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Genetic polymorphism in dopamine receptor D4 is associated with early body condition in a large population of greater flamingos, *Phoenicopterus roseus*

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Abstract

Body condition is an important determinant of fitness in many natural populations. However, as for many fitness traits, the underlying genes that regulate body condition remain elusive. The dopamine receptor D4 gene (DRD4) is a promising candidate as dopamine is known to play an important role in the regulation of food intake and the metabolism of both glucose and lipids in vertebrates. In this study, we take advantage of a large data set of greater flamingos, *Phoenicopterus roseus*, to test whether DRD4 polymorphism predicts early body condition (EBC) while controlling for whole-genome effects of inbreeding and outbreeding using microsatellite multilocus heterozygosity (MLH). We typed 670 of these individuals for exon 3 of the homologue of the human DRD4 gene and 10 microsatellite markers. When controlling for the effects of yearly environmental variations and differences between sexes, we found strong evidence of an association between exon 3 DRD4 polymorphisms and EBC, with 2.2–2.3% of the variation being explained by DRD4 polymorphism, whereas there was only weak evidence that MLH predicts EBC. Because EBC is most likely a polygenic trait, this is a considerable amount of variation explained by a single gene. This is to our knowledge, the first study to show an association between exon 3 DRD4 polymorphism and body condition in non-human animals. We anticipate that the DRD4 gene as well as other genes coding for neurotransmitters and their receptors may play an important role in explaining variation in traits that affect fitness.

Keywords: body condition, candidate gene, dopamine receptor, dopamine receptor D4, greater flamingos, multilocus heterozygosity, single-nucleotide polymorphism

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Introduction

The relative amount of mass in the body not explained by size, that is body condition, is an important determinant of fitness in natural populations (Peig & Green 2009, 2010). In particular, early body condition (EBC) can affect subsequent survival and recruitment into the breeding population in birds (Magrath 1991; Verboven & Visser 1998; Naef-Daenzer *et al.* 2001; Garant *et al.* 2004; Naef-Daenzer & Gruebler 2008), and has been shown to affect dispersal (Edelman 2011) and the development of foraging skills in mammals (Thornton 2008).

As for almost all quantitative phenotypic traits, EBC variability can be explained by both environmental and genetic factors. However, the identification of genes that underlie EBC, as for most phenotypic variations, remains by and large elusive (Phillips 2005; Mitchell-Olds *et al.* 2007; Mackay *et al.* 2009). This is despite the fact that a major goal for evolutionary biologists is to identify genes that underlie the phenotypic variation in natural populations and to understand the consequences of polymorphism of these loci on fitness (Phillips 2005; Mitchell-Olds *et al.* 2007; Mackay *et al.* 2009).

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A difficult challenge for molecular biologists to overcome is that the link between phenotype and genetic variation is often complex (Phillips 2005; Mackay *et al.* 2009). Recently, there has been some success in explaining body condition variation using genetic markers by exploring the correlation between body condition or body fat and genetic markers in controlled crosses (QTL analysis) (e.g. Abasht *et al.* 2006; Moghadam *et al.* 2007; Rao *et al.* 2007; Küttner *et al.* 2011). However, this method is laborious and difficult to achieve in nonmodel organisms that cannot be kept and crossed in laboratory conditions. In addition, QTL approaches have been more successful at identifying genomic regions rather than specific loci which explain phenotypic variation (Phillips 2005; Mackay *et al.* 2009). Direct genotyping of homologous candidate genes which are known to predict traits in model organisms such as the chicken, mouse and humans that are equivalent to fitness traits in natural populations may provide new insights into our understanding of the relationship between phenotype and genetic variation. Recent studies in molecular ecology have started taking that direction (for a review see Fitzpatrick *et al.* 2005).

A promising candidate gene is the dopamine receptor D4 (DRD4) gene that codes for one of five types of dopamine receptors in vertebrates (Callier *et al.* 2003). Neuronal control via various neurotransmitters is known to mediate energy intake and expenditure (for reviews see Meister 2007; Gao & Horvath 2008). Dopamine is one of these important neurotransmitters that regulates many different functions in the central nervous system which in turn affects many traits such as metabolism, food intake, the control of body temperature, and the expression of novelty seeking behaviour (Callier *et al.* 2003). Dopamine seems to play an important role in regulating food intake by modulating food reward via the mesolimbic circuitry of the vertebrate brain (for reviews see Meister 2007; Gao & Horvath 2008; Wang *et al.* 2009). Furthermore, glucose and lipid metabolism, two important determinants of weight gain, are strongly affected by dopaminergic neurotransmission. Indeed, in seasonally obese animals, changes in dopaminergic neural activities are known to play a crucial role in the adjustment of body condition (for a review see Pijl 2003).

In vitro studies in humans suggest that a DRD4 allele (the exon 3 DRD4 7-*repeat* allele) has decreased affinity for dopamine and transmits weaker intracellular signals in comparison with other exon 3 alleles (Asghari *et al.* 1995). In addition, in humans, weight gain has been associated with low brain dopamine activity and different exon 3 genotypes of the DRD4 gene are associated with differences in body mass index (BMI; the equivalent of body condition in human studies) and food intake (Levitan *et al.* 2004a,b, 2006; Sobik *et al.* 2005; Guo *et al.* 2006, 2007; Eisenberg *et al.* 2008). To what extent DRD4 polymorphism is associated with differences in food intake and expenditure, and therefore differences in body condition, in other vertebrate species is still undocumented. Reflecting the many different functions dopamine seems to regulate, several recent studies have also shown an association between DRD4 polymorphism and variation in novelty seeking and exploratory behaviour, in both humans (for reviews see Savitz & Ramesar 2004; Ebstein 2006) and non-human vertebrates (Momozawa *et al.* 2005; Bailey *et al.* 2007; Fidler *et al.* 2007; Hejjas *et al.* 2007; James *et al.* 2007; Flisikowski *et al.* 2009; Korsten *et al.* 2010). Such pleiotropism of the DRD4 gene further validates it as a promising candidate gene to explain variance in body condition because individual variation in behaviour is expected to covary with other life history, physiological and metabolic traits (for reviews see Biro & Stamps 2008, 2010; Houston 2010; Réale *et al.* 2010).

In this study, we take advantage of a large data set of greater flamingos, *Phoenicopterus roseus*, to test whether DRD4 polymorphism predicts EBC. As in many birds, EBC has been found to be an important predictor of postfledging dispersal in flamingos (Barbraud *et al.* 2003). Because postfledging dispersal is an important ecological process with numerous fitness implications (Hamilton & May 1977; Comins *et al.* 1980; Greenwood & Harvey 1982; Clobert *et al.* 2001), EBC may be an important predictor of fitness in flamingos. Indeed, Sanz-Anguilar *et al.* (in press) have found that survival differed according to dispersal strategies in greater flamingos. Long-distance dispersers survival was lower than residents or intermediate-distance dispersers for one- and two-year old birds, while the opposite trend was found for older birds (Sanz-Aguilar *et al.* in press).

The deleterious effects of inbreeding and outbreeding on the whole genome may also have an effect on phenotype variation (David *et al.* 1995; Coltman & Slate 2003; Chapman *et al.* 2009; Szulkin *et al.* 2010), including body condition (e.g.: Lieutenant-Gosselin & Bernatchez 2006). If inbreeding is recurrent in the population then theory predicts a correlation in heterozygosity and/or homozygosity across loci, known as identity disequilibrium (ID) (Szulkin *et al.* 2010). In addition, if there is admixture between populations (outbreeding), alleles at two loci may be preferentially associated in gametes [linkage disequilibrium (LD)] and the random association of gametes yields an excess of double-heterozygous genotypes (therefore also resulting in ID) (Szulkin *et al.* 2009). Thus, if inbreeding and outbreeding are strong in the population, microsatellite multilocus heterozygosity (MLH) may reflect genome-wide heterozygosity as a result of ID (Coltman & Slate 2003; Chapman *et al.* 2009; Szulkin *et al.* 2010). Associations

between MLH and fitness traits are known as heterozygosity-fitness correlation (HFC) (Coltman & Slate 2003; Chapman *et al.* 2009; Szulkin *et al.* 2010). To control for any potential artefactual effects of DRD4 polymorphism on EBC that may be due to inbreeding and/or outbreeding, we also explore the statistical association between MLH and EBC and between MLH and DRD4 heterozygosity.

