Behavioural polymorphism in wintering white-throated sparrows, *Zonotrichia albicollis*

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Behavioural polymorphisms associated with morphs with a known genetic basis are interesting to study since they provide a model system to investigate the molecular basis of complex behaviours. White-throated sparrows are one of such rare models, presenting two morphologies and behavioural types, tan and white, caused by a chromosomal rearrangement. The behavioural differences have been mainly studied during reproduction, defining two morph-specific breeding strategies and different levels of aggression between morphs. Outside of the breeding season, the morphological differences are less apparent, morphs are more difficult to distinguish and the behavioural polymorphism is poorly understood. In this study, we caught wild individuals during the winter, determined their morph by genetic analysis, and analysed the differences in behaviour between morphs when encountering a novel object under standardized conditions in captivity. White morphs are more aggressive during reproduction; therefore, we expected them to be less affected by the presence of the novel object than tan morphs; animal personality research shows that more aggressive individuals are less affected by the presence of novel objects (i.e. neophilic). Contrary to our expectations, white morphs were more neophobic than tan morphs, and within morphs, males were more neophobic than females. Furthermore, white morph individuals and males of both morphs suffered greater weight loss during captivity compared to tan morphs and females, respectively, suggesting that the response to captivity-induced stress differs between morphs and between sexes. Finding behavioural differences between morphs not related to reproduction implies a broader impact of the effects of the chromosomal rearrangement on the behavioural polymorphism in this species.

Behavioural polymorphisms are consistent differences in behaviour found in a population. These polymorphisms show relatively stable frequencies, either by individuals choosing a suitable strategy according to the environmental stimuli or by individual consistency of behaviour over time (Gosling, 2001; Houston & McNamara, 1985; Maynard Smith, 1966). In both cases, evolutionary processes will determine the frequency of the behaviours, resulting in the maintenance of the polymorphism. The first scenario has been studied extensively using game theory. Individuals can choose a strategy depending on the social and physical environment in order to obtain the highest benefit (Hedrick, 1983; Maynard Smith, 1982). Classical examples include optimal foraging strategies where individuals adapt their responses to food availability, and change them when necessary (Caraco, Martindale, & Whittam, 1980). The second scenario contemplates the possibility of individuals behaving in a consistent manner over time. This phenomenon could be explained by local adaptation to the environment or by genetic constraints (reviewed in Dall, Bell, Bolnick, & Ratnieks, 2012). Local adaptation implies that individuals prefer and choose a particular environment; therefore, their behaviour will remain constant and adapted to the local conditions, creating the perception of individual consistency of behaviour over time (Dall, Houston, & McNamara, 2004; Jaenike & Holt, 1991; Maynard Smith, 1966). On the other hand, the consistency of behaviour can be a result of the genetic make-up. Individuals showing similar genetic and physiologic networks will tend to behave in a similar manner in a wide range of environments; thus, differences in the

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genetic background will result in different behavioural types (McGlothlin & Ketterson, 2008).

The field of animal personality studies the individual consistency of behaviour and the genetic mechanisms underlying it. Research in this field has shown that behaviours such as aggression, dominance, risk sensitivity, exploratory behaviour and boldness are often correlated and constant over time in different taxa (Réale, Reader, Sol, McDougall, & Dingemanse, 2007; Verbeek, Drent, & Wiepkema, 1994). In many instances, these behaviours are associated with the individual's melanization (Quesada & Senar, 2007; reviewed in Ducrest, Keller, & Roulin, 2008). The genetic relationship among these behaviours and morphology has been studied through cross-fostering experiments and artificial selection, showing high levels of heritability (Dingemanse, Both, Drent, van Oers, & van Noordwijk, 2002; Drent, van Oers, & van Noordwijk, 2003; van Oers, Drent, de Goede, & van Noordwijk, 2003). Studying correlated and heritable traits from an evolutionary perspective is challenging (Zawistowski & Hirsch, 1983). Nowadays, the expanding availability of genetic tools allows researchers to study the genetic basis of complex behaviours, such as alternative reproductive strategies. Recent studies explained how supergenes are associated with stable reproductive strategies in two bird species, the ruff, *Philomachus pugnax*, and the white-throated sparrow, *Zonotrichia albicollis* (Lamichhaney et al., 2015; Taylor & Campagna, 2016; Tuttle et al., 2016). In both cases, there is a chromosomal rearrangement, which acts as a supergene that causes a behavioural and a morphological stable polymorphism. Interestingly both polymorphisms are associated with melanin-based coloration and distinct reproductive strategies (Grunst et al., 2017; Höglund & Lundberg, 1989; Tuttle, 2003; Widemo, 1998).

