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Behaviour-related *DRD4* polymorphisms in invasive bird populations

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Abstract

It has been suggested that individual behavioural traits influence the potential to successfully colonize new areas. Identifying the genetic basis of behavioural variation in invasive species thus represents an important step towards understanding the evolutionary potential of the invader. Here, we sequenced a candidate region for neophilic/neophobic and activity behaviour – the complete exon 3 of the *DRD4* gene – in 100 Yellow-crowned bishops (*Euplectes afer*) from two invasive populations in Spain and Portugal. The same birds were scored twice for activity behaviour while exposed to novel objects (battery or slice of apple) in captivity. Response to novel objects was repeatable ($r = 0.41$) within individuals. We identified two synonymous *DRD4* SNPs that explained on average between 11% and 15% of the phenotypic variance in both populations, indicating a clear genetic component to the neophilic/neophobic/activity personality axis in this species. This consistently high estimated effect size was mainly due to the repeated measurement design, which excludes part of the within-individual nongenetic variance in the response to different novel objects. We suggest that the alternative alleles of these SNPs are likely introduced from the original population and maintained by weak or antagonistic selection during different stages of the invasion process. The identified genetic variants have not only the potential to serve as genetic markers of the neophobic/neophilic/activity personality axis, but may also help to understand the evolution of behaviour in these invasive bird populations.

Keywords: dopamine receptor D4, *Euplectes afer*, genotype–phenotype association, multiple measurements, personality, Yellow-crowned bishop

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Introduction

Deliberate and unintentional introductions of non-native species represent an ideal situation in which to study adaptation to novel environments (Sax *et al.* 2007). The invading organisms often experience dramatic environmental changes at the newly colonized location in comparison with the native habitat, but also extreme conditions during transportation. This has led to the idea that certain phenotypes are selectively 'filtered' while passing through each stage of the invasion process (i.e. trapping/entering transport vectors, transport, escape/introduction, establishment and spread) (Carrete *et al.* 2012; Chapple *et al.* 2012).

For animals, behaviour is considered an essential component of invasion success as it mediates how individuals interact with their environment (Holway & Suarez 1999; Duckworth 2009). For instance, exploratory behaviour and boldness may enhance the likelihood of

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uptake into transport vectors or of trapping and the subsequent establishment and spread in the recipient region, but may be accompanied with higher detection and mortality risks during transportation (Carrete *et al.* 2012; Chapple *et al.* 2012). Indeed, it has been shown that variation in exploratory and risk-taking behaviour results in a sampling bias of wild animals (Biro & Dingemanse 2009; Biro 2013), including birds (Stuber *et al.* 2013), influences range expansion after the introduction into non-native areas (Russell *et al.* 2010; Liebl & Martin 2012) and helps exploring novel food resources (Sol *et al.* 2011). In addition, other behavioural and life-history traits such as neophobia, baseline activity, aggression, sociability and dispersal, which are important for invasiveness, are often linked to exploratory and risk-taking behaviour in many species, including invasive ones (Duckworth & Badyaev 2007; Reale *et al.* 2007; Cote *et al.* 2010).

Obtaining knowledge of the genetic basis of repeatable variation in behaviour ('personality') in a newly invading species represents an important step towards understanding the evolutionary potential and constraints of the subsequent stages of spread and adaptation to the novel environment. Selective filtering of specific behavioural types during the initial stages of introduction might deplete its underlying genetic variance and concomitantly its adaptive potential. On the other hand, if selection on behavioural variation is weak and/or antagonistic between the different stages of introduction, then combined with sufficiently large numbers of introduced individuals, this could maintain or restore the original genetic variation in the newly colonized populations. The outcome can have consequences for the subsequent invasion potential and speed of the invasion front as shown from empirical (Duckworth & Badyaev 2007; Cote *et al.* 2011) and theoretical (Fogarty *et al.* 2011) studies.

