Non-autosomal genetic variation in carotenoid coloration

SIMON R. EVANS[1,2]*, HOLGER SCHIELZETH[3], WOLFGANG FORSTMEIER[4], BEN C. SHELDON[2] & ARILD HUSBY[5]

1. Department of Animal Ecology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, 752 36 Uppsala, Sweden.
2. Edward Grey Institute, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, United Kingdom.
3. Department of Evolutionary Biology, Bielefeld University, Morgenbreede 45, 33615, Bielefeld, Germany.
4. Department of Behavioural Ecology and Evolutionary Genetics, Max Planck Institute for Ornithology, 82319 Seewiesen, Germany.
5. Centre for Biodiversity Dynamics, Department of Biology, Norwegian University of Science and Technology, N-7491, Trondheim, Norway.

* Corresponding author

E-mail: simon.evans@ebc.uu.se, holger.schielzeth@uni-bielefeld.de, forstmeier@orn.mpg.de, ben.sheldon@zoo.ox.ac.uk, arild.husby@ntnu.no

Keywords: Cytoplasmic inheritance, Great tit, Mitochondria, W-linkage, Z-linkage, Zebra finch.

This manuscript is submitted for consideration as an Article and is accompanied by a Supplementary Materials document.

ABSTRACT

Carotenoid-based coloration plays an important role in signaling, is often sexually dimorphic, and is potentially subject to directional and/or sex-specific selection. To understand the evolutionary dynamics of such color traits, it is essential to quantify patterns of inheritance, yet non-autosomal sources of genetic variation are easily overlooked by classical heritability analyses. Carotenoid metabolism has recently been linked to mitochondria, highlighting the potential for color variation to be explained by cytoplasmically inherited factors. In this study, we used quantitative genetic animal models to estimate the importance of mitochondrial and sex chromosome-linked sources of genetic variation in coloration in two songbird populations, in which dietary carotenoids are either unmodified (great tit plumage) or metabolized into alternative color forms (zebra finch beak). We found no significant Z-linked genetic variance in great tit plumage coloration, whilst zebra finch beak coloration exhibited significant W-linkage and cytoplasmic inheritance. Our results support cytoplasmic inheritance of color in the zebra finch, a trait based on endogenously metabolized carotenoids, and demonstrate the potential for non-autosomal sources to account for a considerable share of genetic variation in coloration. Although often overlooked, such non-autosomal genetic variation exhibits sex-dependent patterns of inheritance and potentially influences the evolution of sexual dichromatism.

INTRODUCTION

Intraspecific variation in color has long attracted the attention of evolutionary biologists (Wallace 1881), with conspicuous color patches serving as model traits for ornamentation and for signal traits more generally (Hill 2006a; Hill 2006b). Carotenoid-based colors provide many examples of such prominent coloration and, since Endler's (1980; 1983) seminal work on the guppy (*Poecilia reticulata*), have received considerable attention (Svensson and Wong 2011). The inability of vertebrates to synthesize carotenoid pigments *de novo* means that carotenoids must be sequestered from the environment via the diet. This environmental dependence was assumed to ensure honesty of carotenoid-based signals as predicted by theoretical models (Grafen 1990; Zahavi 1975), though this reasoning, founded on physiological mechanisms, has since been challenged (Griffith et al. 2006).

Carotenoid-based colors are often sexually dichromatic and biologists have long been interested in the mechanisms of inheritance that permit sex-specific expression (Darwin 1871; Winge 1927). However, despite advances in analytical methods, the evolutionary genetics of sexually dimorphic traits remain poorly understood (Fairbairn and Roff 2006). Sex-linked genetic variance is one of several candidate mechanisms that can facilitate the evolution of sexual dimorphism in response to sexually antagonistic selection (Chenoweth et al. 2008; Fairbairn and Roff 2006) and experimental evidence suggests that sexually antagonistic genes accumulate on the sex chromosomes (Gibson et al. 2002; Rice 1992). The response to selection for sex-linked genetic variance will differ from that of autosomally linked genetic variance, due to the lower effective population size of sex chromosomes (Charlesworth et al. 1987). For example, the effective population size of the W chromosome is a quarter that of the autosomes in populations with a 1:1 sex ratio, making W-linked variation more susceptible to genetic drift. Similarly, mitochondrial genetic variation, which is cytoplasmically inherited, exhibits particular evolutionary dynamics since responses to selection are limited to females (Partridge and Hurst 1998). Clearly, knowledge of the distribution of genetic variation must be developed if we are to explain its maintenance in phenotypes exposed to selection – a major aim of evolutionary biology (Charlesworth and Hughes 2000; Pomiankowski and Møller 1995) but one that has proved challenging. Quantitative genetics provides an analytical framework to quantify the contribution of different modes of inheritance.

Recent studies have shown that carotenoid-based colors exhibit moderate heritability (Evans and Sheldon 2012; Hadfield et al. 2007; Schielzeth et al. 2012). However, estimates of heritability may be biased if non-autosomal sources of heritable variation are present but not modeled, leading to erroneous conclusions about the evolvability of a trait. Based on the shared biochemical pathways of intracellular energy production and the oxidation of carotenoids, Johnson and Hill (2013) recently suggested that carotenoid metabolism takes place at the inner mitochondrial membrane, highlighting the potential for functional sequence variation in the mitochondrial genome (Lu et al. 2010) to impact directly on coloration, much as it impacts aerobic performance

(Harrison and Turrion-Gomez 2006; Niemi and Majamaa 2005). Given that mitochondria are maternally inherited (Brown 2008; though paternal leakage of mitochondrial DNA has been reported in birds: Kvist et al. 2003), this raises the possibility that variation in carotenoid-based coloration is associated with extranuclear genetic variation. To our knowledge, this has not been explored in natural populations, although the methodology for estimating mitochondrial contributions to phenotypic variation has been applied within the animal breeding literature (e.g., Garmyn et al. 2011; Harrison and Turrion-Gomez 2006; Pritchard et al. 2008).

Sex chromosome-linked genetic variance might also bias the autosomal genetic variance estimate, leading to inaccurate predictions of evolutionary change (Brooks and Endler 2001). This possibility was demonstrated by Husby et al. (2013), who reported Z-linked heritability of the sexually selected wing patch of collared flycatchers (*Ficedula albicollis*) that was estimated to represent more than three-quarters of the total additive genetic variation; Z-linked color variation is also reported in barn owls (*Tyto alba*; Roulin et al. 2010). W-linked effects are also possible and have been linked to expression of carotenoid-based colors (Schielzeth et al. 2012), though W-linkage has not been quantified independently of other maternally derived factors in this system.

Here, we examine whether non-autosomal sources of inheritance – Z- and W-linked genetic variation, and cytoplasmic inheritance of mitochondria – contribute to color variation in two species in which carotenoid coloration has been studied in detail. The ventral plumage coloration of the great tit (*Parus major*) has been studied in populations across Europe (Evans et al. 2010) and is a characteristic yellow color due to the deposition of dietary carotenoids, these being used as colorants without endogenous chemical modification (Partali et al. 1987). The red beak of the zebra finch is another popular model for studies of animal coloration and is based on the deposition of endogenously metabolized carotenoids into beak tissue (McGraw 2004; McGraw et al. 2002). Husby et al. (2013) reported a maternal effect that explained approximately 3.4% of phenotypic variation in beak coloration, and the inheritance of mitochondrial variants is a possible route by which this effect could be realized (though there are alternative explanations, e.g., Tschirren et al. 2003). Indeed, Schielzeth et al. (2012) report that matrilineal identity explained a significant amount of phenotypic variation in beak coloration in the same population, though W-linked effects could also be responsible, as the W chromosome also follows a matrilineal pattern of inheritance.

METHODS

Great tits

We sampled mature individuals from a nestbox-breeding great tit population at Bagley Wood, near Oxford, UK, from 2008 to 2012. Sampling was conducted from mid-September to June (i.e., excluding the time-period when great tits are undergoing their annual moult: Jenni and Winkler 1994). Sex and age-class (first-year or adult) were assessed according to standard plumage

characteristics (Svensson 1994). Prior to fledging, all nestlings were ringed: if they recruited to the population we therefore had information on their natal origin and parentage. The pedigree is based on behavioral observations (provisioning of nestlings) and will thus assign erroneous parentage for any offspring sired by an extra-pair male. However, extra-pair paternity rates in this species (13% for a nearby population: Patrick et al. 2012) are expected to have only minor impacts on the accuracy of quantitative genetic parameters (Charmantier and Réale 2005). We used dummy coding to represent unknown parents as unrelated founders so that sibship information was retained in the pedigree. The informative pedigree (Morrissey and Wilson 2010) contained 1635 individuals, with 541 maternities, 528 paternities and 441 full sibships, and mean maternal and paternal sibship sizes of 2.11 and 2.12, respectively.