The white-throated sparrow presents a large rearrangement in chromosome 2, resulting in two different plumage morphs, tan and white. Individuals that are homozygotes without the chromosomal rearrangement are tan, heterozygotes for the rearranged and non-rearranged chromosome are white, and homozygotes for the rearrangement are phenotypically white but scarcely found (Lowther, 1961; Thorneycroft, 1966, 1975). The plumage differences are limited to the birds' head and upper chest plumage and are more conspicuous during the breeding season (Falls & Kopachena, 2010). Tan birds have tan and brown pheomelanic stripes, and white birds have pure white and black eumelanic stripes (Grunst et al., 2017; Lowther, 1961; Rathbun et al., 2015). Both morphs are present in both sexes and mate disassortatively, creating pairs formed by a tan male (TM) with a white female (WF), and a white male (WM) with a tan female (TF) (Knapton & Falls, 1982; Lowther, 1961; Tuttle, 1993; Tuttle et al., 2016). The disassortative pairing during reproduction does not allow associating reproductive strategies to morph; instead, the reproductive strategies are associated with a morph—sex type. Tan males tend to perform more mate guarding, ensuring their within-pair paternity (Tuttle, 2003). White males sing more and pursue extrapair copulations, resulting in within-nest paternity loss (Lowther, 1962; Tuttle, 2003). White females sing and display more sexual solicitations, while tan females do not sing (Lowther, 1962; Tuttle, 2003). The behavioural polymorphism is not only reduced to mating strategies but also includes habitat choice and aggression levels. White males are more aggressive and breed in high-density areas, while tan males are more parental and establish their territories in low-density areas (Formica, Gonser, Ramsay, & Tuttle, 2004; Horton, Moore, & Maney, 2014; Kopachena & Falls, 1993a, 1993b; Lowther, 1962). After the breeding season, birds undergo postnuptial moult when their morphological differences become less apparent and hinders visual morph recognition (Atkinson & Ralph, 1980; Vardy, 1971). At that time, both morphs flock together and migrate south for wintering.

The behavioural polymorphism in this species has been mainly studied during the breeding season. Due to the diversity of behaviours that can be regulated by the genes included in the rearrangement, it is possible that behaviours not associated with reproduction are also affected by the supergene. Studies comparing behavioural differences between morphs in wintering birds are not conclusive (Dearborn & Wiley, 1993; Ficken, Ficken, & Hailman, 1978; Hailman, 1975; Harrington, 1973; Piper & Wiley, 1989; Schlinger, 1987; Schneider, 1979; Schwabl, Ramenofsky, Schwabl-benzinger, Farner, & Wingfield, 1988; Watt, Ralph, & Atkinson, 1984; Wiley, Piper, Archawaranon, & Wyrick, 1993). To our knowledge, all studies used wintering foraging flocks to examine differences in dominance rank among individuals, leading to different results. Watt et al. (1984) found that, during winter, tan females were dominant over white females, but dominance in males was age related. Moreover, no morph differences associated with dominance rank in wintering flocks were found in other studies (Dearborn & Wiley, 1993; Piper & Wiley, 1989; Schlinger, 1987; Schneider, 1979; Schwabl et al., 1988; Wiley et al., 1993). Studies of dominance conducted in spring migrant flocks showed differences in dominance and aggression, with white birds being more aggressive and dominant than tan birds (Harrington, 1973; Ficken et al., 1978; but see Hailman, 1975).

