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Linking intronic polymorphism on the CHD1-Z gene with fitness correlates in Black-tailed Godwits *Limosa l. limosa*

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We show that variation in an intronic length polymorphism in the CHD1-Z gene in Black-tailed Godwits *Limosa l. limosa* is associated with fitness correlates. This is the second example of the CHDZ-1 gene being correlated with fitness, a previous study having established that Moorhens *Gallinula chloropus* carrying the rare Z* allele have reduced survival. In Godwits, however, carriers of the Z* allele (374 bp) fared better than those with the more frequent Z allele (378 bp) with respect to body mass, plumage ornamentation, reproductive parameters and habitat quality. The Z* allele was found in 14% of 251 adult birds from nature reserves, but was absent from 33 birds breeding in intensively managed agricultural lands. Males and females with the Z* allele had less extensive breeding plumage, and females had a higher body mass, bred earlier and had larger eggs. There were no significant differences in annual survival between birds with and without the Z* allele. DNA isolated from museum skins demonstrated that this polymorphism was present at low frequency in 1929. We speculate that strong asymmetrical overdominance may explain the low frequency of the Z* allele and that genetic linkage to causal genes might be an explanation for the phenotypic correlations. Our findings suggest a degree of cryptic genetic population structuring in the Dutch Godwit population.

Keywords: breeding plumage coloration, intronic polymorphism, molecular sexing, population structure, shorebirds.

Molecular methods of avian sex assignment make use of intronic DNA (Griffiths *et al.* 1996, 1998, Ellegren & Sheldon 1997, Fridolfsson & Ellegren 1999). In birds, males are the homogametic sex (ZZ), whereas females are heterogametic (ZW). Avian sexing methods use PCR-amplification of a non-coding, supposedly neutral fragment of an intron on the conservative CHD1 gene located on both sex chromosomes, labelled CHD1-Z and CHD1-W, which conveniently differ in base pair (bp) length. Males have two fragments of the same length (ZZ genotype), whereas females have two fragments of unequal length (genotype ZW).

However, studies on five auklet species, one rail and three shorebird species report within-sex length variation in the CHDZ locus (Dawson *et al.* 2001, Lee & Griffiths 2003, Schroeder *et al.* 2008a, Casey *et al.* 2009). In some cases, this complicates band interpretation and can lead to incorrect sex assignment (Dawson *et al.* 2001, Robertson & Gemmel 2006). Only one study has examined fitness correlates of this polymorphism:

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in Moorhens *Gallinula chloropus*, Lee *et al.* (2002) reported increased mortality in male chicks with the polymorphism on CHD1-Z. The authors propose that CHD1-Z may have hitchhiked with the causal gene(s).

In Black-tailed Godwits *Limosa l. limosa*, PCR products originating from the Z-chromosome are either 374 bp (the rare type CHD1-Z*) or 378 bp (CHD1-Z) in length. Male Godwits could thus have one of three genotypes: 378/378 bp (CHD1-Z/CHD1-Z, hereafter abbreviated as ZZ), 378/374 bp (ZZ*) or 374/374 bp (Z*Z*). The PCR product of the W-chromosome is 393 bp long and females could have one of two different genotypes: 378/393 bp (ZW) or 374/393 bp (Z*W). Schroeder *et al.* (2008a) found 29% of 70 sexed male Godwits to be of genotype ZZ*, none had the Z*Z* genotype, and 9% of 64 females had the Z*W genotype. Furthermore, ZZ* males had paler breeding plumage than homozygous ZZ males, and this genetic polymorphism is correlated with phenotypic differences (Schroeder *et al.* 2008a).

Here, we present an analysis of correlations between length variation in an intronic amplicon used for molecular sexing and fitness-related traits in Black-tailed Godwits. Because type I statistical errors can never be excluded, we repeated the analysis of Schroeder *et al.* (2008a) on covariation of CHD1-Z with plumage traits with a larger sample size. We then test for covariation of CHD1-Z with the fitness-related variables of presumed quality of the breeding site: body mass, condition, correlates of reproductive success (egg volume and laying-date) and adult survival. We additionally test for the occurrence of this variation in archived DNA from museum specimens from the beginning of the 20th century and discuss possible explanations of the observed patterns.