Ventral plumage reflectance was sampled across the bird-visible spectrum (320-700nm: Andersson and Prager 2006) using a photospectrometer. Three measurements were made at the mid-point of a line between the sternum and the right shoulder, using a coincident normal geometry (Andersson and Prager 2006), and the mean of these used as the reflectance spectrum for each record (further details in Evans et al. 2010). Chromatic variation was quantified using *SWS ratio*, a continuous color scale that estimates chromaticity from an avian perspective. Full details are provided elsewhere (Evans et al. 2010) but SWS ratio compares the contrast between regions of high versus low reflectance (reflectance spectra of carotenoid-based colours have a characteristic peak-trough-plateau form: Shawkey et al. 2006), as perceived via the avian visual system (Hadfield 2005). Thus, SWS ratio quantifies spectral purity (saturation) from the perspective of an avian receiver, with large scores representing highly chromatic plumage (raw data: mean = 0.518, standard deviation = 0.172).

We used an 'animal model' to partition individual variation into additive genetic and environmental components, based on the phenotypic similarities of relatives, as well as modeling contributions to phenotypic variance of other included factors. Patterns of sex chromosome inheritance differ from those of autosomes, allowing Z-linked genetic effects to be discriminated from autosomal contributions in some, but not all, familial relationships. For example, the coefficient of relatedness in mother-daughter dyads is 0.5 for the autosomes but zero for the Z chromosome, because females inherit their single Z chromosome from their father (males being the homogametic sex). See Fairbairn & Roff (2006) for further discussion of the contrast between autosomal and sex-linked genetic relatedness matrices.

Based on the model structure of Evans & Sheldon (2012), we partitioned phenotypic variance (V_P) in plumage coloration into eight components:

$$V_P = V_A + V_Z + V_{PE} + V_{NE} + V_M + V_{CP} + V_{YR} + V_R$$

where V_A is autosomal additive genetic variance, V_Z is Z-linked genetic variance, V_{PE} is the permanent environmental variance (other sources of individual-level variation), V_{NE} is the natal environment (brood-of-rearing), V_M represents maternal effects, V_{CP} represents variance attributable to spatial variation at the time of sampling (i.e., a contemporary effect), V_{YR} models annual variance and V_R represents residual deviance. Previous quantitative genetic analyses of plumage coloration in this population reported that the maternal effect estimate was bound at zero (Evans and Sheldon 2012) but we retained this term in our model, following Fairbairn & Roff (2006; see Results for exploration of the potential for a non-zero maternal effect). Due to their maternal route of inheritance, W-linked and cytoplasmic genetic effects are expected to contribute to the maternal effect if not explicitly modeled. The absence of a quantifiable maternal effect therefore suggests that the dataset is not amenable to partitioning matrilineal variance into potential component effects (see also tables A1-A3); W-linkage and cytoplasmically inherited variation were thus not modeled for great tit coloration.

Year was defined relative to the annual moult cycle (*moult-year*). Contemporary plot (CP) refers to the location (aggregated to 12 spatially separated plots within the study site) where the individual was caught. This effect is record-specific, so if individuals are sampled within multiple plots this is incorporated into the model, although, in actuality, a large majority of individuals was only ever caught within a single plot (Evans and Sheldon 2012). The natal environment is defined as the brood-of-origin: each breeding attempt is assigned a unique label, used to define factor levels. Some individuals were first captured after they had fledged, so we had no information on their natal location (66% of all subjects). These individuals are therefore uninformative with respect to the natal environmental effect but they were retained in the dataset because they contribute to estimation of other model parameters. Their natal environment identity was treated as a missing value using the !MVINCLUDE argument in ASReml (Gilmour et al. 2009). This applies similarly to the maternal effect, defined by maternal identity. However, the errors associated with each variance estimate will reflect the information available. In addition to the random effects, we included fixed effects to account for sex, age-class and measurement date (*Septemberday*: Evans et al. 2010; Evans et al. 2012).

Zebra finches

Data for zebra finches derive from a captive population in Seewiesen, Germany (Bolund et al. 2007). The pedigree for this population is genetic and free of errors, as verified by genotype data from a large number of SNP markers (Schielzeth et al. 2012). The pedigree comprises eight generations: a grandparental generation without phenotypic information, followed by three generations (P, F1 and F2) comprising 875 phenotyped individuals (with 1.97 ± 0.80 SD beak reflectance measures per individual), of which 98% were cross-fostered at the egg-stage, and another four generations (F3 to F6) comprising 1786 non-cross-fostered individuals with a single beak reflectance measurement. The data from generations P to F3 have been used in earlier studies (Husby et al. 2013; Schielzeth et al. 2012) and the details of the breeding of the following

generations (F4 to F6) are described elsewhere (Mathot et al. 2013). The informative pedigree contained 2770 individuals, with 2665 maternities, 2681 paternities and 6683 full sibships, and mean maternal and paternal sibship sizes of 6.10 and 6.31, respectively.

Beak reflectance was recorded by photospectrometry, as described elsewhere (Bolund et al. 2007). Reflectance was measured across the spectral range of 260-820nm, with measurements made at five locations on the upper mandible and the mean of these used as the reflectance spectrum for each record. Beak coloration in this species exhibits continuous variation with respect to hue, from orange-yellow to red (Burley and Coopersmith 1987). Thus, to describe chromatic variation requires an index that incorporates information on spectral location. This is particularly important for the current study, given that carotenoid metabolism is a central issue: in zebra finches, metabolism of dietary carotenoids shifts their absorbance spectra to longer wavelengths (i.e., redder), by converting yellow dietary carotenoids to red forms (McGraw et al. 2002; McGraw et al. 2003). A measure such as SWS ratio, which quantifies spectral purity (i.e., saturation), is insufficient as it fails to capture the dimension of spectral variation we are particularly interested in. We therefore summarized chromatic variation in beak reflectance using *beak redness*, a composite metric calculated via linear discriminant analysis (males and females treated as separate groups) that summarizes the variation in six hue variables and has previously been applied to this trait (Husby et al. 2013; Schielzeth et al. 2012). This metric thus condenses spectral location information into a single variable, and correlates strongly with color rankings based on human visual assessment (Bolund et al. 2007). The raw data had a mean of -0.498 and standard deviation of 1.69. Note that this dataset includes >1100 sampling records not included by Husby et al. (2013).

The environmental random effects included in our model of zebra finch beak coloration were similar to those in the great tit analysis, with the exception that contemporary environment was defined by experiment-specific time periods (i.e. measurement sessions of cohorts of birds; 19 levels in total), rather than the separate temporal and spatial effects applied to great tits; this difference reflects the artificial versus natural environments experienced by the populations. However, in contrast to the situation for great tit coloration (Evans and Sheldon 2012), previous work on the zebra finch population has reported significant maternal effects on beak redness (Schielzeth et al. 2012). We examined whether two potential sources of maternally inherited genetic variation contribute to this effect by explicitly including terms representing W-linked additive genetic effects and cytoplasmically inherited effects (i.e., mitochondrial genetic variation). The founders of our zebra finch population are of known parentage so the maternal lineage of all individuals was known. All members of a matriline were assigned the same identifier as they are expected to share a common mitochondrial haplotype.

In birds, females are the heterogametic sex, so both mitochonria and the W chromosome are maternally inherited. W-linkage and cytoplasmic inheritance are thus potentially confounded.