Here, we test whether there is genetic variation for neophobic/neophilic behaviour in populations of the Yellow-crowned bishop (*Euplectes afer*) that recently invaded and established in Spain and Portugal. The Yellow-crowned bishop is a small songbird that naturally occurs in sub-Saharan Africa, but has established populations in the USA, Jamaica, Puerto Rico, Venezuela, Japan, Italy, Portugal and Spain (Lever 2005). Spanish and Portuguese populations originate from escaped wild-caught cage birds (Carrete & Tella 2008). At our Spanish population in the Doñana marshlands (close to Seville, SW Spain), the first individuals were recorded in 1984, while the first Portuguese records were obtained in 1992 in marshlands close to Santarem (65 km northeast from Lisbon) (own data). Both localities currently hold well-established populations with a population size of several thousands of individuals confirmed in the case of Doñana (own observations). We selected the dopamine receptor D4 gene (*DRD4*), a key element in the dopaminergic reward system and a prime candidate for investigating the genetic basis of novelty seeking and activity behaviour in vertebrates (Ebstein 2006) and in birds in particular (Fidler *et al.* 2007; Korsten *et al.* 2010; Kluen *et al.* 2012; Mueller *et al.* 2013). Our primary objective is to test whether there is variation in the *DRD4* gene and whether this variation is associated with neophobic/neophilic/activity behaviour in the two Iberian populations. We thus sequenced the complete exon 3 of the *DRD4* (the exon showing the most consistent association signals in birds; Mueller *et al.* 2013) in 100 Yellow-crowned bishops sampled in marshlands close to Seville (Spain) and Lisboa (Portugal) and identified single nucleotide polymorphisms (SNPs). The same birds were scored twice for activity behaviour during exposure to novel objects in the laboratory. A consistent association between specific SNPs and these scores across both populations would be compatible with the hypothesis that there is genetic variation for this behavioural trait and that this variation was not completely depleted by strong selection during multiple filters of the invasion process or was recovered during and after the introduction of Yellow-crowned bishops in Iberia.

Methods

Sampling, capture and housing

The Spanish birds (SPA, $N = 53$) were caught with mist nets in January/February 2010 and transferred to the laboratory within a few hours. Recently wild-caught Portuguese birds (POR, $N = 47$) were legally purchased in March 2010 on the Spanish pet market and transferred to the laboratory within 3 days. They were all initially housed in a communal outdoor aviary of 4 m^3 and after 2 days of acclimatization were randomly transferred to identical individual cages ($35 \times 35 \times 40$ cm) within the same room where they stayed until and during the experiments (8 April until 7 May 2010) under natural light and temperature regimes. Each cage was fitted with a feeding station (ad libitum standard tropical finch seed mixture), a drinking station and two perches, always in the same configuration. Cages did not allow visual contact among birds.

All individuals were molecularly sexed after the experiments, using primers CHD-P2 TCTGCATCGCTAAATCCTTT and CHD-P8 CTCCCAAGGATGAGRAAYTG (Griffiths *et al.* 1998), allowing us to test for sex differences in behaviour. Male-to-female ratios were 1.2 and 1.7 in the POR and SPA population, respectively. At the time of sampling, adults and young individuals had finished a complete moult and could

not be distinguished by plumage; we were consequently unable to quantify age.

Behavioural tests

Each individual was tested for its response to two novel objects, a 1-cm-thick slice of apple and a standard AA-sized battery, both presumably unknown in their natural Iberian environment and not encountered before in their captive environment. The major habitats of Yellow-crowned bishops in Iberia are rice fields and marshlands, so it is highly unlikely that these birds gained experience with apples. We placed the novel object in the cage, re-treated behind a screen and recorded the number of movements (hops or flights within or between perches) for 1 min. The permanent screen (already in place at the time the birds were placed in the cages) was mounted at a distance of 70 cm from the cages, and the birds were observed through a hole covered with black meshing. During control sessions prior to exposure to novel objects, all birds quickly (after a few seconds) returned to normal activity levels after any disturbance and appeared to be undisturbed when watched from behind this screen as some would sunbath, preen or sleep.

When exposed to the novel objects, most individuals greatly increased their rate of movement, moved away from the novel object and sometimes appeared to be looking for escape possibilities at the backside of the cage; only a few birds would pause, observe, and approach and touch the novel objects (e.g. by means of a quick flight and split-second perching on the novel object). The observed behaviour suggests that individuals were generally rather afraid of our novel objects (see also Gallup 1977; Verbeek *et al.* 1994; Feenders & Bateson 2011; Edelaar *et al.* 2012), but that some individuals were interested enough in investigating the objects that they overcame their fear and moved less. We hence used the rate of activity (1-min records of number of hops and flights within and between perches) when exposed to a novel object as a measure of neophilic/neophobic behaviour, with more neophobic individuals moving more. The two different novel objects were sequentially tested (first apple, then battery in 80% of the individuals) with on average 2.8 ± 2.0 days between the tests. The exposures to two different novel objects were statistically treated as independent replicates of the same behavioural assay (cf. Drent *et al.* 2003). Tests were performed between 9:30 and 14:30 h.