METHODS

From 2004 to 2007, we captured 121 adult male and 163 female Godwits on their nests in southwest Friesland, The Netherlands. Of these birds, 203 were sampled in our core study area, the Workumerwaard (52°59′44′N, 5°24′45′E), which is described in detail by Schroeder *et al.* (2008a) and by van den Brink *et al.* (2008). The other 81 individuals were caught on surrounding farmlands and in nature reserves. Overall, 251 individuals (109 males and 142 females) were from nature reserves with restricted agricultural management schemes and 33 birds (12 males and 21 females) from intensively managed agricultural land. Birds were captured at the end of incubation (Schroeder *et al.* 2008a), were weighed to the nearest gram, and tarsus + toe length (± 1 mm) was measured. Each individual bird received an individual combination of four colour-rings plus a flag on their tibia.

To quantify plumage, digital pictures were taken of each captured bird with a resolution of 2272 × 1704 pixels using Nikon CoolPix 4500 digital cameras. Seven plumage variables were scored by visual inspection of the pictures: (i) bar score, the extent of black bars on the belly on a scale from one to five; (ii) orange score, the intensity of orange a bird displays on the breast; (ii) white on the head, the percentage of white feathers covering the head in side profile, with an accuracy of 5%; (iv) white spots score, the percentage of the neck covered with white feathers, with an accuracy of 10%; (v) black spots score, the percentage of the neck covered with black spots with an accuracy of 10%; (vi) back score, the extent of breeding feathers covering the back of a bird, on a scale from one to five; and (vii) a count of the absolute number of breeding feathers on the back of a bird. For a more detailed description of these scores and their repeatability see Schroeder *et al.* (2008a).

Length and width of all eggs in the nests were measured (± 1 mm) and egg volume was calculated using the formula $0.52 * \text{length} * \text{width}^2$ (Romanoff & Romanoff 1949). Black-tailed Godwits have an invariate clutch size of four eggs (Cramp & Simmons 1983). Hence, if a female opts for a high investment in a clutch she has to increase the volume of the eggs. The chicks are precocial and for the first few days of their lifes they rely on energy stores left at hatching, which also affects chick survival during the first weeks after hatching (Bolton 1991, Blomqvist *et al.* 1997, Schekkerman *et al.* 2008, J. Schroeder unpubl. data). Once Godwit chicks fledge, annual survival is relatively high (0.70, J. Schroeder unpubl. data). Therefore, we consider egg volume to be a reliable indication of chick survival and hence reproductive success. We do not have a more direct measure of reproductive success because individual fledging success can only be determined reliably with radiotransmitters in Black-tailed Godwits (see Roodbergen & Klok 2008, Schekkerman *et al.* 2008). Recruiting individuals were too few to be used in a statistical analysis. The start of incubation was

estimated by measuring the degree of buoyancy of the eggs in water, as this is related to incubation stage (van Paassen *et al.* 1984, Liebezeit *et al.* 2007).

A blood sample of 20 μL was drawn from the brachial vein of each bird with a sterilized micro-capillary tube. DNA was extracted using the chelex extraction method (Walsh *et al.* 1991). Birds were sexed following the PCR-amplification protocol of Griffiths *et al.* (1998). Fluorescently labelled PCR products were separated on an ABI 377 automatic sequencer and subsequently their exact length was determined using the software GENESCAN 3.1 (Schroeder *et al.* 2008a).

We collected small ($\sim$1 mm^3) skin samples from toe-pads of museum skins of 34 Godwits collected between 1901 and 1931, housed in the Zoological Museum in Copenhagen. The skins were all collected at sites in Denmark. DNA from the skin samples was extracted with DNeasy Tissue Kits (QIAGEN) following the manufacturer's protocol in an archive-DNA clean laboratory at the Royal Ontario Museum (see e.g. Baker *et al.* 2005). Birds were sexed with the primers M5 (Bantock *et al.* 2008) and P8 (Griffiths *et al.* 1998), which prime for a shorter amplicon of the intron than the combination P2 and P8 (Bantock *et al.* 2008). The benefit of this method is that it has a higher chance of success in PCR-amplifying partially degraded DNA isolated from museum specimens. More importantly, it was shown to contain the same genetic polymorphism of the CHD1-Z locus in Moorhens (Bantock *et al.* 2008). We ran negative controls during both DNA extraction and PCR-amplification to exclude artefacts. To verify that the genetic polymorphism observed with this new primer is the same as the one measured with the method of Griffiths *et al.* (1998), we additionally genotyped seven female (two with the Z* allele) and six male (three of them with the Z* allele) contemporary DNA samples with known genotypes as controls using this method.