However, these two effects can be discriminated if analyses combine males and females: maternal inheritance of the W chromosome is, by definition, limited to females, whilst cytoplasmic factors are inherited by sons and daughters alike. In an univariate model in which male and female data are combined, defining an appropriate relatedness matrix for W-linked inheritance (i.e., accounting for non-inheritance by males) is non-trivial. To circumvent this issue we constructed a bivariate model in which we concurrently analyzed beak coloration in each sex. Within each random effect, variance and covariance components were constrained, such that the model mimicked an univariate model, with the exception that estimation of the W component (defined by the same matrilineal identifiers as for cytoplasmic inheritance) was limited to females. For all other variance components, estimates in males and females were constrained to equality and intersexual correlations to unity (if covariance estimates were not fixed to zero by design; see Supplementary Materials). The additive genetic correlation between the sexes has previously been estimated to be very large (r_{MF} = 0.93), justifying this approach (Schielzeth et al. 2012). Given these constraints, each random effect in the model accounted for one degree of freedom, as in a standard univariate model. Thus, phenotypic variance in beak coloration is effectively partitioned into nine components for females and eight components in males (see Supplementary Materials for more details):

$$\text{In females: } V_P = V_A + V_Z + V_W + V_C + V_{PE} + V_{NE} + V_M + V_{CE} + V_R$$

$$\text{In males: } V_P = V_A + V_Z + V_C + V_{PE} + V_{NE} + V_M + V_{CE} + V_R$$

Notation is as for the great tit model but with V_W representing W-linked additive genetic variance (in females), V_C representing variance attributable to cytoplasmic sources and V_{CE} representing the contemporary environment (i.e., measurement session). The maternal effects term (V_M; represented by maternal identity) is retained, to control for single-generation effects that would otherwise be confounded with both sex-linked genetic variation (Fairbairn and Roff 2006) and variance due to cytoplasmic inheritance. To ensure that conclusions were not dependent upon model definition, we also constructed two alternative univariate models, in which all male data were either: (i) assigned unique factor levels for the W component (hence all male records have zero relatedness to all other records; table A4); or (ii) assigned the same factor level (representing their shared, 'null' status with respect to the W chromosome; table A5). Both models produced qualitatively similar results to those we present in the main article.

Up to 120 days of age, beak color undergoes an approximately linear change (Burley and Coopersmith 1987), so we followed previous studies (Husby et al. 2013; Schielzeth et al. 2012) by including subadult age as a continuous fixed effect in our model, with samples of individuals older than 120 days assigned a subadult age-value of 120 because beak color is stable after this time (Burley and Coopersmith 1987; Schielzeth et al. 2012). Inbreeding coefficient was also included as a fixed effect (Husby et al. 2013).

Significance testing of variance components

All analyses were conducted in ASReml v.3 (VSN International, Hemel Hempstead, UK) and based on restricted maximum likelihood estimation. Random effects were tested by comparing the full model to one in which the focal effect had been excluded. The test statistic was defined as twice the difference in log-likelihood between the two models and was assumed to follow a mixture of two chi-squared distributions with zero and one degree of freedom, respectively (Self and Liang 1987). Note that this applies to the bivariate zebra finch model as well as the great tit model: for the former, constraints imposed on (co)variance terms of all random effects (see Supplementary Materials) mean that each effect accounted for a single degree of freedom. To facilitate comparison, we also modeled the variance composition of carotenoid coloration via models in which heritable variation was assumed to be wholly autosomal (table A6).

RESULTS

Great tits

Our dataset of ventral plumage coloration in the great tit consisted of 2783 records of 1559 individuals, collected across five consecutive years. Incorporating an effect describing Z-linked genetic variance had little impact on the environmental components included in the model but did reduce the additive genetic variance estimate (figure 1). Z-linked genetic variance explained 4.9 ± 6.9% (estimate ± SE) of phenotypic variance in plumage coloration (conditioned upon fixed effects), though this estimate was non-significant (table 1). As a result, the autosomal heritability estimate was reduced to 23.4 ± 7.5% (in the absence of a random effect estimating Z-linked genetic variance, the heritability estimate was 27.3 ± 5.3%: table A6). Thus, Z-linked effects (although non-significant) were estimated to represent 17% of the total additive genetic variance in plumage coloration. The maternal effect estimate was bound at zero, suggesting that there may be little potential for matrilinearly inherited effects on plumage coloration in our great tit population. However, as currently defined, the natal environment and maternal effects in this model are partly confounded because many known siblings (both full and maternal half siblings) share the same natal environment (brood-of-origin) as well as the same mother. We therefore examined the possibility for maternal effects in this system in greater detail. Firstly, we excluded the natal environment effect from the model, which resulted in a significant maternal effect (5.5 ± 3.3%, $\chi^2 = 3.60$, $P = 0.029$; table A1). However, since this might have erroneously inflated the maternal effect estimate, we used an alternative approach to parameterize the natal environment by defining it at the level of the nestbox plot in which the natal nestbox is sited (*natal plot*; see Evans and Sheldon 2012). Including natal plot in our model reduced the maternal effect estimate to less than half its previous value (2.6 ± 3.1%, $\chi^2 = 0.80$, $P = 0.186$; table A2) and gave strong support for natal environmental variation, as defined at the level of the nestbox plot (4.1 ± 2.6%, $\chi^2 = 6.84$, $P = 0.004$). This suggests that the natal environment effect of our primary model (table 1) is not merely an artifact of confounding with an extant maternal effect. Thus, whilst the dataset does not allow firm conclusions to be drawn, we lack strong evidence for a maternal effect on color expression after the post-fledging moult in this population. If we nonetheless include an

effect representing matrilineal variance (defined as for zebra finches), which will combine any variance effects via W-linkage or mitochondrial variation, we obtain an estimate that lacks statistical support (3.6 ± 3.7%, χ^2 = 0.98, P = 0.161; table A3) and which is bound at zero if we retain only those maternal lines represented by more than one individual (other variance estimates are identical to those listed in table A2).

Zebra finches

The zebra finch dataset contained 3508 records, representing 2661 individuals. With the exception of the W-linkage effect (which was fixed to zero in males), all variance components were constrained to be equal in males and females. Given these model restrictions, phenotypic variance (conditioned upon fixed effects) was smaller for males ($V_{P.male}$ = 0.918 ± 0.051; $V_{P.female}$ = 0.943 ± 0.052). As a consequence, the shared variance parameters are larger in males when expressed as a proportion (table 2); here we present the variance composition for female beak coloration (figure 2). Z-linked genetic variance explained 4.1 ± 3.0% of phenotypic variance, though this was marginally non-significant. The W-linked effect explained 2.6 ± 1.5% of phenotypic variance and was highly significant. Together, the sex chromosomes account for an estimated 6.7 ± 3.2% of phenotypic variance and represent 20.2 ± 9.3% of the total nuclear genetic variance. There was also support for cytoplasmically inherited variance (2.4 ± 1.5% of phenotypic variance), consistent with an influence of mitochondrial variation on carotenoid-based color expression. Since a maternal effect term was included in the model, the cytoplasmic inheritance effect is not expected to be confounded with single-generation, maternally inherited factors (e.g., egg size and composition: Tschirren et al. 2012). Overall, non-autosomal sources of heritable variation were estimated to account for 9.1 ± 3.5% of phenotypic variance and to represent 25.4 ± 9.4% of the total heritable variance in beak coloration.

DISCUSSION

Based on large datasets from two pedigreed populations, we quantified the contribution of non-autosomal sources of genetic variation – Z-linkage, W-linkage and mitochondrial variation – to phenotypic variation in two forms of carotenoid-based color: plumage coloration and beak coloration. We identified a non-significant estimate of Z-linked genetic variance in great tit plumage coloration, the incorporation of which reduced the estimate of autosomal genetic variance from 27% to 23%. For zebra finch beak coloration we modeled multiple sources of non-autosomal genetic variation. We found statistical support for W-linked genetic variance and, in combination, the sex chromosomes represented a considerable share of the total heritable variation in color expression. Further, a significant share of variation in beak coloration was consistent with cytoplasmic inheritance, as expected if mitochondrial variation impacts carotenoid metabolism. Our results thus demonstrate that extranuclear sources of genetic variation can be important to consider when examining factors determining coloration.

The estimate of Z-linked genetic variance in great tit plumage coloration was small (< 10%), though its relative magnitude is similar to estimates of Z-linked genetic variance in other traits in collared flycatchers and zebra finches (Husby et al. 2013). Statistical support was lacking, though this may result from relatively low statistical power: parameter estimation relies on the sampling of relatives for which coefficients of relatedness differ between Z-linked and autosomal genes. Despite such difficulties, it is essential to understand the location (i.e., autosomes versus sex chromosomes) of genetic variation in color traits if we are to improve our understanding of their evolution (Roulin and Ducrest 2013). For genes located on the homogametic sex chromosome (i.e., the Z chromosome in birds), the effective population size is, at most, three-quarters that of autosomal loci. If sexual selection drives higher fitness variation in males (Clutton-Brock 2007) then this discrepancy will be even greater. Reducing the effective population size increases the strength of drift relative to selection and thus alters the expected evolutionary dynamics (Bachtrog et al. 2011).