Sequencing and genotyping

Several weeks before the exploration tests, blood was sampled for DNA extraction. We amplified the complete exon 3 of the *DRD4* homologue in the Yellow-crowned bishop (621 bp including small pieces of flanking introns using the primers DRD4_I2F CACCACACCAGGACTGACT and DRD4_I3R GTGKGACAAGSTGGCACATTT). The PCR products of all 100 birds were directly sequenced using both primers as sequencing primers and genotyped for all identified SNPs. We identified 16 SNPs in the *DRD4* exon 3 that were polymorphic in at least one of the two populations. Information about allele names, minor allele frequencies, coding status and Hardy–Weinberg equilibria is given in Table 1.

Table 1 Allele names, coding status, minor allele frequencies (MAF) and *P*-values of tests for Hardy–Weinberg disequilibrium (HWD) for each *DRD4* SNP in the Portuguese (POR) and Spanish (SPA) populations

Locus	Major/minor allele	Coding status	MAF_POR	MAF_SPA	HWD_POR	HWD_SPA
SNP449	G/A	S	0.39	0.40	0.13	0.78
SNP458	C/T	S	0.16	0.17	0.31	0.15
SNP515	C/T	S	0.02	0.07	1.00	1.00
SNP662	G/A	S	0.40	0.56	0.36	0.06
SNP698	A/G	S	0.45	0.67	0.14	0.21
SNP705	C/T	NS Arg/Trp	0	0.01	—	—
SNP724	G/A	NS Gly/Asp	0.01	0	—	—
SNP727	G/T	NS Cys/Phe	0	0.03	—	1.00
SNP763	C/A	NS Pro/Gln	0.06	0.01	1.00	—
SNP781	A/G	S	0.02	0.01	1.00	—
SNP821	C/T	S	0.01	0.03	—	1.00
SNP822	G/A	NS Ala/Thr	0.23	0.27	1.00	0.17
SNP852	G/A	NS Val/Met	0.18	0.09	0.62	1.00
SNP858	A/G	NS Ser/Gly	0.17	0.13	0.60	1.00
SNP911	C/T	S	0.18	0.22	1.00	0.23
SNP971	C/T	S	0.48	0.74	0.56	0.07

S, synonymous coding SNP; NS, nonsynonymous coding SNP.

As population structure within the set of individuals used for association tests can lead to confounded associations (Balding 2006), we quantified population structure not only between but also within the two populations using 11 random autosomal microsatellites GCSW31, 35, 51, 55, 57 (McRae *et al.* 2005), WBSW1, 7 (McRae & Amos 1999), INDIGO 7, 29, 30, 41 (Sefc *et al.* 2001) (Table S1, Supporting information).

Data analyses

Overall genotyping quality and independence of markers were evaluated by exact tests for Hardy–Weinberg disequilibrium and genotypic linkage disequilibrium in each population sample using *Genepop* (Rousset 2008). Allelic correlations between *DRD4* SNPs were calculated using *Haploview* (Barrett *et al.* 2005). Allelic differentiation between the two populations was tested by exact tests using *Genepop*. We explored potential genetic substructuring within the populations, which could confound the genotype–phenotype associations, using S*tructure* with default settings of the underlying model, that is, allowing for admixtured individuals and correlated allele frequencies between genetic clusters (Pritchard *et al.* 2000; Falush *et al.* 2003). The web tool *Structure Harvester* was used to combine the *Structure* output of 10 independent runs (Earl & von Holdt 2012).

We considered that the two activity measures (one for each novel object test) were expressions of repeatable, consistent variation among individuals (Drent *et al.* 2003). To test this, following the recommendations of Nakagawa and Schielzeth (2010), the mode and 95% credible interval for repeatability of square-root-transformed and observer-corrected (see below) activity were estimated using the Bayesian *R* library MCMCglmm (Hadfield 2010; R Development Core Team 2012), using prior settings that were confirmed by simulation to be noninformative for repeatability ($V = 0.5$, nu = 2), and based on 3000 uncorrelated effective samples.