For each live bird, only data from one capture occasion were used, to prevent pseudoreplication. An individual bird's body mass can vary as a consequence of variation in size or variation in nutritional stores (van der Meer & Piersma 1994). To differentiate between these two possibilities we estimated size-corrected body mass (hereafter called 'condition'). Stepwise linear regression was carried out with body mass as the dependent variable and tarsus to toe length as the predictor variable with sex as a fixed factor. The standardized residuals of this analysis were used as an index of condition ($F_{2,275} = 300.2$, $r^2 = 0.69$, $P < 0.001$). Data on all plumage traits (bar score, orange score, white head, white spots, black spots, back score, breeding feathers) were combined in a principal components analysis. We extracted only factors with eigenvalues > 1. The first two principal components (PC1, PC2) explained 63% of the variation in plumage traits (PCA: Kaiser–Meyer–Olkin measure = 0.74, $\chi^2 = 631.96$, $P < 0.001$). Birds that scored high on PC1 had more breeding feathers on their back, were more orange and had a larger extent of black bars on their belly; they also had less white plumage on the head and neck. Birds that scored high on PC2 had more black spots on their neck. Principal component scores were normally distributed. We found no significant effects of PC2 and therefore do not report on this component from here onwards.

To confirm the results from Schroeder *et al.* (2008a), we first tested univariately for within-sex plumage differences between Z* and Z birds using non-parametric Mann–Whitney *U*-tests. We then performed Generalized Linear Models (GLMs) on PC1, body mass and condition. Sex and Z* were modelled as explanatory factors, and the interaction between them was used to detect differences between the sexes. Birds carrying the more frequent Z allele were coded as 0, and birds with genotype including the Z* allele as 1. Females were coded as 0 and males as 1. As plumage may fade over the course of the season and nutritional status may change over time, we included date of capture as a covariate in the models.

We assessed whether average egg volume per nest and laying-date differed between nests of which at least one parent had a Z* allele and nests of which none of the parents had the Z* allele. Even if the genes of the male partner may not affect these parameters, females may decide on reproductive investment depending on the perceived genetic quality of their mates. Godwits are socially monogamous, mating with the same partner over many years, and share parental care (Beintema *et al.* 1995, J. Schroeder unpubl. data). Related species show low or no extra-pair paternity (Wallander *et al.* 2001, Blomqvist *et al.* 2002) and we have (with respect to the Z genotype) not found evidence of extra-pair paternity in Godwits to date (J. Schroeder unpubl. data). We therefore believe that the majority of our birds are genetically monogamous. As the genotype of both

partners may influence the reproductive parameters, for the control group of that analysis we only used nests for which the genotypes of both parents were known not to contain the Z* allele. This considerably reduced sample size, and to account for all birds, we additionally performed analysis on individual birds (ignoring the genotype of the partner) to determine whether average egg volume per nest and laying-date differed between the sexes and genotypes. A GLM was performed with male and female genotype as explanatory factors (ZZ or ZW was coded as 0, ZZ* or Z*W as 1). For two males of the ZZ* genotype, we only had data on one variable of reproductive output, which explains differences in sample sizes. Egg volume may decline over the course of the season, and egg volume and laying-date also may vary between years (J. Schroeder unpubl. data). Therefore, laying-date was modelled as a covariate with egg volume, and year as a fixed factor in both models. Laying-date, season and year were not significant in any model and we therefore do not report statistics for these variables.

To determine the likelihood of missing a homozygous male (Z*Z*) in a sample the size of our dataset we used a simple randomization model. Genotypes for 121 male birds (92 from the core study area only) were drawn with the expected frequencies for being homozygous Z* or not, and iterated 1000 times.

For the survival analysis, we assembled resighting histories of 190 individuals ringed as adults on the breeding grounds between 2004 and 2008. Individuals were recorded as being alive if caught or observed at least twice during the breeding period from February until July. Model notation follows Lebreton *et al.* (1992). We first set up an *a priori* global model with the parameters that were deemed important (sex, time). Goodness of fit (GOF) of this global model was tested with bootstrap procedures. We calculated the variance inflation factor by dividing the model deviance by the bootstrapped deviance. The model fitted the data well ($P = 0.20$). We used Akaike's information criterion (*AIC*) to select the most parsimonious model (Akaike 1973). As there was no evidence for strong overdispersion ($\hat{c} = 1.08$), we adjusted *AIC* values to allow for the extent of overdispersion measured by $\hat{c}$, using quasi-likelihood (*QAIC*). Preference for one model over another was based on $\Delta QAIC$ larger than two (Burnham & Anderson 2002). To test for the effect of genotype on annual apparent survival (φ), we changed the most parsimonious model and made survival probability dependent on genotype and genotype * sex and report the change in $\Delta QAIC$.