In the present study, we analysed the differences in behaviour outside of the breeding season in wild white-throated sparrows caught during winter. Our aim was to determine whether the behavioural differences between morphs found during the breeding season are also present in a consistent manner during the wintering period. Personality studies in other species have found correlations in different behavioural responses across seasons (Highcock & Carter, 2014; Thys et al., 2017). We applied similar methodology to study behavioural differences between white-throated sparrow morphs in response to a novel object. We already know that white males sing more and are dominant over tan males during the breeding season and in spring migrant flocks (Ficken et al., 1978; Lowther, 1962), and furthermore, that white females sing more and are more aggressive than tan females (Kopachena & Falls, 1993a; Lowther, 1962). Since animal personality studies in other species show that more aggressive and dominant individuals are bolder when facing novel situations, we expected white birds to be less affected by the presence of a novel object (i.e. neophilic) in winter. Our model species is ideal for this study due to the presence of two discrete morphs that present differences not only in behaviour but also in plumage coloration. Our findings will allow us to determine whether the behavioural differences between morphs are due to hormonal changes associated with the breeding season, or whether the two morphs show a behavioural polymorphism that can be distinguished across seasons. Having a full understanding of the behavioural differences between and within morphs across seasons will help to better characterize the behavioural polymorphism of this species. Due to the importance of the white-throated sparrow as a model species in behavioural and molecular ecology (Formica et al., 2004; Grunst et al., 2017, 2018; Maney, 2008; Thomas et al., 2008; Tuttle et al., 2016), having a deeper understanding of their behaviour and genetics will keep building the bridges between ecology and molecular biology, unravelling the puzzle of the evolution of behaviour.

METHODS

We captured wintering white-throated sparrows ($N = 41$; 11 TM, 3 TF, 15 WM, 12 WF) in Terre Haute, Indiana, U.S.A. (39°28′N, 87°24′W), during the winter season (from late November to early February) from 2013 to 2015. Birds were captured using mist nets, either by passive netting or by flushing the birds into the nets. At

capture, each bird received an aluminium leg band and one or more plastic colour leg bands for individual identification. In addition, we took body measurements and blood samples by brachial ven-epuncture for posterior laboratory analysis. After capture, birds were immediately transported to the experimental facilities at Indiana State University, where they were kept individually in wire cages ($45 \times 45 \times 50$ cm) with food at the base (white-throated sparrows are ground foragers) and two feeders on the sides: one with water and one with mealworms. Two mealworms were offered once per day every day. Three to four days after capture (in order to avoid the peak of stress resulting from the first hours of captivity), we measured the behavioural response when facing a novel object (novel object experiment). While in captivity, all birds ate the mealworms at least once, ate the food provided and drank, ensuring they did not start the trials food deprived. On the release day, up to 7 days after capture, all birds were weighed and released unharmed at the same capture location.

Novel Object Experiment

The birds were subjected to a novel object experiment following Verbeek et al. (1994). The experimental trials consisted of the addition of a novel object into the bird's known environment. We added a red marker hanging from the side of the feeder containing the mealworm. In order to get the mealworm, the birds had to get near the novel object and reach inside the feeder. Birds were randomly assigned to start with a control (absence of the novel object) or experimental (presence of the novel object) treatments. The trial started after placing a mealworm in the feeder; the behaviour of the bird was videorecorded for 30 min. After that time, the opposite treatment (presence/absence of the novel object) was applied; we added another mealworm in the feeder, and recorded another 30 min of video. The recordings were made using a Sony HandyCam compact camera. The camera was placed on a tripod in front of the cage from the first day the birds were held captive, to avoid adding any novelty on the day of the experiment. All birds were tested only once since the purpose of the experiment was to observe birds' responses to an object that they had never encountered before.