For the zebra finches, we employed an analytical approach that allowed us to separate the different contributions to the matrilinear inheritance of beak coloration reported previously (Schielzeth et al. 2012) and provided statistical support for both W-linkage and cytoplasmic inheritance. Despite the widespread use of avian models in evolutionary ecology, the possibility of W-linked genetic variation has received little attention and we are not aware of other studies quantifying W-linked heritability of color traits. Y-linked genetic variation in color expression (the Y chromosome in taxa with XX-XY sex determination being the equivalent of the W chromosome in ZW-ZZ taxa; Mank 2012) has been described in guppies (Brooks and Endler 2001; Lindholm et al. 2004; Postma et al. 2011; Winge 1927) but the evolutionary consequences of genetic variation in ornamental color traits being linked to W versus Y chromosomes may differ, at least for non-sex-role reversed species, due to the higher variance in reproductive success we typically expect for males (Bateman 1948). Being present only as a single copy in each sex, the effective population size of the W/Y chromosome is one-quarter that of the autosomes, such that W/Y-linked loci will be particularly susceptible to genetic drift. Additionally, alleles on the W chromosome will always be co-inherited alongside the same mitochondrial alleles, giving rise to linkage disequilibrium that will reduce the efficacy of selection on both mitochondrial and W-linked genes (Berlin et al. 2007; Dowling et al. 2008). Clearly, if our findings are typical of carotenoid colors more generally, the existence of sex chromosome-linked genetic variation will be an important issue to consider in ongoing attempts to improve understanding of the evolutionary dynamics of animal coloration (Roulin and Ducrest 2013).

The localization of carotenoid metabolism to the mitochondria (Johnson and Hill 2013) does not necessitate cytoplasmic inheritance of color variation, as functional mitochondrial variation that impacts on carotenoid metabolism may be absent. However, our analysis of carotenoid-based coloration in the zebra finch suggests that the potential for mitochondrial variation to impact upon color expression is realized and, as such, presents evidence of extranuclear heritable

variation in the color phenotype. Previous work demonstrated the existence of distinct mitochondrial haplotypes within the ancestral population of our study animals (Mossman et al. 2006) and one avenue for future work would be to map color variation to loci within the mitochondrial genome, an approach that has previously been applied to relate variation in human athletic performance to mitochondrial genotypes (Niemi and Majamaa 2005). Mitochondrial genetic variation has been linked to aerobic performance in other species, including birds (Shen et al. 2009; Toews et al. 2014), but consideration of its impact on other phenotypes is more limited (though see Dowling et al. 2008 and references therein). Thus, the extent to which cytoplasmic inheritance is a phenomenon particularly associated with carotenoid coloration is unknown. Heritable variation in the mitochondrial genome might respond more slowly to selection than autosomal genetic variation since: (i) the effective population size is around a quarter that of autosomal genes due to uniparental inheritance and haplodiploidy (Lynch 1997); (ii) negative Hill-Robertson effects arise through co-transmittance with the W chromosome (Berlin et al. 2007; Dowling et al. 2008), as detailed above; and (iii) only selection on females can drive evolution (Partridge and Hurst 1998).

Given the diversity of carotenoid coloration strategies employed by even closely related bird species (Hill and Johnson 2012), we might predict that the importance of functional mitochondrial variation will vary across species. The dietary carotenoids available to songbirds are typically yellow, with some species using these as colorants without modification, while others metabolize diet-derived carotenoids into new forms prior to deposition in the integument (Brush and Power 1976; Hill 1996; Hill and Johnson 2012). These metabolically-derived carotenoids may still be yellow, or may instead be orange or red (Hill and Johnson 2012; McGraw 2006). Given this diversity in the degree of carotenoid processing, we might expect expression of color patches based on metabolized carotenoids (e.g., red patches), to be influenced more strongly by mitochondrial variation than colors based on dietary carotenoids. This is potentially consistent with our results: great tits deposit dietary carotenoids into their plumage without metabolic modification (Partali et al. 1987) whilst zebra finches metabolize dietary carotenoids into alternative forms prior to use as colorants (McGraw et al. 2002). However, due to the limited availability of suitable datasets, our study is restricted to a single comparison and alternative factors could explain the observed difference (e.g., different integumentary tissue types, captive versus free-ranging status of populations). The great tit population is open and many individuals of unknown origin recruit to the population every year, resulting in a very large number of maternal lines being identified, the majority (871 of 1058 matrilines: 82.3 %) represented by a single individual. This population structure, typical of passerine birds, greatly reduces the power to partition out mitochondrial effects, such that wild populations may not be amenable to quantifying matrilineal effects within an animal model framework unless individual-based population records are particularly long running. In contrast, the 2661 phenotyped individuals in our zebra finch population represented 64 identified maternal lineages, and significant W-linked and cytoplasmically inherited effects were quantified. Given these

differences, we are not in a position to draw conclusions on the potential discordance between species using unmodified dietary carotenoids as colorants versus those that metabolize ingested carotenoids prior to deposition in the integument; this may be a worthwhile pursuit for future research efforts.

Acknowledgements

This study was inspired by a discussion with Geoffrey Hill at the 2012 ISBE conference in Lund. We are grateful to A. Roulin, an anonymous reviewer and the editors for comments on previous versions of the manuscript. Great tit study: we are grateful to St. John's College (Oxford) for allowing us to conduct research at Bagley Wood and to their staff for accommodating us; N. Milligan for extensive assistance with data collation; H. Edwards, C. Hinde, J. Howe, C. Isaksson, S. Jones, A. Mennerat, N. Milligan, A. Mouchet, S. Roberts, T. Wilkin, H. Wilkinson and S. Will for fieldwork during breeding seasons; S. Pickett for providing breeding records from previous years; and the National Environment Research Council (NE/D011744/1; to BCS), the European Research Council (AdG 250164; to BCS) and Zoologisk Stiftelsen (to SRE) for funding. Zebra finch study: we thank U. Knief, E. Bolund and K. Martin for help with data collection, which was enabled by an Emmy Noether fellowship (FO340/1-1, FO340/1-2, FO340/1-3; to WF) and by the Max Planck Society (WF). SRE acknowledges the Swedish Research Council (grant to Lars Gustafsson) and Zoologisk Stiftelsen for funding; HS was supported by the German Research Foundation (DFG; SCHI 1188/1-1); and AH acknowledges support from the Norwegian Research Council (214553).

References

Andersson, S., and M. Prager. 2006. Quantifying colors, Pages 41-89 *in* G. E. Hill, and K. J. McGraw, eds. Bird Coloration. Volume I: Mechanisms and measurements. Cambridge, Massachusetts, Harvard University Press.

Bachtrog, D., M. Kirkpatrick, J. E. Mank, S. F. McDaniel, J. C. Pires, W. R. Rice, and N. Valenzuela. 2011. Are all sex chromosomes created equal? Trends in Genetics 27:350-357.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity 2:349-368.

Berlin, S., D. Tomaras, and B. Charlesworth. 2007. Low mitochondrial variability in birds may indicate Hill-Robertson effects on the W chromosome. Heredity 99:389-396.

Bolund, E., H. Schielzeth, and W. Forstmeier. 2007. Intrasexual competition in zebra finches, the role of beak colour and body size. Animal Behaviour 74:715-724.

Brooks, R., and J. A. Endler. 2001. Direct and indirect selection and quantitative genetics of male traits in guppies (*Pecilia reticulata*). Evolution 55:1002-1015.

Brown, K. H. 2008. Fish mitochondrial genomics: sequence, inheritance and functional variation. Journal of Fish Biology 72:355-374.

Brush, A. H., and D. M. Power. 1976. House finch pigmentation: carotenoid metabolism and the effect of diet. Auk 93:725-739.

Burley, N., and C. B. Coopersmith. 1987. Bill color preferences of zebra finches. Ethology 76:133-151.