The R.2.7.1 statistical software (R Development Core Team 2008) was used to compute statistics. The *lm*( ) function was used for constructing models and the *step*( ) function (both in the base package) to select the most parsimonious model by *AIC* (Akaike 1973, Burnham & Anderson 2002). We report parameter estimates ± se for all effects that remained significant in the most parsimonious model, with covariates for correction (year, date of season, laying-date) included in the model, and *F*-statistics for each presented parameter as well as the final model. For the survival analysis, the program MARK (White & Burnham 1999) was used.

RESULTS

Eighteen of 121 male (15%) and 15 of 163 female (9%) Black-tailed Godwits carried the Z* allele. We found no homozygous males with the Z*Z* genotype. No deviation from Hardy–Weinberg equilibrium was detected in the dataset ($\chi^2_{\text{males}} = 0.78$, $P_{\text{males}} = 0.93$, $\chi^2_{\text{females}} = 0.38$, $P_{\text{females}} = 0.95$). Given the frequency of the Z* allele in the population (8% of Z-alleles were Z*), we expected 0.7% of all males to be of the Z*Z* genotype, which of our 121 genotyped males would amount to fewer than one individual. The probability of not detecting a homozygous male in a dataset of this size is 0.38.

Data on reproductive success were available for 37 nests where both adult birds were of the more frequent ZZ or ZW genotypes, and for 38 nests where one bird was ZZ* or Z*W. No nest was incubated by two birds with the Z* allele. All adult birds with the Z* allele were caught breeding in nature reserve areas (33 of 251), whereas none of them was caught on intensively managed agricultural land (33 birds; $P = 0.02$, Fisher exact test).

In general, ZZ* males had a paler breeding plumage than ZZ males. ZZ* males had significantly fewer black bars on the breast and more white in the neck plumage than ZZ males, consistent with our earlier results (Table 1). There was no such effect of the Z* allele in female Godwits (Table 1). The first principal component (PC1) of male plumage traits differed between ZZ* and ZZ males, the latter being more ornamented (Fig. 1). The interaction of sex * genotype was removed from the

Table 1. Univariate analyses of the effect of genotypic variation (ZZ, ZZ*, ZW, Z*W) on breeding plumage in male and female Black-tailed Godwits breeding in the Netherlands.

	Males			Females		
	n_{ZZ}/n_{ZZ*}	*Z*	*P*	n_{ZZ}/n_{ZZ*}	*Z*	*P*
Bars	81/14	−2.49	0.01	111/14	−0.26	0.79
Orange	85/15	−0.06	0.96	114/14	−1.74	0.08
White head	85/15	−0.30	0.77	115/14	−0.01	0.99
White	85/15	−2.91	0.004	116/14	−0.08	0.94
Black	85/15	−1.33	0.18	116/14	−0.80	0.42
Back	82/15	−0.45	0.65	113/14	−0.28	0.78
Feathers	71/14	−1.04	0.30	109/11	−0.43	0.67

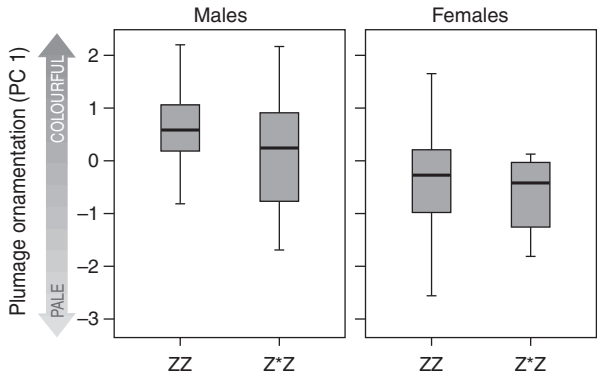

Figure 1. Phenotypic variation in plumage ornamentation of Black-tailed Godwit males and females with different genotypes on the CHD1 gene: ZZ, ZZ*, ZW and Z*W. Plumage ornamentation is presented as PC1 scores; birds scoring higher on PC1 are more ornamented than birds scoring low. Boxes depict the lowest and highest quartiles, lines through the boxes indicate the median and whiskers the range of the observations.

final model (Table 2, parameter estimate when in model: $\beta \pm se_{sex*genotype} = -0.21 \pm 0.39$, $F_{1,187} = 0.28$, $P = 0.60$): thus although the effect seemed more prominent in males than in females (Fig. 1), we could not recover this sexual difference statistically.