To quantify the behavioural response towards the novel object, we used The Observer XT v. 12.0 software (Noldus Information Technology, Wageningen, the Netherlands). The behavioural scoring was blind since at that time we did not know the morph and sex of the individuals, which was determined a posteriori by molecular analyses (detailed below). For the behavioural quantifi-cation, we visually 'divided' the cage into two sides: the side containing the feeder with the mealworms (where the novel object would be placed) and the side containing the feeder with water. For each trial, control and experimental, we obtained four behavioural measures: (1) number of hops between different locations in the cage ('number of hops'); (2) percentage of time the birds spent on the side of the cage containing the novel object ('percentage of time close'); (3) whether the bird approached the feeder ('Feeder Y/N'); and (4) whether the bird ate the mealworm ('Worm Y/N'). The first variable gives us a measure of the birds' activity during the experiment, while the later three variables give us a measure of direct response to the novel object and can be used as proxy for boldness/neophilia.

Statistical Analysis

We analysed the behavioural variables by general (or general-ized when necessary) linear models (GLM). All analyses were car-ried out with R 3.4.3 (R Core Team, 2017). Type III sums of squares and orthogonal contrasts were used in all cases.

We used repeated measures analysis of variance (RM-ANOVA) to test for the effect of the experiment (presence versus absence of the novel object) within subjects on the number of hops and the percentage of time close, controlling for the predictor variables sex and morph, and their interaction. To meet normality assumptions, number of hops was transformed to its logarithm. However, we were unable to normalize the percentage of time close. Therefore, the *P* values estimated in a RM-ANOVA would not be reliable, as we were violating some basic assumptions of the model. To solve this issue, we built ad hoc the distribution for all statistics under the null hypothesis by Monte Carlo simulations. We permuted the recorded percentages of time among individuals within the experimental and control observations to keep the block structure of the data. We carried out 10 000 simulations and estimated *P* values from the resulting empirical cumulative distribution functions for the *F* statistic of each predictor included in the model.

We distinguished between neophilic and neophobic individuals according to whether or not they approached the feeder (Feeder Y/ N) and whether or not they ate the worm (Worm Y/N). We tested for differences in the probability of approximation between experimental and control observations while controlling for the effect of sex and morph. We could not include the interaction be-tween the predictors because one of the combinations (tan fe-males) was not represented. We used a GLM with binomial distribution and logit link function to analyse the data.

Finally, we assessed the effect of captivity on the physical con-dition of individuals by studying changes in bird body mass at capture and release. We used a RM-ANOVA with sex, morph, number of days in captivity and their interactions as predictors. We did not have data for four individuals on the capture day.

Laboratory Analysis

White-throated sparrows do not show obvious morphological differences in plumage coloration or any other sex-linked attributes after postnuptial moult (at the end of the breeding season). To identify the morph and sex of the individuals, we used microsat-ellite genetic techniques following Griffiths, Double, Orr, and Dawson (1998) to determine sex, and Michopoulos, Maney, Morehouse, and Thomas (2007) to determine morph. The DNA was obtained from blood samples stored in Longmire's solution (Longmire, Gee, Hardekopf, & Mark, 1992). We extracted genomic DNA using DNA IQ magnetic extraction (Promega Corporation, Madison, WI, U.S.A.) and sequenced the amplified samples using the ABI PRISM 310 Genetic Analyzer (Applied Biosystems Corpo-ration, Foster City, CA, U.S.A.).