We tested for associations between genotype and raw activity score over both novel object tests (response variable) in both populations using a generalized linear mixed model in the *R* package glmmADMB (Fournier *et al.* 2012). The random factor 'bird identity' accounted for the twin measurements on the same individual. The gender of the birds, novel object type (apple or battery) and observer identity were included as fixed factors in the model, to account for potential differences in the response to these factors. It turned out that sex was nonsignificant ($P > 0.2$) and observer identity was significant ($P < 0.05$: the five observers differed slightly in scoring smaller movements within perches) in all models, whereas novel object type was only significant ($P < 0.05$) in models on the data of the Portuguese population. We applied a negative binomial error structure, because of the characteristics of count data and the detected overdispersion in the model (overdispersion parameters were estimated between 7.1 and 8.3). We used two different allele effect models to test for association with all SNPs: the additive and the overdominant (heterozygote) effect model. These two widely complementary single-marker tests (with 1 degree of freedom) cover all potential allele effect models (additive, recessive, dominant and overdominant) with a reasonable power (Balding 2006; Ioannidis *et al.* 2009). The overall significance of finding replicated associations at specific markers in both populations was evaluated by a permutation procedure with 500 permutations. This was performed by comparing the observed number of replicated associations with the distribution of replicated associations calculated on data sets for which the genotype data as a whole were randomly permuted to the activity scores and cofactor values within each population and novel object type (Manly 1997). With this procedure, we therefore maintained any linkage disequilibrium structure among the genetic markers in the permutated data sets. We performed the permutations with all loci, and for the common loci only (SNPs with minor allele frequency >0.05 in both populations). Standardized effect sizes as partial correlation coefficients r of individual SNPs were calculated from test statistics following formula 11 of Nakagawa and Cuthill (2007) and approximate degrees of freedom (Pinheiro & Bates 2000). Phenotypic variance explained by single SNPs was calculated as the square of these coefficients (r^2). Specific tests for potential effects of test order and novel object type were performed for the group of significantly associated SNPs by adding the interaction term between novel object type and genotype or by including the order of novel object experiments and its interaction with genotype to the standard model. To allow comparisons with other studies, we also calculated explained variances from generalized linear models with negative binomial error structure on response to single novel object types.

For graphical purposes, we plotted standardized mean activity for each genotype of the associated SNPs. Square-root-transformed activity values were adjusted for observer, averaged over both novel object tests and standardized to their mean and standard deviation prior to their usage in the graph.

Results

Behavioural measurements

Activity scores were significantly repeatable within individuals (posterior mode: $r = 0.41$, 95% credible

interval: $r = 0.23$–0.55). Activity scores did not differ appreciably between sexes (credible interval of activity score differences: −0.43 to 0.24) nor populations (credible interval: −0.33 to 0.36).

Genotyping quality and population structure

None of the exon 3 SNPs in the *DRD4* gene deviated from Hardy–Weinberg equilibrium in either population, suggesting no ancient population substructure (Table 1). Significant genotypic disequilibrium was found for 18 of the 105 pairwise *DRD4* SNP combinations in the Spanish population and for 19 of the 91 pairwise combinations in the Portuguese population, indicating some linkage disequilibrium in the *DRD4* exon 3 region of the Yellow-crowned bishop. However, estimated allelic correlations between *DRD4* SNPs are generally weak with most r^2 values below 0.5 except for the locus pairs SNP852-SNP858 ($r^2 = 0.79$), SNP458-SNP822 ($r^2 = 0.54$) in the Portuguese population and SNP727-SNP821 ($r^2 = 1.00$) in the Spanish population. In each population, two of 11 random microsatellites showed significant Hardy–Weinberg deviations (Table S1, Supporting information), suggesting among others (e.g. presence of null alleles) the possibility of shallow within-population genetic substructure. We therefore performed a formal cluster analysis using all microsatellites. Posterior probabilities of models assuming more than one genetic subcluster per population were not higher than the model probabilities assuming no substructuring (Fig. S1, Supporting information). We thus conclude that there is no evidence for substantial substructuring within the Portuguese or Spanish population and no detectable risk of confounding any genotype–phenotype associations. Further information against confounding effects came from the finding that only two random microsatellites (GCSW35 in Spain and GCSW51 in Portugal) showed significant associations with the focal phenotype, when all 11 microsatellites were tested for additive major allele effect associations. This is similar to the number of significant test results expected by chance at $\alpha = 0.05$ for 22 tests (1.1). However, nine of the 11 random microsatellites showed significant allele frequency differentiation between the Portuguese and Spanish populations (all loci $F_{ST} = 0.017$; standardized all loci F_{ST}' (Meirmans 2006) = 0.049), indicating restricted gene flow between the two populations and/or differential origins of the founders. This means that the genotype–phenotype association tests in the two populations can be seen as relatively independent in comparison with two samples coming from the same population and therefore serve as replicates not only in the typical sense of independent replications to avoid type 1 error due to testing of multiple markers, but additionally to a certain extent as evolutionary replicates that help guard against any unrecognized confounding factors. It is indeed very unlikely that the association tests were similarly confounded by a similar, undetected substructure in both populations.