Long-term monitoring of the Camargue population of greater flamingos (Johnson & Cézilly 2007) has revealed that environmental conditions, such as water levels around the breeding colony, significantly affect chick body condition (Cézilly *et al.* 1995; Béchet & Johnson 2008). Another important factor that may influence body condition is differential allocation of nutritional resources towards growth and storage between males and females. Therefore, we statistically controlled for the effects of both sex and annual environmental conditions (over four consecutive years) when investigating whether DRD4 polymorphism influences EBC.

Methods

Study area and species

Greater flamingos have bred every year at the Fangassier's lagoon (43°25′N, 4°37′E; Salin de Giraud, Camargue, southern France) since 1969 (except in 2007; Béchet *et al.* 2012). Between 1995 and 1998, blood samples were collected from chicks during ringing operations in July/August and preserved in a blood buffer (Seutin *et al.* 1991). Chicks were caught on average 106 days after the start of laying just before the oldest chicks fledged, by herding the crèche into a corral. Thus, a random sample of chicks, which included early- and late-hatching birds, were captured (approximate range 35–77 days) (Johnson & Cézilly 2007). During the ringing operation, chicks were marked individually with PVC plastic rings engraved with a four-digit code and measured (tarsus length to the nearest 1 mm and body weight to the nearest 50 g using a 5-kg Pesola spring balance; see Johnson & Cézilly (2007) for details). Descriptive statistics of mean chick weight and tarsus length according to sex and cohort year are provided in Table 1. As greater flamingos only lay one egg per season and switch mates systematically between consecutive breeding seasons, there is no sibship relationships in this study (Cézilly & Johnson 1995; Johnson & Cézilly 2007).

DNA extraction and sexing

DNA was extracted using a standard phenol–chloroform method (Hillis *et al.* 1996). The quality and concentration of DNA extracted was estimated by UV spectrophotometry (Spectramax plus 384, Molecular devices) to ensure that a final dilution of approximately 50 ng/μL was achieved. Molecular sexing was based on CHD gene sequences polymorphism and was carried out as described by either Bertault *et al.* (1999) or Balkız *et al.* (2007).

Table 1 Mean and standard deviation of chick weight (g) and chick tarsus length (mm) according to cohort and sex

Year	Sex	Sample size	Mean weight (SD)	Mean tarsus length (SD)
1995	Female	68	1877 (348)	198 (20)
	Male	60	2152 (432)	216 (21)
1996	Female	40	2673 (343)	228 (13)
	Male	38	2869 (411)	243 (20)
1997	Female	127	2311 (331)	210 (15)
	Male	132	2585 (383)	221 (17)
1998	Female	120	2044 (411)	199 (21)
	Male	85	2303 (434)	214 (22)
Total		670	2311 (472)	213 (22)

DRD4 genotyping

Six hundred and seventy individuals belonging to four cohorts: 1995 (n = 128), 1996 (n = 78), 1997 (n = 259) and 1998 (n = 205) were sequenced for DRD4 exon 3. DRD4 exon 3 sequences from *Gallus gallus* DRD4 (NM001142849 and FJ217173), *Parus major* (DQ006802) and *Taeniopygia guttata* (GQ359780) were aligned manually using MEGA version 5 (Tamura *et al.* 2011) allowing the definition of two degenerated primers: F1-E3-DR4D (5′-CCRCTSAACTACAACCGGCG-3′) and R1-E3-DR4D (5′-YTCCCGGCCGTTGATCTTGG-3′). These primers amplify 486bp of DRD4 exon 3. Polymerase chain reactions (PCRs) were performed with approximately 40 ng of extracted DNA in 25 μL reactions containing 50 μM of each dNTP (Euromedex), 200 nM of each primer, 0.15 units of Manual HotMaster™ *Taq* DNA polymerase (5′) and 2.5 μL of the supplied 10× buffer. PCR programme comprised an initial denaturation step of 3 min at 95 °C, 35 cycles at 94 °C for 45 s, 60 °C for 60 s and 65 °C for 60 s, followed by a final extension step at 65 °C for 5 min. Prior to direct sequencing PCR products were purified by using exonuclease 1 and shrimp alkaline phosphatase (Fermentas Life Sciences). Products were then sequenced using the forward primer F1-E3-DR4D at the Macrogen Sequencing Service (Macrogen Inc., South Korea). Sequence editing, BLAST searches and multiple sequence alignments carried out using clustalw were executed in MEGA. Six synonymous single-nucleotide polymorphism (SNP) sites were identified.

A total of 23 DRD4 exon 3 genotypes were identified at varying frequencies (Fig. 1a).

Thirteen heterozygote individuals (Fig. 1a) encompassing all putative alleles were cloned to enable the characterization of every DRD4 exon 3 allele. Amplicons were cloned using the pGEM-T Easy Vectors (Promega) kit and JM109 competent cells (Promega) by following manufacturers' guidelines. At least seven clones were sequenced from each individual and each allele was sequenced in at least two individuals. Eight alleles, named a to h (GenBank Accession entries for alleles are JQ425594, JQ425595, JQ425596, JQ425597, JQ425598, JQ425599, JQ425600 and JQ425601, respectively), were identified from cloned sequences. Genotypes of all remaining directly sequenced individuals were inferred based on a combination of these eight alleles, allowing the definition of the 23 genotypes. Although we cannot be certain that all alleles were identified, the fact that all of the eight alleles identified (each sequenced in at least two individuals) allowed the definition of the 23 observed genotypes would suggest that they were. Furthermore, when analysing the association of DRD4 polymorphism and EBC, we used only the five most common genotypes (see below), which were either cloned (ac and bc), homozygotes (aa and bb) or a heterozygote genotype of the two most common alleles in the