Z*W females were on average 13 g heavier than the more frequent ZW females (Fig. 2, Table 2). This was not the case in males; the interaction between sex * genotype remained in the most parsimonious model (Table 2). There was a trend for Z*W females to be heavier in relation to their size, as evidenced by their better condition (Fig. 2, Table 2), whereas we found no effect in males. Although the effect was not significant, the interaction of sex * genotype remained in the final model to explain condition (Table 2). There was no difference in body

Table 2. Model results of the final GLM explaining Black-tailed Godwit breeding plumage ornamentation (measured as PC1), body mass and condition by genotypic variation on the CHD1 gene during late incubation. Date of capture during the season was added to the most parsimonious model as a covariate. The *F*-statistics are for the final model including the (non-significant) date covariate (not presented). Coding: females = 0, males = 1; Z = 0; Z* = 1. PC1: $R^2 = 0.20$; $F_{3,188} = 17.27$; $P < 0.001$. Body mass: $R^2 = 0.62$; $F_{4,277} = 111.2$; $P < 0.001$. Condition: $R^2 = 0.03$; $F_{4,268} = 2.93$; $P = 0.02$.

	$\beta \pm$ se	*F*	*P*
	Plumage ornamentation (PC1)		
Genotype	−0.39 ± 0.20	3.81	0.05
Sex	0.91 ± 0.13	55.51	< 0.001
	Body mass (g)		
Genotype	11.64 ± 2.75	1.34	0.25
Sex	−52.69 ± 2.75	440.53	< 0.001
Genotype * sex	−13.48 ± 8.07	2.80	0.09
	Condition (residuals)		
Genotype	0.43 ± 0.27	0.61	0.44
Sex	−0.31 ± 0.13	9.03	0.003
Genotype * sex	−0.54 ± 0.38	2.06	0.15

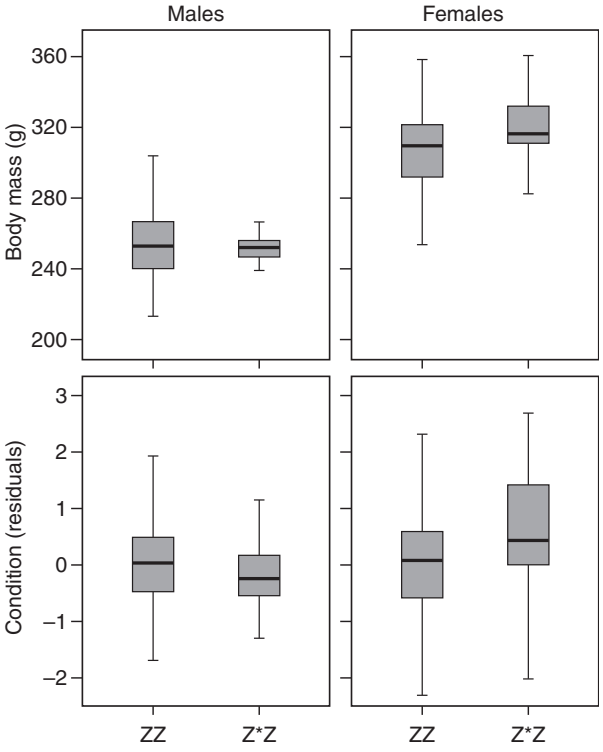

Figure 2. Body mass (g) and condition (residuals of a linear regression of body mass on tarsus–toe length, see text for statistics) of male and female Black-tailed Godwits in relation to genotypic variation on the CHD1 gene. Boxes depict the lowest and highest quartiles, lines through the boxes indicate the median and whiskers extend to the range of the observations.

Table 3. Results of the final model explaining Black-tailed Godwit average egg volume per nest and laying-date as a function of genotypic variation on the CHD1-Z gene of the parents (whether or not a parent carries the Z* allele). Year was added to the most parsimonious model as fixed factor and, in the model with egg volume, laying-date as covariate. The *F*-statistics are of the final model including (non-significant) year and laying-date as main effects (not presented). Egg volume: $R^2 = 0.14$; $F_{5,67} = 2.26$; $P = 0.06$; Laying-date: $R^2 = 0.11$; $F_{4,70} = 2.06$; $P = 0.09$.