Genotype–phenotype associations

We found significant additive allele effect associations with activity during the novel object test for the two *DRD4* SNPs SNP449 and SNP698 in the Portuguese population ($r = 0.31$, $P = 0.030$ and $r = -0.44$, $P = 0.001$, respectively) and for the SNP449 in the Spanish population ($r = 0.40$, $P = 0.002$) (Fig. 1A). It is striking that SNP449 showed the same positive association in both populations. Furthermore, the second significant SNP in the Portuguese population (SNP698) showed a marginally nonsignificant association in the same negative direction in the Spanish population ($r = -0.24$, $P = 0.086$). At a significance level of $\alpha = 0.10$ (a level covering the observed significances of the two consistent SNPs), the data permutation procedure on all loci yields a *P*-value of 0.014 that two (or more) association signals with the same allele effect relationships are replicated in two populations by chance alone. The corresponding *P*-value based on permutations of common SNPs only is 0.008. As an alternative approach, we also tested the additive allele effect associations of the common SNPs with activity scores in single models for both populations and obtained the same overall result: SNP449 and SNP698 significant, and its interactions with population nonsignificant (Table S2, Supporting information). For both SNPs, the heterozygotes showed activity levels that were intermediate between the two homozygotes (Fig. 2), except for SNP449 in the Portuguese population. According to the graphs, the average change per genotype was about 0.5 standardized activity scores in most SNPs and populations. For the Portuguese population, 10% of the variance in activity scores is explained by SNP449 and 19% by SNP698, whereas for the Spanish population, 16% of the variance was explained by SNP449 and 6% by SNP698. The effects of the two SNPs are not completely independent, because the two SNPs were weakly linked with allelic correlations of 0.13 and 0.15 in the Portuguese and Spanish populations, respectively. The explained variance of the response to single novel object types by SNP449 or SNP698 ranged from an average of 2% (battery) to 18% (apple) in the Portuguese population and was around 11% (battery and apple) in the Spanish population.

For the two significantly associated SNPs (SNP449 and SNP698), we further extended the models to test the effect of experiment order and the interaction between experiment order and SNP genotype (this only in the Portuguese population, in which the order of