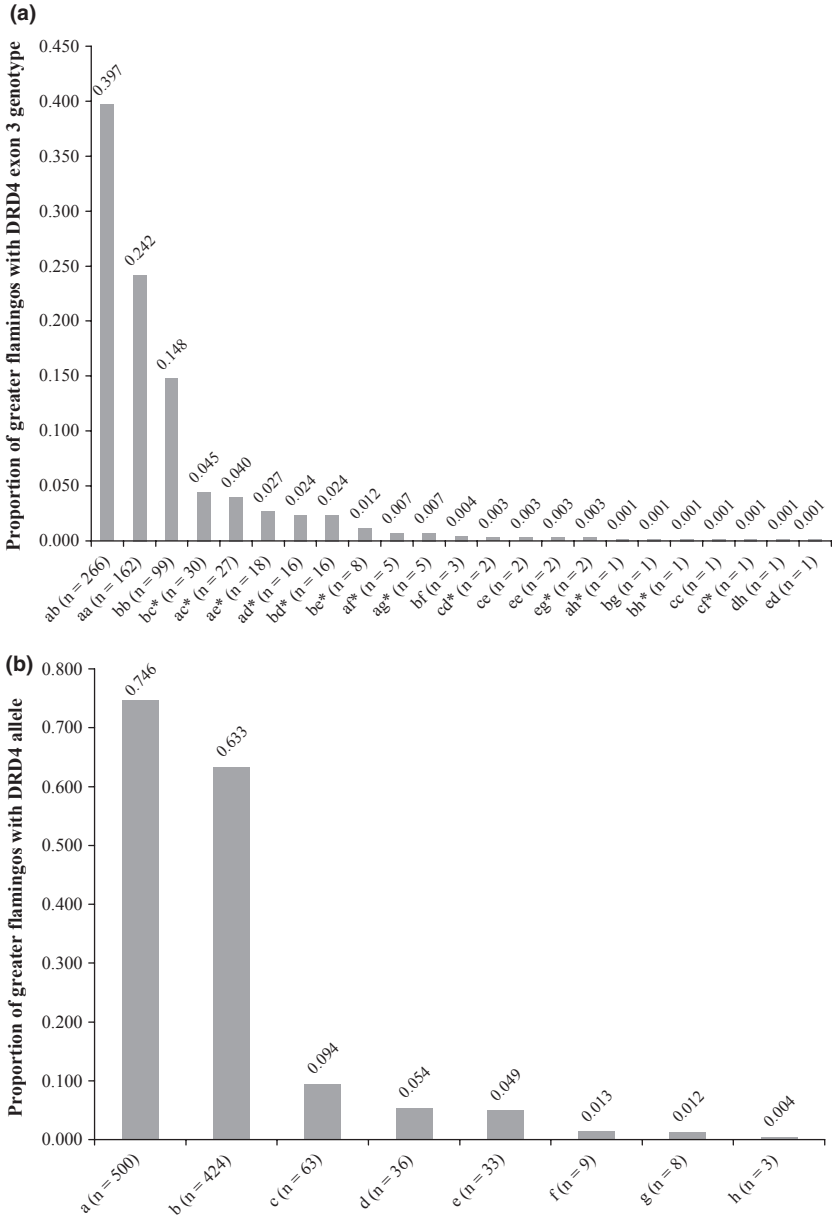

Fig. 1 Proportion of greater flamingos in the sampled population with DRD4 exon 3 genotype (a) and DRD4 exon 3 allele (b). Lower case font represents DRD4 exon 3 alleles and number of individuals with DRD4 genotype and alleles are indicated within brackets. An individual was chosen for cloning for genotypes marked with an *.

population (ab). Deviations from Hardy–Weinberg equilibrium were tested with Genepop 4.0.10. (Rousset 2008).

Microsatellite genotyping

Genotyping of microsatellite markers was performed at 10 microsatellite loci (PrD3, PrD4, PrD5, PrD9, PrA110, PrA113, PrC109, PrD108, PrD121 and PrD126) developed for the Greater flamingo (An *et al.* 2010) (PCRs conditions can be found at http://tomato.bio.trinity.edu/manuscripts/10-2/mer-09-0396.pdf; GenBank Accession entries for microsatellites are GF101824, GF101825, GF101826, GF101828, GF101835, GF101837, GF101842, GF101847, GF101892 and GF101849 respectively). Electrophoresis was performed on a 96 capillary sequencer ABI3730XL (GENTYANE, INRA, France). Alleles were scored using GeneMapper 4.0 (Applied Biosystems). For each cohort, the program MICRO-CHECKER (Van Oosterhout *et al.* 2004) was used to test for genotyping errors. Identity disequilibrium, predicted to occur whether inbreeding is common in a population, was estimated from parameter g_2, using the RMES software available at http://ftp.cefe.cnrs.fr (David *et al.* 2007). A significant departure of g_2 from 0 is a powerful way of detecting ID in the population (David *et al.* 2007; Szulkin *et al.* 2010).

Multilocus heterozygosity

Multilocus heterozygosity was measured as the sum of heterozygote loci divided by the number of loci (Chapman *et al.* 2009; Szulkin *et al.* 2010). We also calculated three other measures of MLH, namely internal relatedness (IR, Amos *et al.* 2001), standardized heterozygosity (SH, Coltman *et al.* 1999) and heterozygosity by loci (HL, Aparicio *et al.* 2006) using the R function GENHET (Coulon 2010). All MLH measures were highly correlated and results were highly similar to analyses using MLH (Appendix S1, Supporting information). We only present here the results using MLH following recommendations from Chapman *et al.* (2009).

Calculating body condition

When estimating body condition, chick tarsus length was used as a body size indicator as in previous studies (Cézilly *et al.* 1995; Barbraud *et al.* 2003; Béchet & Johnson 2008), which significantly correlates with chicks' mass [standardized major axis (SMA) regression of the log-transformed mass–length relationship; $R^2 = 0.72$; $P < 0.001$, slope = 2.01]. We used the scaled mass index to calculate EBC (Peig & Green 2009, 2010), in order to control for the effects of body size on both the independent and dependent variables. In addition, the scale mass index retains the original units of measurement. However in our study, using more traditional methods of measuring body condition, that is, the residuals from an ordinary least squares (OLS) or SMA regressions of the log-transformed mass–length relationship, were highly collinear with the scale mass index and results were highly similar to analyses using the scale mass index (Appendix S2, Supporting information). Student residuals from an OLS regression of log-transformed mass and log-transformed tarsus length were used to test for significant outliers with the R function CAR in the software package R-2.14.2 (http://www.R-project.org). Three significant outliers (Bonferroni adjusted $P < 0.05$) were removed from the data set (from a total of 670 individuals) and were considered to be the result of measurement errors at ringing.

When calculating the scaled mass index, we used the slope of the SMA of log-transformed mass against log-transformed tarsus length for all cohorts and both sexes confounded as the scaling exponent using the R function SMATR. By confounding all cohorts and sex, we can test whether EBC varied significantly between cohorts (1995, 1996, 1997 and 1998) and sexes. This was legitimate because of a lack of difference in slopes ($P > 0.05$) when fitting a SMA regression of log-transformed mass against log-transformed tarsus within each cohort and sex. Therefore, despite morphological differences between sexes and cohorts, we assumed the same morphogenetic pattern for males and females between each cohort.

Statistical analysis

All statistical analyses were carried out using the software package R-2.14.2 (http://www.R-project.org). To investigate the effect of DRD4 polymorphisms on EBC, genotypes that were found in fewer than 20 individuals were excluded from the data set, thus avoiding false interpretation because of small sample sizes (type I and type II errors). As a result, only five genotypes (aa, bb, ab, ac and bc) and three alleles (a, b and c) were considered when analysing the association of DRD4 genotypes with EBC. We chose to investigate whether DRD4 genotypes rather than individual SNPs predict EBC because of a lack of independence between SNPs within DRD4 exon 3 (Appendix S4, Supporting information). However, using SNPs instead of DRD4 genotypes gave virtually identical results (Appendix S4, Supporting information). Therefore, 581 of 670 individuals genotyped for DRD4 exon 3 were included in this analysis. Model selection was achieved through information-theoretic (I-T) model selection and multimodel inference approach (Burnham & Anderson 2002), because recent literature recommends using this statistical approach

for observational studies (see the following reviews for I-T model selection in behavioural ecology: Johnson & Omland 2004; Garamszegi *et al.* 2009; Hegyi & Garamszegi 2011; Richards *et al.* 2011; Symonds & Moussalli 2011). Burnham & Anderson (2002) provide a complete description of model selection methods. All possible candidate models were constructed using the predictor variables cohort (1995, 1996, 1997 or 1998), sex (female or male), DRD4 genotype (aa, bb, ab, ac or bc) and the two-way interaction between sex and cohort. As both inbreeding and outbreeding may generate HFC, a complex relationship between MLH and body condition can be expected if effects are pulled by extreme values of MLH (i.e. among the very inbred and/or very outbred individuals). Therefore, we initially tested for a nonlinear association between MLH and body condition using general additive modelling (GAM) fitted with a Gaussian distribution with the R package MGCV (Wood & Augustin 2002), which automatically tests for nonlinear response shapes by fitting smoothed functions for the predictor variables (Guisan *et al.* 2002; Zuur *et al.* 2009). MLH was fitted with a smoothed function with an upper limit of five degrees of freedom. As all GAM models did not report significant nonlinear relationships and gave equivalent Akaike's Information Criterion (AIC) to linear models, we continued model selection using linear regression models and concluded that there was no evidence of a nonlinear association between MLH and body condition. AIC weights (ω) were used to assess the relative strength of support for models (Burnham & Anderson 2002; Johnson & Omland 2004). Only the 10 top ranked models based on AIC are presented. Parameter estimates were calculated using model averaging whereby regression coefficients are based on the full suite of models described previously. This procedure is more robust when several models have similar support (Burnham & Anderson 2002; Johnson & Omland 2004). The relative importance of each predictor variable is estimated by summing the AIC weight in which that variable appears across supported models ($\Sigma AIC\omega$) (Burnham & Anderson 2002). A summed Akaike weight value tends towards 1 if a particular predictor appears in all of the top models. Conversely, a summed Akaike weight value tends towards 0 if a particular predictor appears only in models with low support (Burnham & Anderson 2002; Symonds & Moussalli 2011).