	$\beta \pm$ se	*F*	*P*		$\beta \pm$ se	*F*	*P*
	Average egg volume				Laying-date		
Male genotype	1.68 ± 0.87	6.05	0.02	Female genotype	−4.38 ± 2.15	4.27	0.04

dimensions (tarsus + toe length) between the different genotypes. The interaction between sex and genotype, and genotype was removed from the final model and only sex remained (parameter estimates when in model: $\beta \pm se_{sex*genotype} = 0.41 \pm 1.82$, $F_{1,278} = 0.05$, $P = 0.82$; without interaction: $\beta \pm se_{genotype} = 0.77 \pm 0.91$, $F_{1,279} = 0.14$, $P = 0.75$).

Nests of a bird with one Z* allele had a higher average egg volume compared with nests in which both of the incubating birds only had the Z allele ($t = -2.09$, $P = 0.04$; $n_Z = 31$, $n_{Z^*} = 36$). This was mainly due to an effect of ZZ* males incubating at nests that contained larger eggs than those of ZZ males ($t = -2.33$, $P = 0.03$; $n_Z = 103$, $n_{Z^*} = 18$), whereas we did not find such an effect in Z*W females ($t = -0.44$, $P = 0.66$; $n_Z = 148$, $n_{Z^*} = 15$; Fig. 3). A nest-independent GLM of individual genotype confirmed that eggs incubated by ZZ* males were 2 cm^3 larger than eggs incubated in nests by ZZ males, and female genotype did not remain in the most parsimonious model (Table 3). There was no effect of nests with at least one parent having the Z* allele on timing of breeding ($t = 0.75$, $P = 0.46$; $n_{ZZ} = 36$, $n_{ZZ^*} = 31$). At the individual level, Z*W females initiated their clutches earlier ($t = 2.52$, $P = 0.02$; $n_{ZZ} = 148$, $n_{ZZ^*} = 15$), but there was no effect of male genotype on timing of breeding ($t = -0.40$, $P = 0.69$; $n_{ZZ} = 103$, $n_{ZZ^*} = 18$, Fig. 3). The GLM of individual genotypes confirmed that Z*W females initiated their clutches on average 4 days earlier than ZW females, and male genotype was removed in the most parsimonious model (Table 3). As in this model we did not distinguish between nature reserves and regular agricultural habitat, we repeated all the above analyses (plumage, body mass, condition, egg volume and laying-date as response variables) on birds caught only in the core study area, the largest nature reserve with the greatest sample size ($n = 203$, $n_{ZZ} = 75$, $n_{ZZ^*} = 17$, $n_{ZW} = 102$, $n_{Z^*W} = 9$). These analyses gave qualitatively the same results as the full dataset, with lower significance values (all $P < 0.05$). Similar results were obtained when excluding birds from outside of nature reserves (i.e. without the occurrence of Z*), but for laying-date we detected no significant effect ($n = 254$, $n_{ZZ} = 94$, $n_{ZZ^*} = 18$, $n_{ZW} = 127$, $n_{Z^*W} = 15$). This indicates that the links between genotype and fitness correlates do not arise due to a bias of the Z* allele occurring

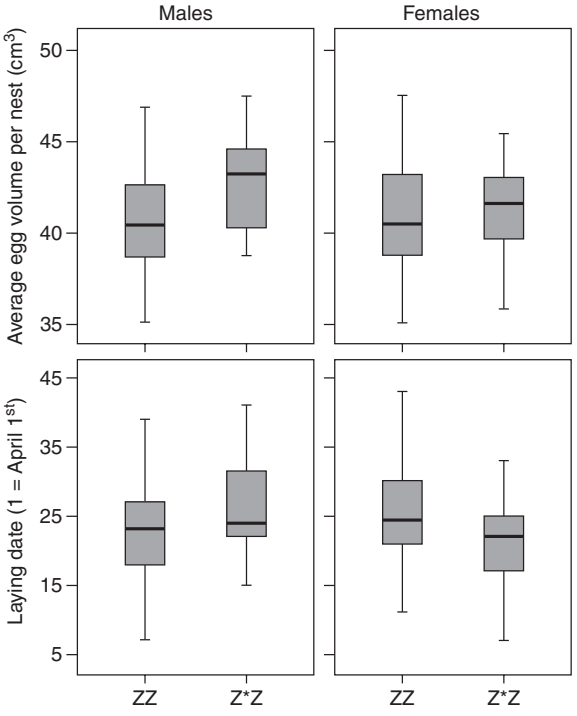

Figure 3. Average egg volume (cm^3) and laying-date in relation to the genotypic variation at the CHD1 gene of male and female Black-tailed Godwits. Boxes depict the lowest and highest quartiles, lines through the boxes indicate the median and whiskers extend to the range of the observations.

only in nature reserves where fitness is higher (R. Kentie unpubl. data).