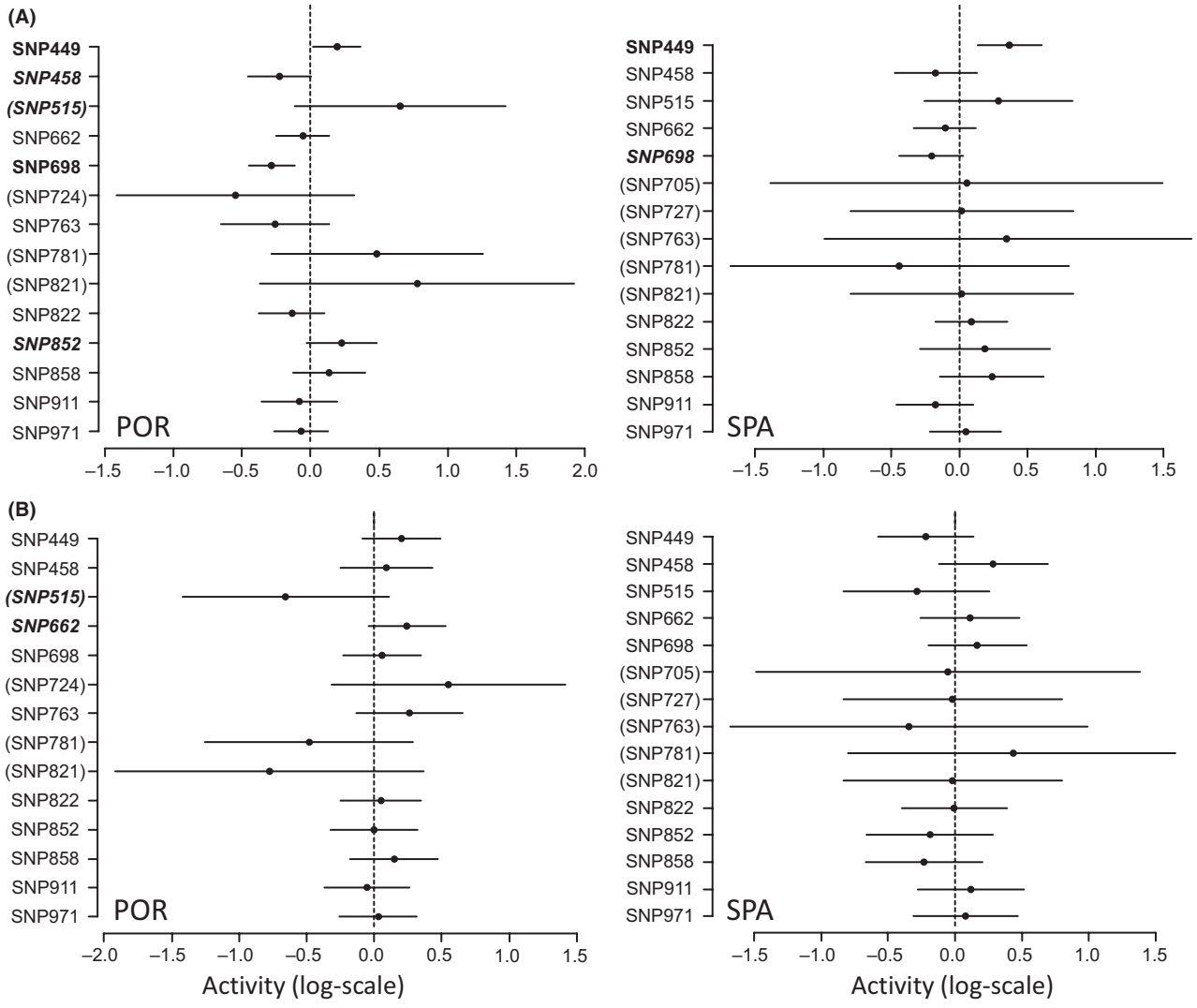

Fig. 1 Effect sizes with 95% confidence intervals of *DRD4* SNP genotypes for raw activity scores (log scale) in the populations of Portugal (POR) and Spain (SPA). SNPs in bold and SNPs in bold italics have significant effects at the 5% and 10% significance level, respectively. SNPs in brackets have minor allele frequency lower than 0.05 and are shown for the sake of completeness. The underlying generalized linear mixed effects models include the sex of individuals, novel object type and observer ID. (A) additive allele effect models and (B) overdominant allele effect models.

tests was balanced, i.e. 24× apple test first and 23× battery test first; in the Spanish population, the apple test was always first). Both effects were nonsignificant for both SNPs ($P > 0.1$). The interaction between novel object type and genotype was also nonsignificant for the two associated SNPs in both populations ($P > 0.1$).

No significant overdominant allele effect on activity score was found in the Portuguese or Spanish population, Fig. 1B.

Discussion

Evidence for a heritable component of behaviour can be derived from selective breeding experiments or using pedigrees, but also by evaluating direct genotype–phenotype relationships. We found that the Yellow-crowned bishop responded to two different novel objects moderately consistently with a repeatability of 0.41. This value is in line with results from a recent meta-analysis of repeatability (Bell *et al.* 2009), which reported a mean repeatability of 0.37 across all sorts of animal behaviours. Further, we provide evidence for a direct genetic association between *DRD4* polymorphisms and the activity scores during the exposure to novel objects. These findings indicate a strong genetic component to the activity behaviour in introduced populations of the Yellow-crowned bishop.