Heterozygosity-fitness correlation may be generated independently from inbreeding and outbreeding if there is overdominant linkage between one or more microsatellite loci to one or more functional molecular markers [referred to as a local effect (Szulkin *et al.* 2010)]. Although, as argued by Szulkin *et al.* (2010), this is highly unlikely particularly if HFC is weak (as is the association between MLH and EBC, see results), we nonetheless tested for a significant local effect of MLH using the methods described by Szulkin *et al.* (2010). Briefly, an *F*-ratio test was used to test whether a model containing each single loci heterozygosity (SLH; expressed as 0 or 1, missing values replaced by the mean at that locus) explained more of the variance than a model containing MLH (calculated for this test as the sum of SLH, missing values replaced by the mean at that locus). The best-ranked model with MLH (model 2 in Table 2a) was used for the local effects test. Thus, a model containing the predictor variables cohort, sex, DRD4 genotypes and MLH was compared to a model with predictor variables cohort, sex, DRD4 genotypes and the 10 SLH to test for significant local effects. We used the top ranked model (model 1 in Table 1a) to test whether DRD4 heterozygosity (DRD4 homozygote or heterozygote) rather than DRD4 genotype predicted EBC. We also evaluated whether MLH predicted DRD4 heterozygosity using a GLM with a binomial distribution, DRD4 heterozygosity as the response variable and MLH as the explanatory variable.

To check whether a significant association in DRD4 polymorphism was not driven by rare genotypes, the analysis was repeated after excluding all genotypes with fewer than 99 individuals. As a result, only three genotypes (aa, bb and ab) and two alleles (a and b) were considered, which represents 79% of the sampled greater flamingo population. Consequently, 526 of 670 individuals genotyped for DRD4 exon 3 were included in this second analysis.

Results

DRD4 exon 3 genotyping

Amplification of exon 3 DRD4 was confirmed through BLASTP search of the GenBank (nr database) using the predicted 161 residue greater flamingo DRD4 protein which returned *E*-values of $4 \times e^{-99}$, $2 \times e^{-98}$, $7 \times e^{-98}$, $1 \times e^{-96}$ and $2 \times e^{-94}$ for alignments with the chicken, *Gallus gallus* (NP001136321), the wild turkey, *Meleagris gallopavo* (XP003206209), the blackcap, *Sylvia atricapilla* (AEC22814), the zebra finch, *Taeniopygia guttata* (ACT99861) and the great tit, *Parus major* (AAY56686) DRD4 protein sequences, respectively. The DRD4 exon 3 protein showed 87% identity with the chicken DRD4 protein, 63% with the mouse DRD4 protein and 61% with the human DRD4 protein.

A total of six synonymous SNPs were identified, defining eight alleles (named a–h), these alleles being combined in 23 observed genotypes (Fig. 1). There was no evidence that DRD4 genotypes significantly deviated from Hardy–Weinberg equilibrium ($n = 670$; P-value = 0.1673;

Table 2 Top 10 models of early body condition of greater flamingos with the five most common DRD4 exon 3 genotypes included in the data set (a; $n = 581$) and the three most common DRD4 exon 3 genotypes included in the data set (b; $n = 526$), showing number of parameters (k), log-likelihood (LL), AIC of the models, change in AIC compared with the best-ranked model (Δ AIC), and Akaike model weights (ω). The base model included sex (male or female), cohort, microsatellite heterozygosity, DRD4 genotype and the interaction between sex and cohort

Model rank	Model	k	LL	AIC	Δ AIC	ω
(a) Models with only the five most common DRD4 genotypes ($n = 581$)						
1	Cohort + DRD4 genotype + Sex	10	−3955.29	7930.6	0	0.287
2	Cohort + DRD4 genotype + Sex + MLH	11	−3954.50	7931.0	0.43	0.231
3	Cohort + DRD4 genotype + Sex + Cohort × Sex	13	−3952.77	7931.5	0.96	0.177
4	Cohort + DRD4 genotype + Sex + Cohort × Sex + MLH	14	−3952.02	7932.0	1.47	0.138
5	Cohort + DRD4 genotype	9	−3957.81	7933.6	3.05	0.062
6	Cohort + DRD4 genotype + MLH	10	−3956.97	7933.9	3.36	0.054
7	Cohort + MLH + Sex	7	−3961.05	7936.1	5.52	0.018
8	Cohort + Sex	6	−3962.09	7936.2	5.6	0.017
9	Cohort + Sex + Cohort × Sex + MLH	10	−3959.45	7938.9	8.32	0.004
10	Cohort + Sex + Cohort × Sex	9	−3960.47	7938.9	8.37	0.004
(b) Models with only the three most common DRD4 genotypes ($n = 526$)						
1	Cohort + DRD4 genotype + Sex	8	−3582.46	7180.9	0	0.257
2	Cohort + DRD4 genotype	7	−3583.91	7181.8	0.88	0.165
3	Cohort + DRD4 genotype + Sex + MLH	9	−3582.10	7182.2	1.27	0.137
4	Cohort + DRD4 genotype + Sex + Cohort × Sex	11	−3580.37	7182.7	1.8	0.104
5	Cohort + DRD4 genotype + MLH	8	−3583.55	7183.1	2.17	0.087
6	Cohort + Sex	6	−3585.84	7183.7	2.76	0.065
7	Cohort + DRD4 genotype + Sex + Cohort × Sex + MLH	12	−3579.95	7183.9	2.97	0.058
8	Cohort	5	−3587.35	7184.7	3.78	0.039
9	Cohort + Sex + MLH	7	−3585.52	7185.0	4.11	0.033
10	Cohort + Sex + Cohort × Sex	9	−3583.90	7185.8	4.87	0.023

SE = 0.0196). DRD4 genotypes were found at an uneven frequency with for example the heterozygote genotype ab present in almost 40% of birds, and the homozygote genotypes aa and bb in 24% and 15% of birds, respectively (Fig. 1a). Alleles a and b were present in almost 75% and 63% of birds, respectively, with the next most common allele c present in only 9% of birds (Fig. 1b). None of the alleles differed by more than four base pairs, with a mean of 2.1 and a standard deviation of 0.91 (Appendix S3, Supporting information). Furthermore, the three most common alleles (a, b and c) that defined the five most common genotypes (aa, bb, ab, ac and bc) only differed by two base pairs (from three SNPs; Appendix S3, Supporting information).

Association between EBC and DRD4 polymorphism

Four models were retained as equivalent (ΔAIC < 2), all of which retained an effect of DRD4 polymorphism, cohort and sex (Table 2 a). Model averaging revealed very strong support of an association between DRD4 genotypes and EBC ($\Sigma AIC\omega = 1$; Table 3a) and DRD4 genotypes explained between 2.2 and 2.3% of EBC variance according to the 4 equivalent models. Chicks carrying genotypes aa, bc and ab were of lower body condition than those carrying genotypes ac and bb, with individuals of genotype ac in higher body condition than those of genotype bb (Fig. 2). Indeed, EBC of genotypes ac and bb were on average 5.7% and 2.8% higher, respectively, than individuals carrying genotype aa (the genotype with the lowest mean adjusted EBC; Fig. 2). Chicks carrying genotypes aa, bc and ab were of similar condition (Fig. 2). Virtually identical results were found when using individual DRD4 SNPs, whereby there was strong evidence of an association between EBC and the three DRD4 exon 3 SNPs (although there was a lack of independence between different SNPs; Appendix S4, Supporting information). These combined results are suggestive of a complex interaction between alleles rather than a simple allele dominance interaction. Model averaging also revealed very strong support of variation in EBC between cohorts ($\Sigma AIC\omega = 1$; Table 3a; Fig. 3) and strong support of higher EBC in females compared with males ($\Sigma AIC\omega = 0.88$; Table 3a; Fig. 3). There was only weak support for an interaction between sex and cohort ($\Sigma AIC\omega = 0.33$; Table 3a; Fig. 3).