Charlesworth, B., J. A. Coyne, and N. H. Barton. 1987. The relative rates of evolution of sex chromosomes and autosomes. American Naturalist 130:113-146.

Charlesworth, B., and K. A. Hughes. 2000. The maintenance of genetic variation in life-history traits, Pages 369-392 *in* R. S. Singh, and C. B. Krimbas, eds. Evolutionary Genetics: from Molecules to Morphology. Cambridge, UK, Cambridge University Press.

Charmantier, A., and D. Réale. 2005. How do misassigned paternities affect the estimation of heritability in the wild? Molecular Ecology 14:2839-2850.

Chenoweth, S. F., H. D. Rundle, and M. W. Blows. 2008. Genetic constraints and the evolution of display trait sexual dimorphism by natural and sexual selection. American Naturalist 171:22-34.

Clutton-Brock, T. H. 2007. Sexual selection in males and females. Science 318:1882-1885.

Darwin, C. 1871, The descent of man, and selection in relation to sex. London, UK, Murray.

Dowling, D. K., U. Friberg, and J. Lindell. 2008. Evolutionary implications of non-neutral mitochondrial genetic variation. Trends in Ecology and Evolution 23:546-554.

Endler, J. A. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution 34:76-91.

—. 1983. Natural and sexual selection on color patterns in poeciliid fishes. Environmental Biology of Fishes 9:173-190.

Evans, S. R., A. E. Hinks, T. A. Wilkin, and B. C. Sheldon. 2010. Age, sex and beauty: methodological dependence of age- and sex-dichromatism in great tits. Biological Journal of the Linnean Society 101:777-796.

Evans, S. R., and B. C. Sheldon. 2012. Quantitative genetics of a carotenoid-based colour: heritability and persistent natal environmental effects in the great tit. American Naturalist 179:79-94.

Evans, S. R., A. G. R. Summers, and B. C. Sheldon. 2012. Seasonality of carotenoid-based plumage coloration: modelling wavelength-specific change through spectral reconstruction. Journal of Avian Biology 43:234-243.

Fairbairn, D. J., and D. A. Roff. 2006. The quantitative genetics of sexual dimorphism: assessing the importance of sex-linkage. Heredity 97:319-328.

Garmyn, A. J., D. W. Moser, R. A. Christmas, and J. Minick Mormann. 2011. Estimation of genetic parameters and effects of cytoplasmic line on scrotal circumference and semen quality traits in Angus bulls. Journal of Animal Science 89:693-698.

Gibson, J. R., A. K. Chippindale, and W. R. Rice. 2002. The X chromosome is a hot spot for sexually antagonistic fitness variation. Proceedings of the Royal Society, Series B: Biological Sciences 269:499-505.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, and R. Thompson. 2009. ASReml User Guide Release 3.0. Hemel Hempstead, U. K., VSN International.

Grafen, A. 1990. Biological signals as handicaps. Journal of Theoretical Biology 144:517-546.

Griffith, S. C., T. H. Parker, and V. A. Olson. 2006. Melanin- versus carotenoid-based sexual signals: is the difference really so black and red? Animal Behaviour 71:749-763.

Hadfield, J. D. 2005. The quantitative genetics of plumage colour in the blue tit (*Parus caeruleus*), Imperial College, London, U. K.

Hadfield, J. D., A. Nutall, D. Osorio, and I. P. F. Owens. 2007. Testing the phenotypic gambit: phenotypic, genetic and environmental correlations of colour. Journal of Evolutionary Biology 20:549-557.

Harrison, S. P., and J. L. Turrion-Gomez. 2006. Mitochondrial DNA: An important female contribution to thoroughbred racehorse performance. Mitochondrion 6:53-66.

Hill, G. E. 1996. Redness as a measure of the production cost of ornamental coloration. Ethology, Ecology & Evolution 8:157-175.

—. 2006a. Environmental regulation of ornamental coloration, Pages 507-560 *in* G. E. Hill, and K. J. McGraw, eds. Bird Coloration. Volume I: Mechanisms and measurements. Cambridge, Massechusetts, Harvard University Press.

—. 2006b. Female mate choice for ornamental coloration, Pages 137-200 *in* G. E. Hill, and K. J. McGraw, eds. Bird Coloration. Volume II: Function and Evolution. Cambridge, Massachusetts, Harvard University Press.

Hill, G. E., and J. D. Johnson. 2012. The vitamin A–redox hypothesis: a biochemical basis for honest signalling via carotenoid pigmentation. American Naturalist 180:E127-E150.

Husby, A., H. Schielzeth, W. Forstmeier, L. Gustafsson, and A. Qvarnström. 2013. Sex chromosome linked genetic variance and the evolution of sexual dimorphism of quantitative traits. Evolution 67:609-619.

Jenni, L., and R. Winkler. 1994, Moult and ageing of European passerines. London, Academic Press.

Johnson, J. D., and G. E. Hill. 2013. Is carotenoid ornamentation linked to the inner mitochondria membrane potential? A hypothesis for the maintenance of signal honesty. Biochimie 95:436-444.

Kvist, L., J. Martens, A. A. Nazarenko, and M. Orell. 2003. Paternal leakage of mitochondrial DNA in the great tit (*Parus major*). Molecular Biology and Evolution 20:243-247.

Lindholm, A. K., R. Brooks, and F. Breden. 2004. Extreme polymorphism in a Y-linked sexually selected trait. Heredity 92:156-162.

Lu, J., K. Wang, M. Rodova, R. Esteves, D. Berry, E. Lezi, A. Crafter et al. 2010. Polymorphic variation in cytochrome oxidase subunit genes. Journal of Alzheimer's Disease 21:141-154.

Lynch, M. 1997. Mutation accumulation in nuclear, organelle, and prokaryotic transfer RNA genes. Molecular Biology and Evolution 14:914-925.

Mank, J. E. 2012. Small but mighty: the evolutionary dynamics of W and Y sex chromosomes. Chromosome Research 20:21-33.

Mathot, K. J., K. Martin, B. Kempenaers, and W. Forstmeier. 2013. Basal metabolic rate can evolve independently of morhpological and behavioural traits. Heredity.

McGraw, K. J. 2004. Colorful songbirds metabolize carotenoids at the integument. Journal of Avian Biology 35:471-476.

—. 2006. Mechanics of carotenoid-based coloration, Pages 177-242 *in* G. E. Hill, and K. J. McGraw, eds. Bird Coloration. Volume I: Mechanisms and measurements. Cambridge, Massachusetts, Harvard University Press.

McGraw, K. J., A.-R. E., and R. S. Parker. 2002. Anhydrolutein in the zebra finch: a new, metabolically derived carotenoid in birds. Comparative Biochemistry and Physiology, Part B 132:811-818.

McGraw, K. J., A. J. Gregory, R. S. Parker, and E. Adkins-Regan. 2003. Diet, plasma carotenoids, and sexual coloration in the zebra finch (*Taeniopygia guttata*). Auk 120:400-410.

Morrissey, M. B., and A. J. Wilson. 2010. Pedantics: an R package for pedigree-based genetic simulation and pedigree manipulation, characterization and viewing. Molecular Ecology Resources 10:711-719.

Mossman, J. A., T. R. Birkhead, and J. Slate. 2006. The whole mitochondrial genome sequence of the zebra finch (*Taeniopygia guttata*). Molecular Ecology Notes 6:1222-1227.

Niemi, A.-K., and K. Majamaa. 2005. Mitochondrial DNA and ACTN3 genotypes in Finnish elite endurance and spring athletes. European Journal of Human Genetics 13:965-969.

Partali, V., S. Liaaen-Jensen, T. Slagsvold, and J. T. Lifjeld. 1987. Carotenoids in food chain studies-II. The food chain of *Parus* spp. monitored by carotenoid analysis. Comparative Biochemistry and Physiology 87B:885-888.

Partridge, L., and L. D. Hurst. 1998. Sex and conflict. Science 281:2003-2008.

Patrick, S. C., J. R. Chapman, H. L. Dugdale, J. L. Quinn, and B. C. Sheldon. 2012. Promiscuity, paternity and personality in the great tit. Proceedings of the Royal Society, Series B: Biological Sciences 279:1724-1730.

Pomiankowski, A., and A. P. Møller. 1995. A resolution of the lek paradox. Proceedings of the Royal Society of London, Series B 260:21-29.

