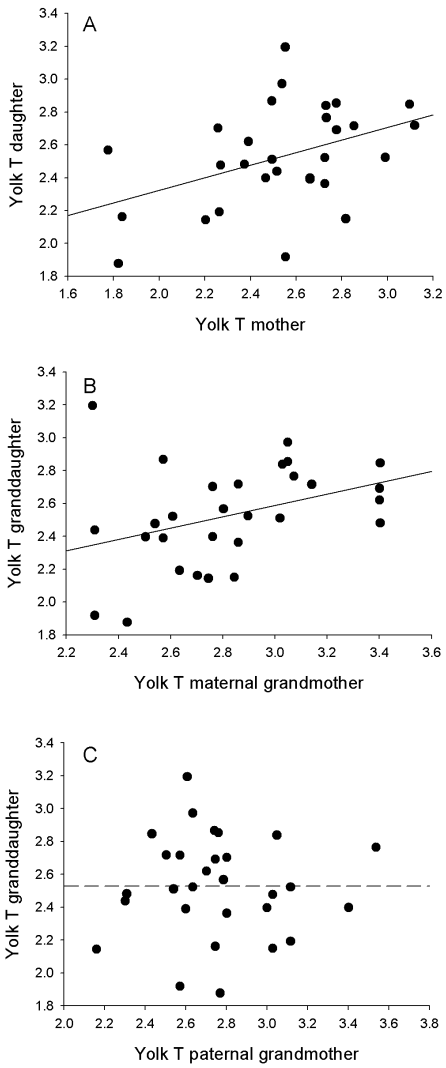

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Fig. 2. Effect of prenatal testosterone manipulation on the transfer of yolk testosterone to the eggs. Shown is the difference between the yolk testosterone concentration (log yolk T pg / mg yolk) in the eggs of females that have experienced an experimentally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