Ethical Note

Birds used in the experiment ($N = 41$) were caught at a local feeder using mist nets (USGS Banding Permit number 22296; Indiana State University Institutional Animal Care and Use Com-mittee (IACUC) protocol number 562154-1:ET/RG). After capture, each bird was placed in an aluminium cage ($40 \times 30 \times 40$ cm) covered with a cloth for immediate transport to the experimental facilities at Indiana State University. Upon arrival, birds were indi-vidually housed in wire cages ($45 \times 45 \times 50$ cm) and provided with food and water ad libitum. During the captive period, birds were only disturbed once per day to provide food and were kept in a room with automatic lights that produced a natural light:dark regime. The birds were only manipulated twice, after capture and before release. During manipulation, we banded them with metal and plastic colour leg bands, took blood samples (up to 80 µl) by brachial venepuncture, and obtained biometric measures. Up to 7 days after capture, avoiding days of bad weather conditions, birds

were released at their capture location, where they had direct access to food and shelter. All birds survived the experimental trials and many of them ($N = 16$) were seen during subsequent netting attempts and/or in subsequent years, suggesting that the experiments did not affect their survival or migratory abilities.

RESULTS

The number of hops, a measure of activity, increased significantly when the novel object was present ($F_{1,37} = 8.653, P = 0.006$), and this increase was similar for both sexes and both morphs (Fig. 1; see Appendix, Table A1).

The percentage of time close, a measure of the birds' direct response to the novel object, differed significantly between males and females (treatment∗sex: $F_{1,37} = 14.45, P < 0.001$; Fig. 2; see Appendix, Table A2). Females spent a greater percentage of time in the area close to the novel object when the novel object was present (increase of 28%). In contrast, males decreased such time by 11%. Furthermore, we recorded a significant difference in behaviour between morphs towards the experimental treatment (treatment∗ morph: $F_{1,37} = 28.66, P < 0.001$; Fig. 2, Table A2). Tan morphs increased the time spent in the area close to the novel object by 37% when the novel object was present, while white morphs reduced their time by 19%. The interaction between morph and sex and the experimental treatment was also statistically significant ($F_{1,37} = 0.08, P = 0.009$), demonstrating that the attraction (or aversion) towards the novel object was sex dependent within the morphs.

The probability of approaching the feeder and the probability of obtaining the worm were not affected by the presence or absence of the novel object (see Appendix, Table A3).

Finally, captivity had a negative effect on the body mass of individuals ($F_{1,32} = 10.74, P = 0.003$; Fig. 3; see Appendix, Table A4). As expected, the effect of captivity became more deleterious as the individuals remained captive for longer periods ($F_{1,32} = 17.87, P < 0.001$); i.e. the longer they were held captive, the greater the

weight difference between capture and release days. Interestingly, males lost more mass than females ($F_{1,32} = 5.16, P = 0.030$; Fig. 3), and white morphs tended to lose more body mass than tan morphs ($F_{1,32} = 3.93, P = 0.056$; Fig. 3).