Postma, E., N. Spyrou, L. A. Rollins, and R. C. Brooks. 2011. Sex-dependent selection differentially shapes genetic variation on and off the guppy Y chromosome. Evolution 65:2145-2156.

Pritchard, T., C. Chalan, and I. Ap Dewi. 2008. Exploration of cytoplasmic inheritance as a contributor to maternal effects in Welsh Mountain sheep. Genetics Selection Evolution 40:309-319.

Rice, W. R. 1992. Sexually antagonistic genes: experimental evidence. Science 256:1436-1439.

Roulin, A., R. Altwegg, H. Jensen, I. Steinsland, and M. Schaub. 2010. Sex-dependent selection on an autosomal melanic female ornament promotes the evolution of sex ratio bias. Ecology Letters 13:616-626.

Roulin, A., and A.-L. Ducrest. 2013. Genetics of colouration in birds. Seminars in Cell & Developmental Biology 24:594-608.

Schielzeth, H., B. Kempenaers, H. Ellegren, and W. Forstmeier. 2012. QTL linkage mapping of zebra finch beak color shows an oligogenic control of a sexually selected trait. Evolution 66:18-30.

Self, S. G., and K.-Y. Liang. 1987. Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under nonstandard conditions. Journal of the American Statistical Association 82:605-610.

Shawkey, M. D., G. E. Hill, K. J. McGraw, W. R. Hood, and K. Huggins. 2006. An experimental test of the contributions and condition dependence of microstructure in yellow plumage colouration. Proceedings of the Royal Society of London, Series B 273:2985-2991.

Shen, Y.-Y., P. Shi, Y.-B. Sun, and Y.-P. Zhang. 2009. Relaxation of selective constraints on avian mitochondrial DNA following the degeneration of flight ability. Genome Research 19:1760-1765.

Svensson, L. 1994, Identification guide to European passerines. Stockhom, Sweden, Svensson.

Svensson, P. A., and B. B. M. Wong. 2011. Carotenoid-based signals in behavioural ecology: a review. Behaviour 148:131-189.

Toews, D. P. L., M. Mandic, J. G. Richards, and D. E. Irwin. 2014. Migration, mitochondria, and the yellow-rumped warbler. Evolution 68:241-255.

Tschirren, B., P. S. Fitze, and H. Richner. 2003. Proximate mechanisms of variation in the carotenoid-based plumage coloration of nestling Great Tits (*Parus major* L.). Journal of Evolutionary Biology 16:91-100.

Tschirren, B., E. Postma, A. N. Rutstein, and S. C. Griffith. 2012. When mothers make sons sexy: maternal effects contribute to the increased sexual attractiveness of extra-pair offspring. Proceedings of the Royal Society of London, Series B: Biological Sciences 279:1233-1240.

Wallace, A. R. 1881, Island life: or, the phenomena and cause of insular faunas and floras. New York, Harper & Brothers.

Winge, Ö. 1927. The location of eighteen genes in *Lebistes reticulatus*. Journal of Genetics 18:1-43.

Zahavi, A. 1975. Mate Selection—A selection for a handicap. Journal of Theoretical Biology 53:205-214.

Table 1. Estimated composition of phenotypic variance (V_P) in ventral plumage coloration of great tits ($n_{records}$ = 2783; $n_{individuals}$ = 1559). Sex, age and measurement date were included as fixed effects.

Variance source	estimate (se)	χ^2	*P*	proportion of V_P (se)
Residual, V_R	0.0108 (0.0004)	–	–	0.395 (0.036)
Year, V_{YR}	0.0027 (0.0019)	256	<0.001	0.098 (0.064)
Contemporary plot, V_{CP}	0.0003 (0.0002)	15.5	<0.001	0.011 (0.007)
Natal environment, V_{NE}	0.0033 (0.0011)	10.5	0.001	0.122 (0.038)
Maternal, V_M	0.0000 (0.0000)	0.00	0.500	0.000 (0.000)
Permanent environment, V_{PE}	0.0025 (0.0013)	3.36	0.033	0.091 (0.049)
Z-linked additive genetic, V_Z	0.0013 (0.0019)	0.44	0.254	0.049 (0.069)
Autosomal additive genetic, V_A	0.0064 (0.0020)	10.5	0.001	0.234 (0.075)

Table 2. Estimated composition of phenotypic variance (V_P) in beak coloration in zebra finches ($n_{records}$ = 3508; $n_{individuals}$ = 2661). W-linked additive genetic variation was fixed to zero in males, such that phenotypic variance is unequal in the two sexes. Measurement date and inbreeding coefficient were included as fixed effects.

640

Variance source	estimate (se)	χ^2	*P*	proportion of V_P (se)	
				females	males
Residual, V_R	0.356 (0.016)	–	–	0.378 (0.026)	0.388 (0.026)
Contemporary environment, V_{CE}	0.160 (0.042)	314	<0.001	0.170 (0.038)	0.175 (0.038)
Natal environment, V_{NE}	0.038 (0.012)	10.6	0.001	0.040 (0.013)	0.041 (0.014)
Maternal, V_M	0.012 (0.009)	4.79	0.014	0.013 (0.010)	0.013 (0.010)
Permanent environment, V_{PE}	0.038 (0.024)	2.74	0.049	0.041 (0.026)	0.042 (0.027)
Cytoplasmic, V_C	0.022 (0.015)	4.78	0.014	0.024 (0.015)	0.024 (0.016)
W-linked additive genetic, V_W (FEMALES)	0.025 (0.014)	9.98	0.001	0.026 (0.015)	–
Z-linked additive genetic, V_Z	0.039 (0.029)	2.26	0.066	0.041 (0.030)	0.042 (0.031)
Autosomal additive genetic, V_A	0.252 (0.040)	51.6	<0.001	0.267 (0.042)	0.274 (0.043)

Figure 1. Composition of phenotypic variance in the ventral plumage coloration of great tits when excluding (*left*) or including (*right*) an estimate of Z-linked genetic variance (V_Z). Maternal effects were bound at zero. See Methods for definitions and explanations of label terms.

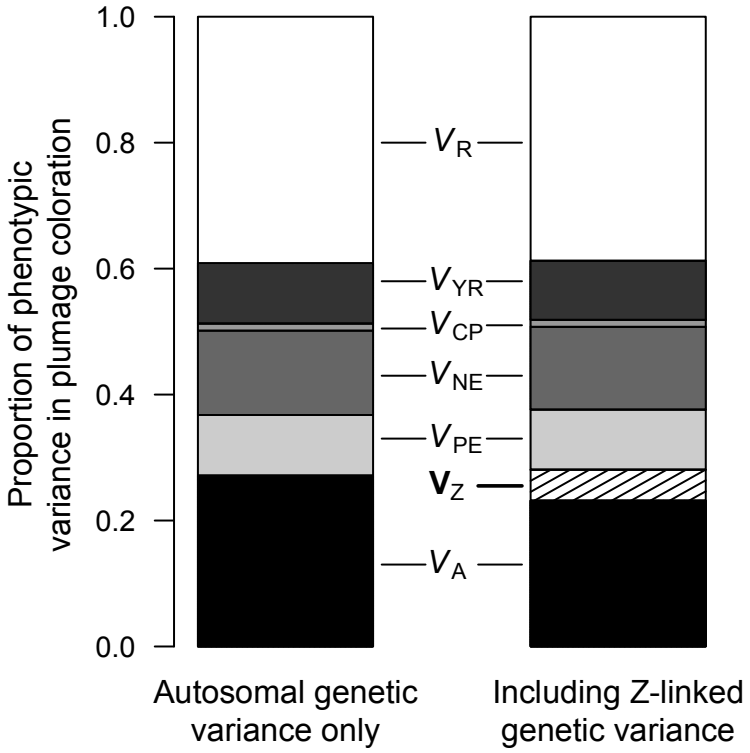
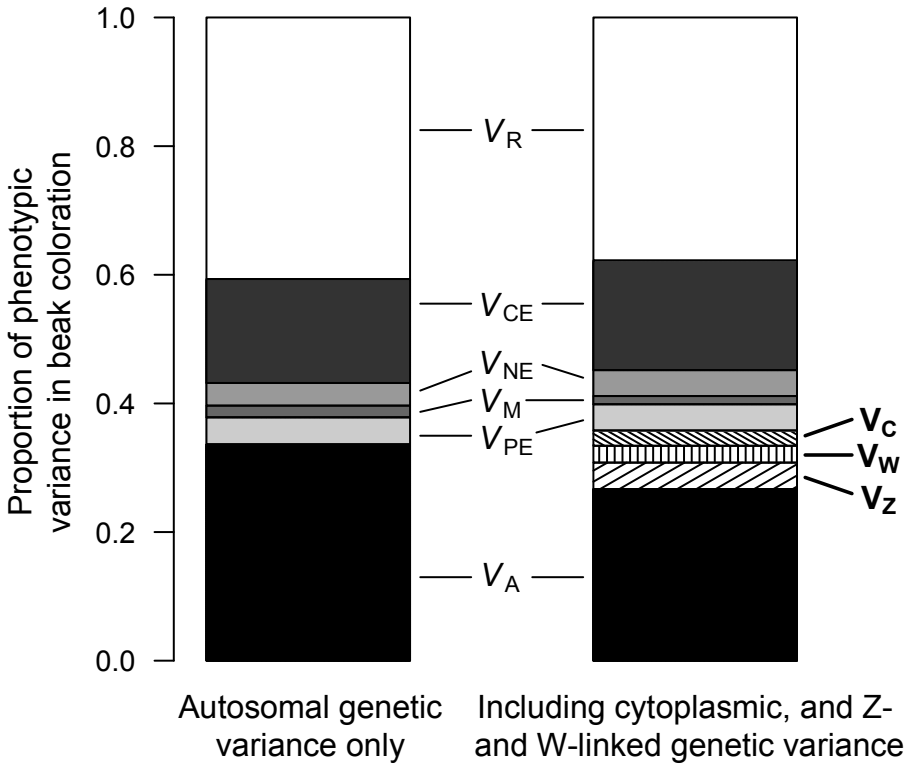