No significant among-locus correlation between microsatellite loci was detected ($g_2 = -0.00004$, SD = 0.002, $P = 0.520$). However, a significant association between

Table 3 Model-averaged parameter estimates of early body condition of greater flamingos with individuals with the five most common DRD4 exon 3 genotypes included in the data set (a; $n = 581$) and the three most common DRD4 exon 3 genotypes included in the data set (b; $n = 526$). Summed AIC weight of each parameter is also shown ($\Sigma AIC\omega$). See Table 3 for descriptions of models

Parameter	Model-averaged estimate	Adjusted SE	95% C.I.	$\Sigma AIC\omega$
(a) Data set with the five most common DRD4 genotypes				
Intercept (females, 1995, aa)	2093.31	52.60	1990.22, 2196.40	
Cohorts				1
1996	191.04	44.08	104.64, 277.44	
1997	274.15	31.74	211.95, 336.35	
1998	193.00	31.57	131.13, 254.87	
Sex				0.88
Males	−49	31.36	−110.47, 12.47	
Cohorts×Sex:				0.33
1996 (Males)	−80.04	68.33	−213.97, 53.88	
1997 (Males)	49.67	51.38	−51.04, 150.38	
1998 (Males)	37.70	53.84	−67.83, 143.23	
DRD4 genotypes				1
ab	−0.38	22.25	−44, 43.23	
ac	131.21	47.67	37.78, 224.64	
bb	63.91	28.28	8.48, 119.35	
bc	17.17	44.81	−70.64, 104.99	
MLH	−82.38	66.64	−213, 48.23	0.45
(b) Data set with the three most common DRD4 genotypes				
Intercept (females, 1995, aa)	2072.86	46.58	1981.57, 2164.16	
Cohorts				1
1996	195.03	43.74	109.30, 280.76	
1997	283.42	29.91	224.80, 342.04	
1998	196.08	30.86	135.59, 256.56	
Sex				0.69
Males	−36.97	28.47	−92.77, 18.83	
Cohorts×Sex:				0.17
1996 (Males)	−90.79	74.11	−236.05, 54.47	
1997 (Males)	37.74	53.62	−67.36, 142.83	
1998 (Males)	36.05	56.21	−74.11, 146.21	
DRD4 genotypes				0.86
ab	−1.09	22.30	−44.79, 42.61	
bb	63.99	28.37	8.38, 119.59	
MLH	−60.88	71.57	−201.14, 79.39	0.33

MLH and EBC may still occur if significant ID is not detected because a slight inbreeding is often more easily detected through its effects on phenotype than through its effect on heterozygosity at a few marker loci (Szulkin *et al.* 2010). However, evidence that MLH predicted EBC was weak ($\Sigma AIC\omega = 0.45$, Table 3a). MLH was not retained in the best-ranked model (Table 2a), and there was weak support for the slope of the term being different from zero (Table 3a). Furthermore, including MLH in the model did not change the strong association of DRD4 polymorphism with EBC. Testing for significant local effects on body condition was achieved as advised by Szulkin *et al.* (2010) and revealed no significant evidence of local effects on EBC ($F_{19,552} = 1.081$, $P = 0.366$). DRD4 homozygotes had a significantly lower MLH than DRD4 heterozygotes ($\chi^2_{1,579} = -4.902$; $P = 0.027$), but DRD4 heterozygosity did not significantly predict EBC ($F_{1,575} = 0.327$; $P = 0.568$).

The analysis of the association of DRD4 genotypes with EBC was repeated with only the three most common DRD4 genotypes aa, bb and ab in the analysis, which represents 79% of the sampled greater flamingo population (at least 99 individuals per DRD4 genotype). Again DRD4 polymorphism was retained by all of the equivalent models (Table 2b), with 1.3% of the variance explained by DRD4 genotypes according to the four equivalent models ($\Sigma AIC\omega = 0.86$; Table 3b).

Discussion

We found strong evidence that EBC in the Greater flamingo was associated with DRD4 exon 3 polymorphism

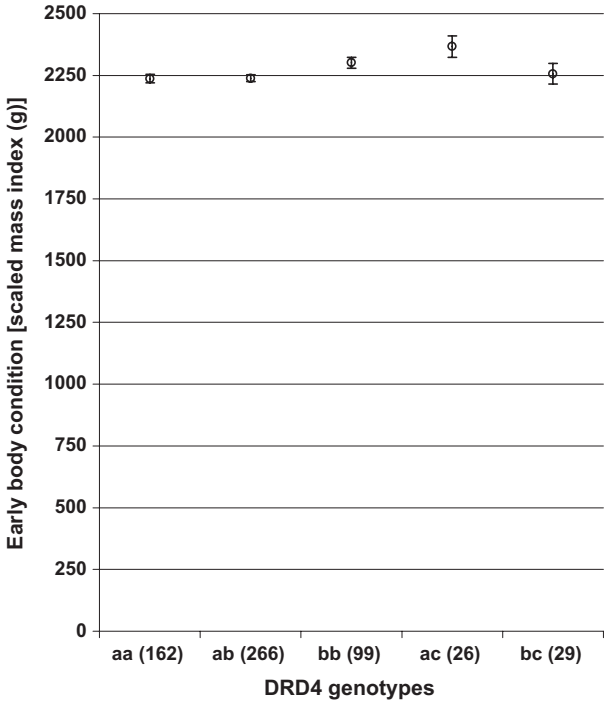

Fig. 2 Adjusted mean early body condition (±SEM) of greater flamingos according to the five most common DRD4 genotypes (corrected for cohort and sex effects using model 1 in Table 2). Sample sizes are indicated within brackets.

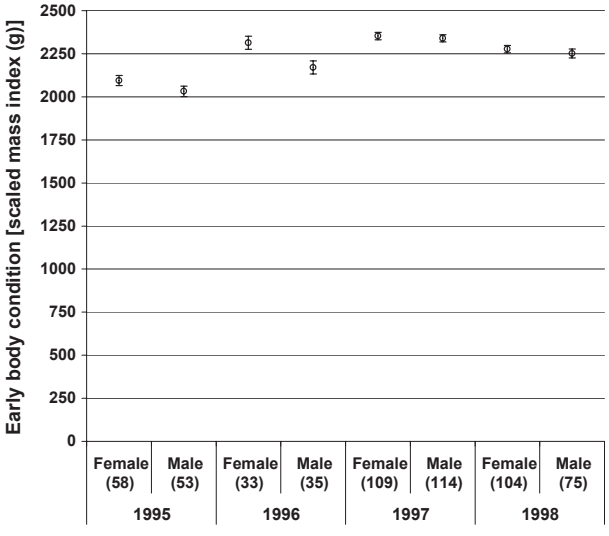

Fig. 3 Adjusted mean early body condition (±SEM) of greater flamingo chicks in relation to cohort and sex (corrected for DRD4 genotypes effect using model 3 in Table 2). Sample sizes are indicated within brackets.

but not with MLH. As expected, we also observed strong evidence of variation in EBC between cohorts that reflects the effects of between-year stochastic environmental conditions. Annual variation (as encompassed by the cohort effect) in EBC explained between 17.3 and 17.6% of the variance (according to the four equivalent models) in our study. This is consistent with previous studies which seem to show that water levels around the breeding colony and number of breeding pairs have a significant effect on EBC (Cézilly *et al.* 1995; Béchet & Johnson 2008). Interestingly, we found for the first time in this species a significant difference in EBC between females and males, with females in a significantly higher condition than males. In our study, between 0.8 and 0.9% (according to the four equivalent models) of the variance in EBC was explained by differences between males and females. Although it is known that there is morphological sexual dimorphism in adult flamingos with males tending to be larger than females (Johnson & Cézilly 2007), no previous study had reported differential body condition between the sexes.