In the most parsimonious survival model, adult survival was time- and sex-independent (Table 4). Resighting probability was high and independent of year (0.90 ± 0.02 se). Annual adult survival estimated over the 4 years was relatively high ($\varphi = 0.95$). We found no support ($\Delta QAIC < 2$) for a statistical difference between this model and a model including genotype or a model including sex (Table 4, model 1 vs. model 2, model 1 vs. model 3). In the model that includes genotype, birds carrying the Z* allele had a statistically non-significant but higher survival rate of 0.02 than birds with the more frequent Z allele (Table 5).

The CHDZ fragment PCR-amplified from the museum skin samples using the M5–P8 method was 266 bp long, the Z* fragment was 262 bp long and the W-amplicon was 282 bp long and included the same length polymorphism as obtained with the P5–P8 primer pair. We successfully sexed 23 of the 34 museum samples (68% success rate). However, most probably due to allelic dropout, the Z-amplicon of three females could not be detected. We found the Z* allele to be present in one female (from the year 1929) among the remaining 20 samples of known genotype (59% success rate for determining genotype with respect to the Z* allele). We found no correlation between genotyping success and age of the skin.

Table 4. Summary of model statistics of sex and genotypic variation on the CHD1-Z gene (Z*) effects on adult annual survival of Black-tailed Godwits marked during late incubation and resighted within the three following breeding seasons in the Netherlands.

No.	Model	No. of parameters	$\Delta QAIC$	*Q* deviance	*QAIC* weight
1	φ(.)P(.)	2	0	70.45	0.38
2	φ(Z*)P(.)	3	1.75	70.17	0.16
3	φ(sex)P(.)	3	1.96	70.38	0.14
4	φ(.)P(t)	5	2.24	66.57	0.13
5	φ(Z*)P(t)	6	3.82	66.10	0.15
6	φ(t)P(t)	7	4.04	64.25	0.05
7	φ(sex)P(t)	6	4.18	66.46	0.05
8	φ(sex × Z*)P(.)	5	5.41	69.75	0.07
9	φ(sex × Z*)P(t)	8	7.45	65.58	0.01

Table 5. Survival estimates for Black-tailed Godwits breeding in the Netherlands for the three best-supported survival models (Table 4).

Model	Group	φ	se	95% CI
1	All adults	0.950	0.019	0.894–0.976
2	With Z* allele	0.968	0.034	0.778–0.996
	Without Z* allele	0.946	0.014	0.907–0.969
3	Males	0.945	0.019	0.892–0.973
	Females	0.952	0.019	0.894–0.976

DISCUSSION

We report correlations between intronic variation on CHD1-Z and fitness correlates in male and female adult Black-tailed Godwits. This is the second species (the other being in Moorhens, Lee *et al.* 2002) in which variation at the CHD1-Z locus has been linked to fitness-correlated traits.

In a previous study of Black-tailed Godwits, paler males paired with females that laid larger eggs, and were in better condition themselves (Schroeder *et al.* 2009). Here we show that part of this effect may be associated with genetic variation of the Z allele: Z* males are also paler and are indeed paired with females producing larger eggs, and we detected a correlation with female body mass. Our estimate for annual survival is comparable with previous ones (Roodbergen *et al.* 2008). There may be a trend for birds of both sexes with the Z* allele to have a higher survival probability than birds with the more frequent allele ($\varphi_{Z^*} = 0.97 \pm 0.03$ se, $\varphi_Z = 0.95 \pm 0.01$ se, Table 5). We speculate that this pattern was not statistically significant at the 5% level due to limited statistical power. As Black-tailed Godwits are long-lived, a slight increase in survival probability can mean a rather large increase in reproductive life. Moreover, Moorhen chicks with the Z* allele were reported to have a lower survival by Lee *et al.* (2002), suggesting that CHD1-Z variation is linked to genes affecting survival in Moorhens and possibly birds in general, although the direction of the effect apparently can vary. However, it is conceivable that such a correlation will eventually be shown to exist in Black-tailed Godwits. Despite the low frequency of the Z* allele, and consequently small sample sizes for the ZZ* and Z*W genotypes, the effect sizes were usually large, and the consistency of the patterns supports the notion that the correlation of genetic variation with fitness is real. All effects are in the same direction, lowering the chance that our conclusion is based on a type I error.