Figure 2. Composition of phenotypic variance in the beak coloration of zebra finches when excluding (*left*) or including (*right*) estimates of variation arising from cytoplasmic (V_C), Z-linked (V_Z) and W-linked (V_W) sources of inheritance. Note that estimates for female beak coloration are presented, as W-linked genetic variance is fixed to zero for male beak coloration; all other variance components were fixed to equality in males and females. See Methods for definitions and explanations of label terms.



Non-autosomal genetic variation in carotenoid coloration: Supplementary Materials

SIMON R. EVANS, HOLGER SCHIELZETH, WOLFGANG FORSTMEIER, BEN C. SHELDON & ARILD HUSBY

Supplementary text explaining the bivariate model of zebra finch beak redness

This bivariate model effectively imitates a univariate model (e.g., the great tit model detailed in the main text) but allows W-linked genetic variance to be limited to females, something that cannot be accommodated within the univariate framework. After conditioning upon fixed effects (subadult age, inbreeding coefficient), phenotypic (*P*) (co)variances in beak redness of male (*brm*) and female (*brf*) zebra finches were partitioned amongst nine random effects as follows:

$$\begin{bmatrix} \sigma^2_{\mathrm{P}\ brm} & \sigma_{\mathrm{P}\ brm,brf} \\ \sigma_{\mathrm{P}\ brf,brm} & \sigma^2_{\mathrm{P}\ brf} \end{bmatrix} = \begin{bmatrix} \sigma^2_{\mathrm{A}\ brm} & \sigma_{\mathrm{A}\ brm,brf} \\ \sigma_{\mathrm{A}\ brf,brm} & \sigma^2_{\mathrm{A}\ brf} \end{bmatrix} + \begin{bmatrix} \sigma^2_{\mathrm{Z}\ brm} & \sigma_{\mathrm{Z}\ brm,brf} \\ \sigma_{\mathrm{Z}\ brf,brm} & \sigma^2_{\mathrm{Z}\ brf} \end{bmatrix} + \begin{bmatrix} 0 & 0 \\ 0 & \sigma^2_{\mathrm{W}\ brf} \end{bmatrix} + \begin{bmatrix} \sigma^2_{\mathrm{C}\ brm} & \sigma_{\mathrm{C}\ brm,brf} \\ \sigma_{\mathrm{C}\ brf,brm} & \sigma^2_{\mathrm{C}\ brf} \end{bmatrix} + \begin{bmatrix} \sigma^2_{\mathrm{PE}\ brm} & 0 \\ 0 & \sigma^2_{\mathrm{PE}\ brf} \end{bmatrix} + \begin{bmatrix} \sigma^2_{\mathrm{NE}\ brm} & \sigma_{\mathrm{NE}\ brm,brf} \\ \sigma_{\mathrm{NE}\ brf,brm} & \sigma^2_{\mathrm{NE}\ brf} \end{bmatrix} + \begin{bmatrix} \sigma^2_{\mathrm{M}\ brm} & \sigma_{\mathrm{M}\ brm,brf} \\ \sigma_{\mathrm{M}\ brf,brm} & \sigma^2_{\mathrm{M}\ brf} \end{bmatrix} + \begin{bmatrix} \sigma^2_{\mathrm{CE}\ brm} & 0 \\ 0 & \sigma^2_{\mathrm{CE}\ brf} \end{bmatrix} + \begin{bmatrix} \sigma_{\mathrm{e}\ brm} & 0 \\ 0 & \sigma_{\mathrm{e}\ brf} \end{bmatrix}$$

Covariances were either (a) fixed to zero (W-linked additive genetic, permanent environment, contemporary environment, residual deviation) or (b) constrained to be equal with the two variance estimates (autosomal and Z-linked additive genetic effects, natal and maternal environments, and cytoplasmic inheritance), based on whether factor levels were nested within sex (e.g., contemporary environment incorporates variation attributable to measurement session and subjects were measured in single-sex batches, so the covariance for this term is fixed to zero). Note that constraining the intersexual covariance to be equal to both the sex-specific variances results in an intersexual correlation of unity (i.e., the same as in a univariate model where both sexes are included).

a) Random effects where covariances were fixed to zero:

i) W-linked additive genetic (*W*)

$\sigma^2_{\mathrm{W}\ brm} = \sigma_{\mathrm{W}\ brm,brf} = 0$

ii) Permanent environment (*PE*)

$\sigma^2_{\mathrm{PE}\ brm} = \sigma^2_{\mathrm{PE}\ brf}$; $\sigma_{\mathrm{PE}\ brm,brf} = 0$

iii) Contemporary environment (CE)

$\sigma^2_{\mathrm{CE}\ brm} = \sigma^2_{\mathrm{CE}\ brf}$; $\sigma_{\mathrm{CE}\ brm,brf} = 0$

iv) Residual deviance (e)

$\sigma^2_{\mathrm{e}\ brm} = \sigma^2_{\mathrm{e}\ brf}$; $\sigma_{\mathrm{e}\ brm,brf} = 0$

b) Random effects where intersexual correlations were fixed to unity:

i) Additive genetic (A)

$\sigma^2_{\mathrm{A}\ brm} = \sigma^2_{\mathrm{A}\ brf} = \sigma_{\mathrm{A}\ brm,brf}$

ii) Z-linked additive genetic (Z)

$\sigma^2_{\mathrm{Z}\ brm} = \sigma^2_{\mathrm{Z}\ brf} = \sigma_{\mathrm{Z}\ brm,brf}$

iii) Cytoplasmic (C)

$\sigma^2_{\mathrm{C}\ brm} = \sigma^2_{\mathrm{C}\ brf} = \sigma_{\mathrm{C}\ brm,brf}$

iv) Natal environment (NE)

$\sigma^2_{\mathrm{NE}\ brm} = \sigma^2_{\mathrm{NE}\ brf} = \sigma_{\mathrm{NE}\ brm,brf}$

v) Maternal (M)

$\sigma^2_{\mathrm{M}\ brm} = \sigma^2_{\mathrm{M}\ brf} = \sigma_{\mathrm{M}\ brm,brf}$

Table A1. Model of great tit coloration (*SWS ratio*) in which natal environment (brood-of-origin) is excluded ($n_{records}$ = 2783; $n_{individuals}$ = 1559). Sex, age and measurement date were included as fixed effects.