When excluding DRD4 exon 3 genotypes with fewer than 20 individuals, 2.2–2.3% of the variation in EBC was explained by DRD4 polymorphisms (although this increased to 4.9% when all genotypes were included in the analysis). Differences in EBC between the two most extreme DRD4 exon 3 genotypes were in the order of 6%. Molecularly this seems to be a substantial effect especially when considering that, as is the case for behavioural traits, body condition is probably to be regulated by multiple genes (reviewed in Snyder *et al.* 2004). Indeed, a minimum of five independent association studies (reviewed in Snyder *et al.* 2004) have provided evidence for at least 15 candidate genes which are probably to regulate body composition in humans. As a point of comparison, in a study in humans investigating the association between changes in body fatness over time and 15 polymorphisms in 10 candidate genes of obesity (although not DRD4 polymorphism in this study), Bouchard *et al.* (2007) found that only 3–5 genes were retained in the model with the strongest candidate gene only explaining 3.3% of the variance. Furthermore, strong evidence of an association between DRD4 polymorphism and EBC remains even when genotypes with fewer than 99 individuals are removed from the analysis. Therefore, although EBC is probably mediated by several genes, our results do provide robust correlative evidence that DRD4 polymorphism, or a gene closely linked to the DRD4 gene, is associated with it. A similar result was found when investigating the association between exploratory behaviour and exon 3 DRD4 polymorphism in a Dutch population of great tits (Fidler *et al.* 2007; Korsten *et al.* 2010). A single synonymous SNP in exon 3 of DRD4 was associated with exploratory behaviour explaining 4.5–5.8% of the variation, although this association was not present in three other wild European populations (Fidler *et al.* 2007; Korsten *et al.* 2010).

Multilocus heterozygosity from a few microsatellites markers is often poorly correlated with pedigree-based values of inbreeding coefficient *F* (Szulkin *et al.* 2010). We cannot exclude the possibility that a lack of association between MLH and EBC may be due to a lack of power in detecting inbreeding depression because of the relatively small number of markers (10) used in this study. Indeed we found that individuals that were DRD4 heterozygotes had significantly lower MLH values than DRD4 homozygotes. This contradictory result may be further suggestive of a lack of power in detecting inbreeding. However, alternatively MLH may have been a poor predictor of whole-genome heterozygosity because the greater flamingo population may be a large panmictic population at equilibrium and at low risk of the effects of inbreeding and outbreeding depression. A recent study on a large (n = 1192), moderately inbred, captive population of zebra finch (*Taeniopygia guttata*) compared the power of 11 microsatellite markers, 1359 SNPs markers and pedigree-based *F* in terms of detecting inbreeding depression on 11 phenotypic traits (Forstmeier *et al.* in press). The authors found that microsatellite and SNP markers produced equally strong HFCs and that both markers produced stronger HFCs than the pedigree-based *F*. Forstmeier *et al.* (in press) concluded that a small panel of microsatellites may reflect better an individual's realized inbreeding depression than previously appreciated. Therefore. our results do at least suggest that the inbreeding and outbreeding effects may be relatively unimportant in explaining the associations between the DRD4 genotypes and EBC. Moreover, EBC was associated with specific DRD4 genotypes rather than DRD4 heterozygosity.

Previous studies in humans have found a significant association between DRD4 exon 3 polymorphism and body condition but to our knowledge this is the first study to find such an association in other vertebrates. In human studies, associations between DRD4 polymorphisms and body condition involve nonsynonymous polymorphisms within exon 3, with studies focusing on a 48 bp repeat (Levitan *et al.* 2004a,b, 2006; Sobik *et al.* 2005; Guo *et al.* 2006, 2007; Eisenberg *et al.* 2008). In our study, all six SNPs were synonymous. However, as argued by Fidler *et al.* (2007) and Korsten *et al.* (2010), a significant association between synonymous SNPs at exon 3 of DRD4 and a phenotypic trait may be biologically meaningful for two reasons. First, synonymous polymorphism within exon 3 may be linked to other polymorphisms either within other exon or promoter regions of the DRD4 gene (e.g. exon 1 which codes for the extracellular domain) and/or to other genes that may also regulate body condition. For example, in chickens, it appears that DRD4 is in LD with deformed epidermal auto regulatory factor one (DEAF1), a gene involved in the regulation of the serotonergic system (Flisikowski *et al.* 2009). Second, there is now a growing consensus that synonymous nucleotide substitutions may alter protein function and can be targeted by natural selection (for a review see Sauna & Kimchi-Sarfaty 2011). Indeed, it has been shown that there is codon usage bias between synonymous codons (Chamary *et al.* 2006; Kimchi-Sarfaty 2007; Plotkin & Kudla 2011). It has recently been demonstrated that codon bias because of silent substitutions can affect protein conformation and have functional consequences (Chamary *et al.* 2006; Kimchi-Sarfaty 2007; Plotkin & Kudla 2011). Moreover, it has been demonstrated that synonymous polymorphisms influence mRNA stability and translation of dopamine receptors (Duan *et al.* 2003). Therefore, synonymous substitutions in DRD4 exon 3 may cause functional differences in protein, changing affinity to dopamine which in turn results in different EBC between genotypes.

Assuming that DRD4 exon 3 polymorphism does result in a differential dopamine affinity in the greater flamingo, we propose three nonmutually exclusive hypotheses that might explain differences in EBC between DRD4 genotypes. First, food intake and energy homoeostasis may be different between DRD4 genotypes. Indeed, dopamine is thought to regulate body weight in vertebrates by playing an important role in the motivational mechanisms associated with the behavioural responses necessary for food intake (for reviews see Meister 2007; Gao & Horvath 2008; Wang *et al.* 2009) as well as the metabolism of glucose and lipids (for a review see Pijl 2003). Second, chick begging behaviour may differ according to exon 3 DRD4 genotype. Recent studies indicate that behavioural types, such as bold and aggressive personalities, are positively related to food intake rates (reviewed in Biro & Stamps 2008) and energy expenditure (Careau *et al.* 2010, 2011). Considering that recent studies suggest a significant association between personalities and DRD4 polymorphism (Momozawa *et al.* 2005; Bailey *et al.* 2007; Fidler *et al.* 2007; Hejjas *et al.* 2007; James *et al.* 2007; Flisikowski *et al.* 2009; Korsten *et al.* 2010), certain DRD4 genotypes might be bolder and more aggressive during begging, resulting in higher food intake and, in turn, higher EBC. However, it is important to emphasize that these potential differences in food intake because of begging behaviour do not result from differences in competitive ability between siblings because greater flamingos only lay one egg per season. Third, an association between EBC and DRD4 genotype may result from an indirect effect of differences in parental foraging behaviour between parental DRD4 exon 3 genotypes. In this scenario, parental rather than chick DRD4 genotypes would be a better predictor of EBC.

Somewhat paradoxically the DRD4 genotypes that were associated with higher EBC were not the most common in the population. Considering the pleiotropic nature of the DRD4 gene, it is possible that DRD4 genotypes associated with higher EBC may have other fitness consequences. Moreover, to what extent differences in mean EBC between DRD4 exon 3 genotypes affect fitness remains to be tested. Future studies should therefore investigate whether DRD4 polymorphisms also predicts survival and recruitment into the breeding population. Recent studies in humans indicate that there is an association between migration and exon 3 DRD4 polymorphisms (Chen *et al.* 1999; Matthews & Butler 2011). Interestingly, Barbraud *et al.* (2003) found that EBC is an important predictor of natal dispersal. Thus, future studies should also focus on investigating a link between dispersal and DRD4 polymorphism in the greater flamingo. So far, a single study has investigated if DRD4 polymorphism is associated with migration (but not dispersal) in non-human animals (blackcaps, *Sylvia atricapilla*) and found no effect (Mueller *et al.* 2011).

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References

Abasht B, Dekkers JCM, Lamont SJ (2006) Review of quantitative trait loci identified in the chicken. *Poultry Science*, **85**, 2079–2096.

Amos W, Wilmer JW, Fullard K *et al.* (2001) The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **268**, 2021–2027.

An JH, Bechet A, Berggren A *et al.* (2010) Permanent genetic resources added to molecular ecology resources database 1 October 2009-30 November 2009. *Molecular Ecology Resources*, **10**, 404–408.

Aparicio JM, Ortego J, Cordero PJ (2006) What should we weigh to estimate heterozygosity, alleles or loci? *Molecular Ecology*, **15**, 4659–4665.

Asghari V, Sanyal S, Buchwaldt S *et al.* (1995) Modulation of intracellular cyclic-amp levels by different human dopamine D4 receptor variants. *Journal of Neurochemistry*, **65**, 1157–1165.

Bailey JN, Breidenthal SE, Jorgensen MJ, McCracken JT, Fairbanks LA (2007) The association of DRD4 and novelty seeking is found in a nonhuman primate model. *Psychiatric Genetics*, **17**, 23–27.