There was evidence that the genetic variation on CHDZ-1 was already present in the Godwit population 80 years ago, indicating that the Z* allele is not a new mutation. This is supported by the fact that this mutation was found in a number of other bird species, which means that it is either old or has arisen independently in many bird lineages. As the sample size of the historical data is small, we are unable to say whether the allele is changing in frequency. However, despite its presence for at least 90 years and its apparent association with fitness, the allele frequency is relatively low. Therefore we suggest that some degree of stabilizing selection must have been present.

Additional support for the notion that fitness consequences of this variation may be strongly asymmetric with respect to different genotypes is that there was no evidence of assortative mating according to genotype. Such could be expected given that Z* females are of high quality and Z* males are able to attract females of high quality. We did not find any homozygous Z*Z* males; however, this might also be due to chance. This suggests that heterozygotes may have a slight advantage over the homozygous ZZ, but that there must have been strong, counteracting selection against the Z*Z*, or on a phenotype expressed by a linked gene, otherwise one would expect to see higher frequencies.

Associations between genetic and phenotypic variation mainly arise for one of three reasons: (i) the polymorphism indeed affects phenotypic variation directly, (ii) the polymorphism is linked (and in linkage disequilibrium) with other loci on the same chromosome, which causally affects the phenotype, or (iii) the polymorphism reflects underlying, probably cryptic, population structure. As the observed CHD1-Z variation is expected to be neutral (located in a non-coding intron), we do not favour a direct causal relationship as an explanation. However, the CHD1-Z locus may be physically linked with a gene(s) coding for or affecting the studied fitness correlates in Godwits, resulting in the observed correlation between CHD1-Z variation and fitness. Even though genes influencing the expression of male plumage traits are most likely located on the Z sex chromosome (Sætre *et al.* 2003, Gunnarsson *et al.* 2007), it is unlikely that the CHD1-Z gene itself is responsible for this effect. This gene is known to have a role in transcription and gene expression, and therefore is expected to be very conservative and most likely not related to the expression of plumage traits (Stokes & Perry 1995). CHD1 supposedly mediates chromatin structure and organization during transcription and is involved in interactions with DNA and RNA (Ellegren 1996). Because all these functions are involved in basic protein synthesis CHD1 is considered a very conservative gene and should not have a fast mutation rate.

The genetic polymorphism may be linked to a different set of genes responsible for the fitness effects by genetic linkage or epistasis (Lee *et al.* 2002). Genetic linkage and epistasis occur more frequently when the linked alleles are on the same chromosome. This is even more likely if there is only one causal gene that affects a whole suite of traits, including plumage ornamentation and body mass change, as recently suggested by Ducrest *et al.* (2008). The differences between the sexes can also be explained by the fact that the Z* polymorphism (including a linked causal gene) is on a sex chromosome. For example, for body mass, the causal allele associated with Z* is recessive and therefore may only be visible in females. Likewise, the causal allele for plumage might not be expressed in Z*W females or suppressed by genes on the W-chromosome. However, as data from families are, due to the low recruitment rates in Godwits, not available, we can neither support nor exclude the possibilities that variation on CHD1-Z may directly or indirectly be linked with genes affecting fitness. It may also be that epigenetic imprinting plays a role, but detection of such effects is usually difficult and involves elaborate laboratory effort.

The differential occurrence of the Z* allele in breeding habitats of different quality indicates some degree of population structuring. Population structure is likely in Black-tailed Godwits as adult birds are highly faithful to their previous nest-site and in the relatively rare cases where they do change nest-sites, dispersal distances are relatively short (Groen 1993, van den Brink *et al.* 2008, but see Schroeder *et al.* 2008b). In a closely related subspecies, the Icelandic Black-tailed Godwit (*L. l. islandica*), it has been shown that nesting birds are partitioned by habitat quality: birds wintering on high-quality foraging grounds are known to also breed in high-quality breeding grounds and have a higher reproductive success (Gunnarsson *et al.* 2005). This may mean that high-quality birds, including those with the Z* allele, are more likely to be found on high-quality breeding areas, and their offspring with the inherited Z* allele are

likely to breed there, too. Using mitochondrial DNA control region sequences, Höglund *et al.* (2009) did not detect any population structure in Godwits breeding in the Netherlands. Although it is currently not possible to distinguish between the three alternative explanations, we suggest that an important next step will be to conduct more extensive studies to determine whether cryptic population structure in the Dutch Black-tailed Godwit population does exist.

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