DISCUSSION

We found empirical support for differences in behaviour approaching a novel object between morphs: white morphs were more neophobic than tan morphs. Interestingly, the results were contrary to our expectations. Since white morphs are found to be more aggressive during the breeding season (Houtman & Falls, 1994; Kopachena & Falls, 1993a; Lowther, 1962), we were expecting them to be more neophilic (i.e. bolder) when facing novel objects. Furthermore, the behavioural response was associated with sex. When presented with a novel object, females were more neophilic (i.e. bolder) than males. To our knowledge, these results are the first experimental trials showing differences in response to novelty between white-throated sparrow morphs in the wintering season. The activity levels, measured as number of hops, did not differ between morphs or between sexes; all individuals similarly increased the number of hops in response to the stimulus. The likelihood of approaching the feeder and the likelihood of eating the mealworm did not differ between morphs or between sexes, and these behaviours were consistent among individuals throughout their period in captivity. Individuals that had eaten the worm consistently during the captive period ate the worm during the experimental trials regardless of the presence/absence of the novel object. Thus, this variable might not be directly measuring boldness per se, but it could be associated with other traits such as food preference, likelihood to feed on unknown food items and/or nutritional status of the individuals. Finally, all individuals lost weight during captivity, and this loss was strongly correlated with the number of days captive. Interestingly, weight loss was also associated with morph and sex. White morphs lost more weight than tan morphs, and males lost more weight than females.
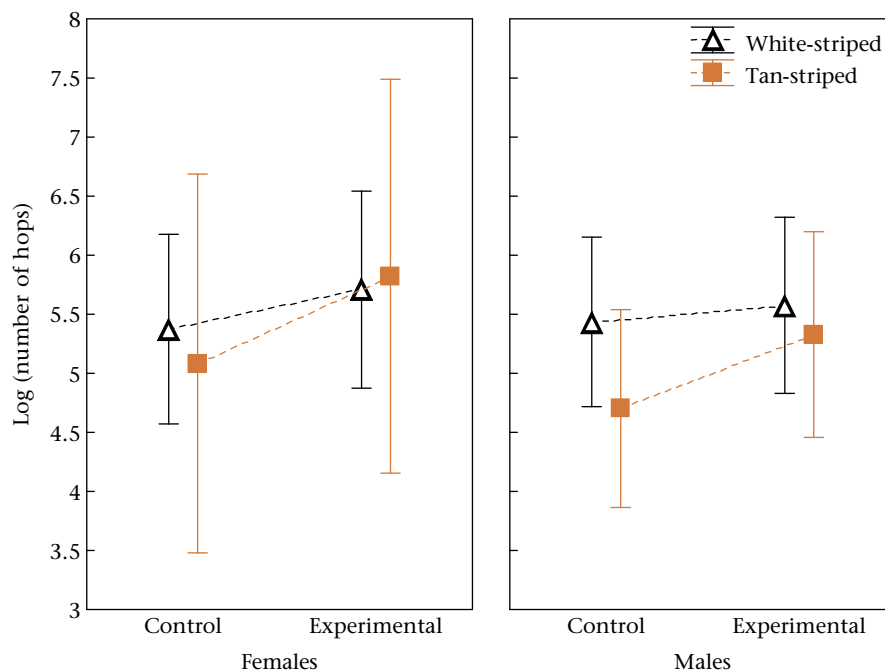
Figure 1. Change in average activity (measured as the logarithm of the number of hops) between control and experimental trials. Error bars represent 95% confidence intervals. $N = 11$ tan-striped males, 3 tan-striped females, 15 white-striped males, 12 white-striped females.
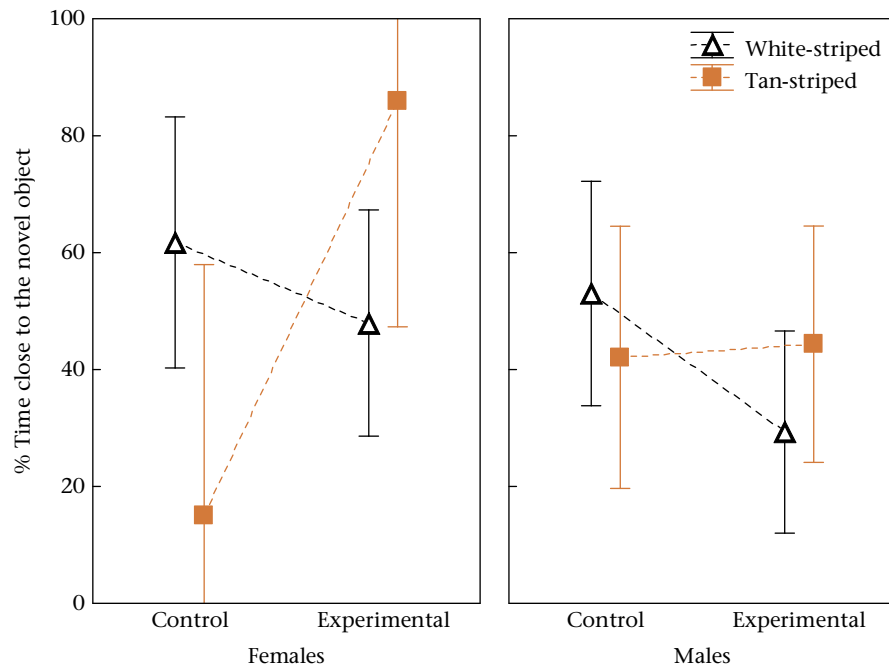
Figure 2. Change in average response to the novel object (measured as the percentage of time close to the feeder containing the novel object) between control and experimental trials. Error bars represent 95% confidence intervals. $N = 11$ tan-striped males, 3 tan-striped females, 15 white-striped males, 12 white-striped females.
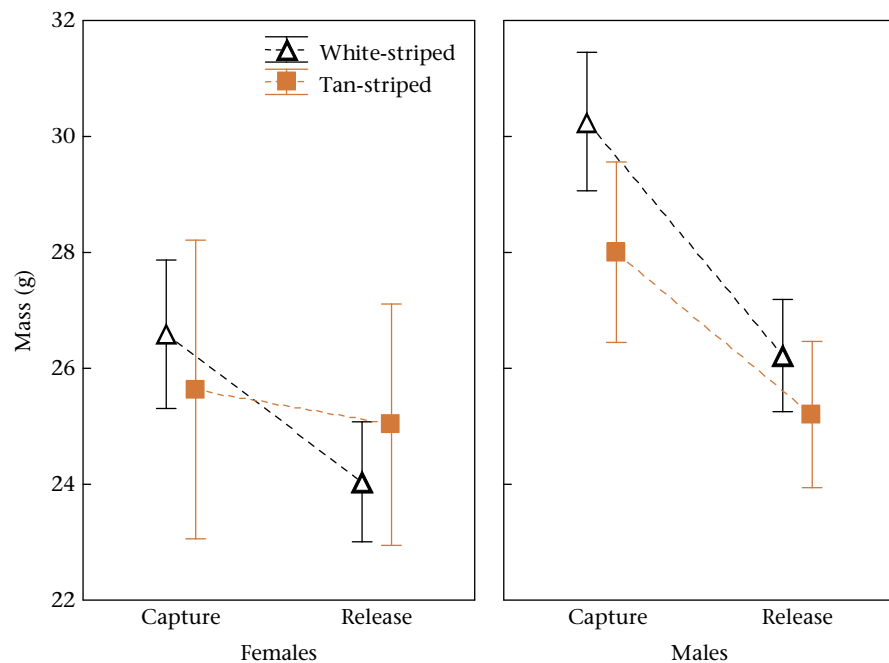
Figure 3. Change in average bird mass at capture and on release days. Error bars represent 95% confidence intervals. $N = 11$ tan-striped males, 3 tan-striped females, 15 white-striped males, 12 white-striped females.