Balkız O, Danol S, Barbraud C *et al.* (2007) Sexing greater flamingo chicks from feather bulb DNA. *Waterbirds*, **30**, 450–453.

Barbraud C, Johnson AR, Bertault G (2003) Phenotypic correlates of post-fledging dispersal in a population of greater flamingos: the importance of body condition. *Journal of Animal Ecology*, **72**, 246–257.

Béchet A, Johnson AR (2008) Anthropogenic and environmental determinants of greater flamingo phoenicopterus roseus breeding numbers and productivity in the Camargue (Rhone delta, southern France). *Ibis*, **150**, 69–79.

Béchet A, Rendón-Martos M, Rendón MA *et al.* (2012) Global economy interacts with climate change to jeopardize species conservation: the case of the greater flamingo in the Mediterranean and West Africa. *Environmental Conservation*, **39**, 1–3.

Bertault G, Joulia D, Johnson AR, Raymond M (1999) Sex determination in greater flamingo chicks through DNA analysis. *Waterbirds*, **22**, 282–284.

Biro PA, Stamps JA (2008) Are animal personality traits linked to life-history productivity? *Trends in Ecology & Evolution*, **23**, 361–368.

Biro PA, Stamps JA (2010) Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology & Evolution*, **25**, 653–659.

Bouchard L, Tremblay A, Bouchard C, Perusse L (2007) Contribution of several candidate gene polymorphisms in the determination of adiposity changes: results from the Quebec Family Study. *International Journal of Obesity*, **31**, 891–899.

Burnham KP, Anderson DR (2002) *Model Selection and Multimodel Inference: A Practice Information-Theoretic Approach*. Springer Verlag, New York.

Callier S, Snapyan M, Le Crom S *et al.* (2003) Evolution and cell biology of dopamine receptors in vertebrates. *Biology of the Cell*, **95**, 489–502.

Careau V, Réale D, Humphries MM, Thomas DW (2010) The pace of life under artificial selection: personality, energy expenditure, and longevity are correlated in domestic dogs. *American Naturalist*, **175**, 753–758.

Careau V, Thomas D, Pelletier F *et al.* (2011) Genetic correlation between resting metabolic rate and exploratory behaviour in deer mice (*Peromyscus maniculatus*). *Journal of Evolutionary Biology*, **24**, 2153–2163.

Cézilly F, Johnson AR (1995) Re-mating between and within breeding seasons in the greater flamingo phoenicopterus-ruber-roseus. *Ibis*, **137**, 543–546.

Cézilly F, Boy V, Green RE, Hirons GJM, Johnson A (1995) Interannual variation in greater flamingo breeding success in relation to water levels. *Ecology*, **76**, 20–26.

Chamary JV, Parmley JL, Hurst LD (2006) Hearing silence: non-neutral evolution at synonymous sites in mammals. *Nature Reviews Genetics*, **7**, 98–108.

Chapman JR, Nakagawa S, Coltman DW, Slate J, Sheldon BC (2009) A quantitative review of heterozygosity-fitness correlations in animal populations. *Molecular Ecology*, **18**, 2746–2765.

Chen CS, Burton M, Greenberger E, Dmitrieva J (1999) Population migration and the variation of dopamine D4 receptor (DRD4) allele frequencies around the globe. *Evolution and Human Behavior*, **20**, 309–324.

Clobert J, Danchin E, Dhondt AA, nichols JD (2001) *Dispersal*. Oxford University Press, Oxford.

Coltman DW, Slate J (2003) Microsatellite measures of inbreeding: a meta-analysis. *Evolution*, **57**, 971–983.

Coltman DW, Pilkington JG, Smith JA, Pemberton JM (1999) Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution*, **53**, 1259–1267.

Comins HN, Hamilton WD, May RM (1980) Evolutionarily stable dispersal strategies. *Journal of Theoretical Biology*, **20**, 5–230.

Coulon A (2010) GENHET: an easy-to-use R function to estimate individual heterozygosity. *Molecular Ecology Resources*, **10**, 167–169.

David P, Delay B, Berthou P, Jarne P (1995) Alternative models for allozyme-associated heterosis in the Marine Bivalve Spisula-Ovalis. *Genetics*, **139**, 1719–1726.

David P, Pujol B, Viard F, Castella V, Goudet J (2007) Reliable selfing rate estimates from imperfect population genetic data. *Molecular Ecology*, **16**, 2474–2487.

Duan JB, Wainwright MS, Comeron JM *et al.* (2003) Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. *Human Molecular Genetics*, **12**, 205–216.

Ebstein RP (2006) The molecular genetic architecture of human personality: beyond self-report questionnaires. *Molecular Psychiatry*, **11**, 427–445.

Edelman AJ (2011) Sex-specific effects of size and condition on timing of natal dispersal in kangaroo rats. *Behavioral Ecology*, **22**, 776–783.

Eisenberg DTA, Campbell B, Gray PB, Sorenson MD (2008) Dopamine receptor genetic polymorphisms and body composition in undernourished pastoralists: an exploration of nutrition indices among nomadic and recently settled Ariaal men of northern Kenya. *BMC Evolutionary Biology*, **8**, 173.

Fidler AE, van Oers K, Drent PJ *et al.* (2007) Drd4 gene polymorphisms are associated with personality variation in a passerine bird. *Proceedings of the Royal Society B-Biological Sciences*, **274**, 1685–1691.

Fitzpatrick MJ, Ben-Shahar Y, Smid HM *et al.* (2005) Candidate genes for behavioural ecology. *Trends in Ecology & Evolution*, **20**, 96–104.

Flisikowski K, Schwarzenbacher H, Wysocki M *et al.* (2009) Variation in neighbouring genes of the dopaminergic and serotonergic systems affects feather pecking behaviour of laying hens. *Animal Genetics*, **40**, 192–199.

Forstmeier W, Schielzeth H, Mueller J, Ellegren H, Kempenaers B (in press) Heterozygosity–fitness correlations in zebra finches: microsatellite markers can be better than their reputation. *Molecular Ecology*, DOI: 10.1111/j.1365-294X.2012.05593.x.

Gao Q, Horvath TL (2008) Neuronal control of energy homeostasis. *FEBS Letters*, **582**, 132–141.

Garamszegi LZ, Calhim S, Dochtermann N *et al.* (2009) Changing philosophies and tools for statistical inferences in behavioral ecology. *Behavioral Ecology*, **20**, 1363–1375.

Garant D, Kruuk LEB, McCleery RH, Sheldon BC (2004) Evolution in a changing environment: a case study with great tit fledging mass. *American Naturalist*, **164**, E115–E129.

Greenwood PJ, Harvey PH (1982) *Annual Review of Ecology and Systematics*, **13**, 1–21.

Guisan A, Edwards TC, Hastie T (2002) Generalized linear and generalized additive models in studies of species distributions: setting the scene. *Ecological Modelling*, **157**, 89–100.

Guo GA, North K, Choi S (2006) DRD4 gene variant associated with body mass: the National Longitudinal Study of Adolescent Health. *Human Mutation*, **27**, 236–241.

Guo G, North KE, Gorden-Larsen P, Bulik CM, Choi S (2007) Body mass, DRD4, physical activity, sedentary behavior, and family socioeconomic status: the add health study. *Obesity*, **15**, 1199–1206.

Hamilton WD, May RM (1977) Dispersal in stable habitats. *Nature*, **269**, 578–581.

Hegyi G, Garamszegi LZ (2011) Using information theory as a substitute for stepwise regression in ecology and behavior. *Behavioral Ecology and Sociobiology*, **65**, 69–76.

Hejjas K, Vas J, Topal J *et al.* (2007) Association of polymorphisms in the dopamine D4 receptor gene and the activity-impulsivity endophenotype in dogs. *Animal Genetics*, **38**, 629–633.

Hillis DM, Mable BK, Larson A, Davis SK, Zimmer EA (1996) Nucleic acids IV: sequencing and cloning. In: *Molecular Systematics* (eds Hillis DM, Mable BK, Moritz C), pp. 321–381. Sinauer, Sunderland, Massachusetts.

Houston AI (2010) Evolutionary models of metabolism, behaviour and personality. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **365**, 3969–3975.