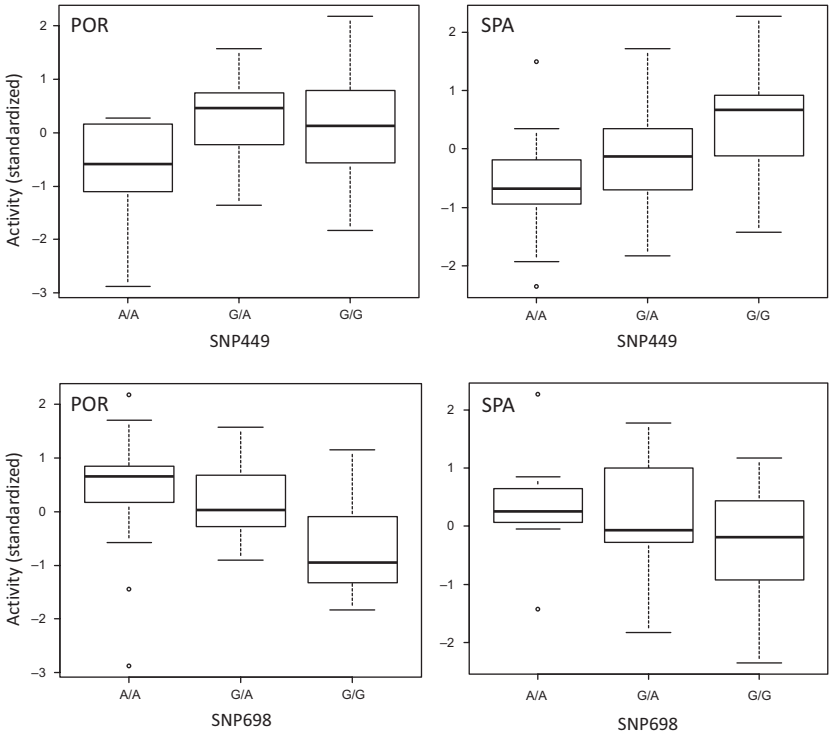

Fig. 2 Relationships between standardized mean activity scores and genotypes of SNP449 and SNP698 in the populations of POR and SPA.

We identified two *DRD4* SNPs (SNP449 and SNP698), which explained on average 11% and 15% of the phenotypic variance in the Spanish and Portuguese population, respectively. These estimates refer to phenotypic variance based on two measurements. In comparison, around 5% of the variance of a single measurement of exploratory behaviour has been explained by a *DRD4* SNP in Great tit populations (Korsten *et al.* 2010; Mueller *et al.* 2013). The explained variance in response to single novel objects by SNPs was highly variable and ranged from 2% to 18% in our study, indicating heterogeneity among genetic associations with similar phenotypes, although there is substantial repeatability between the phenotypic expressions. This strongly advocates the use of repeated measurements in genotype–phenotype analyses when the focus is on the detection of consistent genetic associations with only between-individual variability of traits that also show substantial within-individual variance.

Repeated behavioural tests change the level of experience, which can have a significant effect on the behavioural scores (Dingemanse *et al.* 2012). In general, genetically based contributions to behavioural responses are expected to be stronger in inexperienced birds than in birds with experience with the test situation. However, we did not find a significant effect of the order of experiments or the interaction between order and SNP genotype. Repeated novel object tests specifically also entail the usage of different novel objects in each subsequent test. Response to the apple slice might be different than to the battery if it was recognized as a potential food source, in particular for an invasive bird searching for novel food resources. It has been shown that responses to novel food resources are linked to heritable personality variation in Great tits (Exnerova *et al.* 2010). The interaction between novel object type and genotype was nonsignificant, which indicates that both novel object tests, although potentially to a different extent (see SNP effects of the battery and apple test in the Portuguese population), contribute to the overall genotype–phenotype association.

The two SNPs (SNP449 and SNP698), which showed consistent associations with activity scores in our experiments with two different populations, are synonymous SNPs in the exon 3 of the *DRD4* gene. *DRD4* exon 3 polymorphisms are prime candidates when analysing novelty seeking and related behaviour in mammals and birds (Ebstein 2006; Fidler *et al.* 2007; van Oers & Mueller 2010). Exploratory behaviour, measured as activity in novel environment chambers, was associated with a synonymous exon 3 SNP in some, but not all Great tit populations (Korsten *et al.* 2010; Mueller *et al.* 2013). In a Blue tit population, a synonymous exon 3 SNP was associated with escape behaviour from a cage (Kluen *et al.* 2012). A study on the ecological adaptations of Common waxbills (*Estrilda astrild*), another Iberian invasive bird species, however, did not reveal a significant association between two exon 3 SNPs and