Variance source	estimate (se)	proportion of V_P (se)	χ^2	*P*
Phenotypic, V_P	0.0261 (0.0022)	1		
Residual, V_R	0.0108 (0.0004)	0.416 (0.038)	–	–
Year, V_{YR}	0.0027 (0.0020)	0.104 (0.067)	261	<0.001
Contemporary plot, V_{CP}	0.0003 (0.0002)	0.013 (0.007)	17.2	<0.001
Maternal, V_M	0.0014 (0.0009)	0.055 (0.033)	3.60	0.029
Permanent environment, V_{PE}	0.0036 (0.0012)	0.139 (0.049)	8.48	0.002
Z-linked additive genetic, V_Z	0.0014 (0.0019)	0.055 (0.071)	0.56	0.227
Autosomal additive genetic, V_A	0.0057 (0.0019)	0.217 (0.077)	9.08	0.001

Table A2. Model of great tit coloration (*SWS ratio*) in which an estimate of variation attributable to broader-scale natal environment effects (natal nestbox plot [NP]) is included ($n_{records}$ = 2783; $n_{individuals}$ = 1559). Sex, age and measurement date were included as fixed effects.

Variance source	estimate (se)	proportion of V_P (se)	χ^2	*P*
Phenotypic, V_P	0.0263 (0.0023)	1		
Residual, V_R	0.0108 (0.0004)	0.413 (0.039)	–	–
Year, V_{YR}	0.0027 (0.0020)	0.103 (0.067)	258	<0.001
Contemporary plot, V_{CP}	0.0003 (0.0002)	0.011 (0.007)	15.0	<0.001
Natal plot, V_{NP}	0.0011 (0.0007)	0.041 (0.026)	6.84	0.004
Maternal, V_M	0.0007 (0.0008)	0.026 (0.031)	0.80	0.186
Permanent environment, V_{PE}	0.0044 (0.0012)	0.169 (0.049)	12.1	<0.001
Z-linked additive genetic, V_Z	0.0015 (0.0018)	0.056 (0.068)	0.64	0.212
Autosomal additive genetic, V_A	0.0048 (0.0019)	0.181 (0.074)	6.64	0.005

Table A3. Model of great tit coloration (*SWS ratio*) in which an estimate of variation attributable to matrilineal effects (represented by 1058 maternal lines defined using the pedigree) is incorporated into the model presented above in table A2 ($n_{records}$ = 2783; $n_{individuals}$ = 1559). Sex, age and measurement date were included as fixed effects.

Variance source	estimate (se)	proportion of V_P (se)	χ^2	*P*
Phenotypic, V_P	0.0262 (0.0023)	1		
Residual, V_R	0.0108 (0.0004)	0.414 (0.039)	–	–
Year, V_{YR}	0.0027 (0.0019)	0.103 (0.067)	257	<0.001
Contemporary plot, V_{CP}	0.0003 (0.0002)	0.011 (0.007)	15.2	<0.001
Natal plot, V_{NP}	0.0010 (0.0007)	0.039 (0.025)	6.78	0.005
Matriline, $V_{MATRILINE}$	0.0009 (0.0010)	0.036 (0.037)	0.98	0.161
Maternal, V_M	0.0005 (0.0008)	0.019 (0.032)	0.40	0.180
Permanent environment, V_{PE}	0.0045 (0.0013)	0.173 (0.050)	12.6	<0.001
Z-linked additive genetic, V_Z	0.0017 (0.0018)	0.066 (0.070)	0.84	0.180
Autosomal additive genetic, V_A	0.0036 (0.0023)	0.138 (0.089)	2.58	0.054

Table A4. Composition of phenotypic variance in zebra finch beak coloration, estimated using a univariate (i.e., males and females combined) model, where factor levels for W-linked genetic variance were defined as maternal lineage identity in females, whilst each male record was assigned a unique identity (n_{records} = 3508; $n_{\text{individuals}}$ = 2661). Phenotypic variance was partitioned into nine components, as described in the main article, with sex included as an additional fixed effect.

Variance source	estimate (se)	proportion of V_P (se)	χ^2	*P*
Phenotypic, V_P	0.939 (0.060)	1		
Residual, V_R	0.347 (0.019)	0.369 (0.031)	–	–
Contemporary environment, V_{CE}	0.146 (0.051)	0.155 (0.046)	282	<0.001
Natal environment, V_{NE}	0.033 (0.012)	0.035 (0.013)	8.10	0.002
Maternal, V_M	0.011 (0.009)	0.012 (0.010)	1.73	0.094
Permanent environment, V_{PE}	0.035 (0.024)	0.037 (0.026)	2.14	0.072
Cytoplasmic, V_C	0.026 (0.016)	0.028 (0.017)	6.03	0.007
W-linked additive genetic, V_W	0.039 (0.016)	0.042 (0.017)	14.8	<0.001
Z-linked additive genetic, V_Z	0.043 (0.029)	0.046 (0.030)	3.13	0.038
Autosomal additive genetic, V_A	0.260 (0.040)	0.277 (0.043)	57.9	<0.001

Table A5. Composition of phenotypic variance in zebra finch beak coloration, estimated using a univariate (i.e., males and females combined) animal model, where factor levels for W-linked genetic variance were defined as maternal lineage identity in females, whilst all male records were assigned the same identity (n_{records} = 3508; $n_{\text{individuals}}$ = 2661). Phenotypic variance was partitioned into nine components, as described in the main article, with sex included as an additional fixed effect.

Variance source	estimate (se)	proportion of V_P (se)	χ^2	*P*
Phenotypic, V_P	0.949 (0.061)	1		
Residual, V_R	0.370 (0.017)	0.390 (0.030)	–	–
Contemporary environment, V_{CE}	0.145 (0.051)	0.153 (0.046)	284	<0.001
Natal environment, V_{NE}	0.033 (0.012)	0.035 (0.013)	8.32	0.002
Maternal, V_M	0.011 (0.009)	0.012 (0.010)	1.83	0.088
Permanent environment, V_{PE}	0.034 (0.024)	0.036 (0.026)	2.07	0.075
Cytoplasmic, V_C	0.026 (0.016)	0.027 (0.016)	5.78	0.008
W-linked additive genetic, V_W	0.026 (0.015)	0.028 (0.015)	10.2	<0.001
Z-linked additive genetic, V_Z	0.047 (0.029)	0.049 (0.030)	3.70	0.027
Autosomal additive genetic, V_A	0.256 (0.040)	0.270 (0.042)	55.7	<0.001

Table A6. Estimated composition of phenotypic variance in (A) ventral plumage coloration of great tits and (B) beak coloration in zebra finches, when excluding non-autosomal sources of genetic variation. Note that phenotypic variation in zebra finch beak coloration was analyzed using a univariate model, with sex included as an additional fixed factor.

Variance source	estimate (se)	proportion of V_P (se)	χ^2	P
A. Great tit plumage coloration ($n_{records}$ = 2783; $n_{individuals}$ = 1559)				
Phenotypic, V_P	0.0271 (0.0023)	1		
Residual, V_R	0.0108 (0.0004)	0.399 (0.036)	–	–
Year, V_{YR}	0.0027 (0.0019)	0.099 (0.065)	257	<0.001
Contemporary plot, V_{CP}	0.0003 (0.0002)	0.011 (0.007)	15.9	<0.001
Natal environment, V_{NE}	0.0034 (0.0011)	0.125 (0.038)	10.6	<0.001
Maternal, V_M	0.0000 (0.0000)	0.000 (0.000)	0.00	0.500
Permanent environment, V_{PE}	0.0025 (0.0013)	0.092 (0.049)	3.38	0.033
Autosomal additive genetic, V_A	0.0074 (0.0014)	0.273 (0.053)	28.3	<0.001
B. Zebra finch beak coloration ($n_{records}$ = 3508; $n_{individuals}$ = 2661)				
Phenotypic, V_P	0.906 (0.057)	1		
Residual, V_R	0.368 (0.017)	0.406 (0.031)	-	-
Contemporary environment, V_{CE}	0.147 (0.051)	0.162 (0.048)	285	<0.001
Natal environment, V_{NE}	0.032 (0.012)	0.035 (0.014)	7.49	0.003
Maternal, V_M	0.016 (0.010)	0.018 (0.011)	3.76	0.026
Permanent environment, V_{PE}	0.038 (0.025)	0.042 (0.028)	2.64	0.052
Autosomal additive genetic, V_A	0.305 (0.036)	0.336 (0.038)	190	<0.001