James AS, Groman SM, Seu E *et al.* (2007) Dimensions of impulsivity are associated with poor spatial working memory performance in monkeys. *Journal of Neuroscience*, **27**, 14358–14364.

Johnson AR, Cézilly F (2007) *The Greater Flamingo*. T. & A. D. Poyser, London.

Johnson JB, Omland KS (2004) Model selection in ecology and evolution. *Trends in Ecology & Evolution*, **19**, 101–108.

Kimchi-Sarfaty C (2007) A 'silent' polymorphism in the MDR1 gene changes substrate specificity. *Science*, **333**, 39–39.

Korsten P, Mueller JC, Hermannstadter C *et al.* (2010) Association between DRD4 gene polymorphism and personality variation in great tits: a test across four wild populations. *Molecular Ecology*, **19**, 832–843.

Küttner E, Moghadam HK, Skulason S, Danzmann RG, Ferguson MM (2011) Genetic architecture of body weight, condition factor and age of sexual maturation in Icelandic Arctic charr (*Salvelinus alpinus*). *Molecular Genetics and Genomics*, **286**, 67–79.

Levitan RD, Masellis M, Basile VS *et al.* (2004a) The dopamine-4 receptor gene associated with binge eating and weight gain in women with seasonal affective disorder: an evolutionary perspective. *Biological Psychiatry*, **56**, 665–669.

Levitan RD, Masellis M, Lam RW *et al.* (2004b) Childhood inattention and dysphoria and adult obesity associated with

the dopamine D4 receptor gene in overeating women with seasonal affective disorder. *Neuropsychopharmacology*, **29**, 179–186.

Levitan RD, Masellis M, Lam RW *et al.* (2006) A birth-season/DRD4 gene interaction predicts weight gain and obesity in women with seasonal affective disorder: a seasonal thrifty phenotype hypothesis. *Neuropsychopharmacology*, **31**, 2498–2503.

Lieutenant-Gosselin M, Bernatchez L (2006) Local heterozygosity-fitness correlations with global positive effects on fitness in threespine stickleback. *Evolution*, **60**, 1658–1668.

Mackay TFC, Stone EA, Ayroles JF (2009) The genetics of quantitative traits: challenges and prospects. *Nature Reviews Genetics*, **10**, 565–577.

Magrath RD (1991) Nestling weight and juvenile survival in the blackbird, Turdus-Merula. *Journal of Animal Ecology*, **60**, 335–351.

Matthews LJ, Butler PM (2011) Novelty-seeking DRD4 polymorphisms are associated with human migration distance out-of-Africa after controlling for neutral population gene structure. *American Journal of Physical Anthropology*, **145**, 382–389.

Meister B (2007) Neurotransmitters in key neurons of the hypothalamus that regulate feeding behavior and body weight. *Physiology & Behavior*, **92**, 263–271.

Mitchell-Olds T, Willis JH, Goldstein DB (2007) Which evolutionary processes influence natural genetic variation for phenotypic traits? *Nature Reviews Genetics*, **8**, 845–856.

Moghadam HK, Poissant J, Fotherby H *et al.* (2007) Quantitative trait loci for body weight, condition factor and age at sexual maturation in Arctic charr (*Salvelinus alpinus*): comparative analysis with rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Molecular Genetics and Genomics*, **277**, 647–661.

Momozawa Y, Takeuchi Y, Kusunose R, Kikusui T, Mori Y (2005) Association between equine temperament and polymorphisms in dopamine D4 receptor gene. *Mammalian Genome*, **16**, 538–544.

Mueller JC, Pulido F, Kempenaers B (2011) Identification of a gene associated with avian migratory behaviour. *Proceedings of the Royal Society B-Biological Sciences*, **278**, 2848–2856.

Naef-Daenzer B, Gruebler MU (2008) Post-fledging range use of Great Tit Parus major families in relation to chick body condition. *Ardea*, **96**, 181–190.

Naef-Daenzer B, Widmer F, Nuber M (2001) Differential post-fledging survival of great and coal tits in relation to their condition and fledging date. *Journal of Animal Ecology*, **70**, 730–738.

Peig J, Green AJ (2009) New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos*, **118**, 1883–1891.

Peig J, Green AJ (2010) The paradigm of body condition: a critical reappraisal of current methods based on mass and length. *Functional Ecology*, **24**, 1323–1332.

Phillips PC (2005) Testing hypotheses regarding the genetics of adaptation. *Genetica*, **123**, 15–24.

Pijl H (2003) Reduced dopaminergic tone in hypothalamic neural circuits: expression of a ''thrifty'' genotype underlying the metabolic syndrome? *European Journal of Pharmacology*, **480**, 125–131.

Plotkin JB, Kudla G (2011) Synonymous but not the same: the causes and consequences of codon bias. *Nature Reviews Genetics*, **12**, 32–42.

Rao Y, Shen X, Xia MN *et al.* (2007) SNP mapping of QTL affecting growth and fatness on chicken GGA1. *Genetics Selection Evolution*, **39**, 569–582.

Réale D, Dingemanse NJ, Kazem AJN, Wright J (2010) Evolutionary and ecological approaches to the study of personality. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **365**, 3937–3946.

Richards SA, Whittingham MJ, Stephens PA (2011) Model selection and model averaging in behavioural ecology: the utility of the IT-AIC framework. *Behavioral Ecology and Sociobiology*, **65**, 77–89.

Rousset F (2008) GENEPOP '007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, **8**, 103–106.

Sanz-Aguilar A, Béchet A, Germain C, Johnson A, Pradel R (in press) To leave or not to leave: survival tradeoffs between different migratory strategies in the Greater Flamingo. *Journal of Animal Ecology*, DOI: 10.1111/j.1365-2656.2012.01997.x.

Sauna ZE, Kimchi-Sarfaty C (2011) Understanding the contribution of synonymous mutations to human disease. *Nature Reviews Genetics*, **12**, 683–691.

Savitz JB, Ramesar RS (2004) Genetic variants implicated in personality: a review of the more promising candidates. *American Journal of Medical Genetics Part B-Neuropsychiatric Genetics*, **131B**, 20–32.

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **69**, 82–90.

Snyder EE, Walts B, Perusse L *et al.* (2004) The human obesity gene map: the 2003 update. *Obesity Research*, **12**, 369–439.

Sobik L, Hutchison K, Craighead L (2005) Cue-elicited craving for food: a fresh approach to the study of binge eating. *Appetite*, **44**, 253–261.

Symonds MRE, Moussalli A (2011) A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioral Ecology and Sociobiology*, **65**, 13–21.

Szulkin M, Zelazowski P, Nicholson G, Sheldon BC (2009) Inbreeding avoidance under different null models of random mating in the great tit. *Journal of Animal Ecology*, **78**, 778–788.

Szulkin M, Bierne N, David P (2010) Heterozygosity-fitness correlations: a time for reappraisal. *Evolution*, **64**, 1202–1217.

Tamura K, Peterson D, Peterson N *et al.* (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.

Thornton A (2008) Early body condition, time budgets and the acquisition of foraging skills in meerkats. *Animal Behaviour*, **75**, 951–962.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.

Verboven N, Visser ME (1998) Seasonal variation in local recruitment of great tits: the importance of being early. *Oikos*, **81**, 511–524.

Wang GJ, Volkow ND, Thanos PK, Fowler JS (2009) Imaging of brain dopamine pathways implications for understanding obesity. *Journal of Addiction Medicine*, **3**, 8–18.

Wood SN, Augustin NH (2002) GAMs with integrated model selection using penalized regression splines and applications to environmental modelling. *Ecological Modelling*, **157**, 157–177.

Zuur A, Ieno E, Walker N, Saveliev A, Smith G (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York.

Data accessibility

DRD4 genotypes, microsatellite genotypes, MLH and EBC: DRYAD entry doi:10.5061/dryad.rd45qj52

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1. Correlation between multilocus heterozygosity and other measures of microsatellite multilocus heterozygosity measures.

Appendix S2. Correlation between early body condition estimated as the scale mass index against residuals of an SMA and OLS regression of log (mass) and log (tarsus).

Appendix S3. Mutational distance between different DRD4 alleles.

Appendix S4. Early body condition according to DRD4 SNPs.

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