experimental scores for boldness, tonic immobility, social interaction and exploration (Carvalho *et al.* 2013). An explanation for the lack of association in this study might be the nonrandom selection of individuals according to social reactivity. A recent detailed study suggests multiple functional variants in and around exon 2, 3 and 4 of the *DRD4* gene for exploratory behaviour in Great tit populations (Mueller *et al.* 2013). The two associated SNPs in the Yellow-crowned bishop are good candidates for functional polymorphisms, but it could well be that other strongly linked polymorphisms around exon 3 actually exert the function. Specifically, SNP449, which shows the second strongest conservation among 50 SNP sites in homologous exon 3 regions of 11 bird species (own unpublished data), has a high functional potential. Given the potentially high number of DNA binding sites for regulatory factors (e.g. transcription factors, regulatory RNAs) in gene regions (Mattick *et al.* 2010), an equally high number of genetic variants influencing gene regulation via the binding characteristics of these factors can be expected (Haraksingh & Snyder 2013). Different genetic variants might affect the expression level or the splicing of the *DRD4* gene or might produce different regulatory links with other genes. We can, however, exclude all common coding nonsynonymous polymorphisms in exon 3 as linked drivers for the detected association signals, because we sequenced the complete exon 3 in all individuals and none of these polymorphisms were associated.

Our experiment was designed to measure genetic variation on the personality axis of neophobia/neophilia, which appears to be relevant for predicting the success of invasions. We measured the reaction to novel objects via activity scores, which therefore may also reflect baseline activity. Notably, *DRD4* association studies in humans suggest that neophilic and activity behaviours have a partially common genetic basis, because the best confirmed associations of a *DRD4* exon 3 polymorphism in humans are with novelty seeking behaviour (Ebstein 2006) and attention deficit/hyperactivity disorder (ADHD) (Nikolaidis & Gray 2010). In the context of the evolution of ADHD, it has been suggested that resource-depleted, time-critical or rapidly changing environments might select for individuals with 'response ready' adaptations such as increased motor activity, impulsiveness and scanning behaviour, whereas resource-rich, time-optimal or little-changing environments might select against such adaptations (Jensen *et al.* 1997; Grady *et al.* 2013). Similar microevolutionary processes might be important during an invasion (Carvalho *et al.* 2013). Our detected genotype–phenotype associations suggest that there is ample genetic variation in the reactivity to novel objects/novel food items in newly established and spreading Yellow-crowned bishop populations, because the two associated SNPs (SNP449 and SNP 698) have additive allele effects, are only mildly linked and have high minor allele frequencies. The alleles are likely derived from the original population and may have been maintained by antagonistic selection between the different stages of the invasion process (Chapple *et al.* 2012). Alternatively, they may be restored and maintained by frequency-dependent selection in the new populations. Given the short invasion history of Yellow-crowned bishops in Iberia (*c.* 30 years) and the expected small initial population size(s), it is unlikely that the behaviour-associated SNPs are based on new mutations that occurred after the invasion. However, only a comparison of the allele frequencies between the original African populations and invasive populations of different age will enable a direct tracking of the microevolutionary changes during the invasion process (Carrete *et al.* 2012; Edelaar & Bolnick 2012). Neophilic/neophobic behaviour likely influences dispersal tendencies, and, if linked to aggression, this relationship might perform density dependently (e.g. Duckworth & Badyaev 2007). Empirical and theoretical results predict higher invasion potential and speed of the invasion front for populations with a mix of such behavioural types, because the repeated sequential stages of the invasion process comprise low- and high-density populations as well as variable fitness for individuals with low and high dispersal tendencies (Cote *et al.* 2011; Fogarty *et al.* 2011). Successfully established populations of invasive species are thus likely characterized by a mixture of behavioural types. The observed diversity of underlying genetic variants as those that we report here is in line with this evolutionary scenario and can serve as genetic markers to study the evolution of neophobic/neophilic behaviour.

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Data accessibility

DRD4 exon 3 sequence and polymorphisms information: GenBank Accession no. KJ671448. Genotype and phenotype data: DRYAD entry doi:10.5061/dryad.589t0.

Supporting information

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

Fig. S1 Mean posterior probabilities (±SD) of genotype data for random microsatellites given K subpopulations for the populations of Portugal (POR) and Spain (SPA).

Table S1 Allele numbers, minor allele frequencies (MAF) and *P*-values of tests for Hardy-Weinberg disequilibrium (HWD) for each random microsatellite locus in the Portuguese (POR) and Spanish (SPA) populations.

Table S2 Tests for associations between activity scores and SNP genotypes and its interactions with population (POR) in single models for both populations.