Given the significant interaction between morph and sex in response to the novel object and in weight loss, we can rank morph—sex types as a function of their responses. Tan and female individuals were less affected by the presence of the novel object and lost less weight during captivity, while white and male individuals were more affected by the experimental trials and lost more weight.

As an added twist to the complexity of this system, individuals showing extreme magnitudes of response in our study (white males strongly neophobic and tan females strongly neophilic) form natural pairings during the breeding season (Knapton & Falls, 1982; Lowther, 1961; Tuttle, 1993; Tuttle et al., 2016). In contrast, tan males and white females, the other pair type, showed more balanced responses in our experiment. These findings match with the behavioural differences associated with pair types in our study

species during the breeding season, where parental effort is more balanced in pairs formed by a tan male and a white female and is more female biased in pairs formed by a white male and a tan female (Kopachena & Falls, 1993b; Tuttle, 2003). Along the same lines, in the animal behaviour literature, we can find examples of increased reproductive success in pairs formed by either extreme behavioural types (Both, Dingemanse, Drent, & Tinbergen, 2005; Martin-Wintle et al., 2017) or similar behavioural types (Schuett, Dall, & Royle, 2011).

Our findings appear to contradict the individual consistency of behaviours across seasons found in other species (Highcock & Carter, 2014; Thys et al., 2017). In our system, the differences in behaviour could be associated with morphs showing different levels of stress tolerance. Maintaining animals in captivity increases their stress levels, which affects their behavioural responses (Marra, Lampe, & Tedford, 1995; Morgan & Tromborg, 2007). White morphs lost more weight and seemed to be more affected by captivity (i.e. displaying more stress-like behaviours such as intense preening, stereotypical pacing and crown rising; M. Barcelo-Serra, personal observation) than tan individuals. Stress levels are known to influence behaviour. Carere, Groothuis, Möstl, Daan, and Koolhaas (2003) found that great tits, Parus major, from artificially selected fast and slow personality lines (Drent et al., 2003) differed in corticosterone levels (a hormone related to stress) following a staged aggressive interaction with a conspecific. Individuals from the slow line (i.e. shy individuals) showed increased corticosterone levels, while individuals from the fast line (i.e. bold individuals) did not show a hormonal change after the stimulus. Furthermore, other studies in rodents analysed the effect of stress hormones on risk assessment (Mikics, Barsy, Barsvári, & Haller, 2005; Quartermain, Stone, & Charbonneau, 1996). Individuals challenged by stressful situations or artificially increased levels of stress hormones showed disrupted risk assessment behaviours. High levels of stress hormones resulted in increased risk assessment behaviours in novelty-related nonsocial situations, but resulted in increased aggression in social situations (Mikics et al., 2005). In our study species, Horton and Holberton (2010) found differences in corticosterone levels between white-throated sparrow morphs during the breeding season, with white morphs having higher baseline corticosterone levels than tan morphs. These findings are consistent with behavioural differences observed in the present study (where white morphs were found to be more neophobic) and in other studies performed during the breeding season (where white morphs were found to be more aggressive; Kopachena & Falls, 1993a; Lowther, 1962). Differences in stress tolerance between morphs could be either the cause or the consequence of the differences in behaviour found between the sparrow morphs. Along the same lines, Tuttle et al. (2017) found that white males suffer lower return rates to the breeding grounds after harsh winters, presumably being more susceptible to changes in the environment. Measuring corticosterone levels prior and after captivity outside of the breeding season could help clarify the role of hormones and stress on the behavioural differences found between the morphs.

Another possible explanation to the unexpected results could be associated with risk sensitivity and energetic shortfall. Sensitivity to risk determines the likelihood of engaging in risky situations; individuals that face an energetic shortfall will engage in risky behaviours more often in order to fulfil their energetic demands (Caraco et al., 1980). Under these circumstances dominant individuals will have easier access to food, fulfil their energetic demands faster and be less prone to engage in risky behaviours. Along the same lines, the higher dominance rank of these individuals could lead to maladjustment to stressful situations, resulting in poorer coping strategies (Benus, Koolhaas, & van Oortmerssen,

1987). In our system, during spring migration, white morphs have been found to be dominant over tan morphs (Harrington, 1973; Ficken et al., 1978; but see Hailman, 1975). Furthermore, white morphs and males are, in general, bigger and heavier than tan morphs and females (Falls & Kopachena, 2010). Thus, tan morphs and females would be more sensitive to energetic shortfalls since their lower dominance rank would restrict their access to food (Schneider, 1979), and their reduced body mass could limit their fasting capabilities. It would be expected then that high-ranking white males display poor stress coping strategies and can afford losing more weight, while low-ranking tan birds engage in risky behaviours in order to sustain their energetic demands. This could explain the neophilic response associated with the tan morph and the higher stress levels associated with captivity observed in white males.

The white morph of the white-throated sparrow has customarily been considered more aggressive during the breeding season (Kopachena & Falls, 1993a; Lowther, 1962) and in breeding-induced birds during the winter (Houtman & Falls, 1994). Under this premise, we expected to find white birds to be less affected by the presence of a novel object, although this was not the case. Nevertheless, differences in aggressive behaviour between morphs during the breeding season either have been observational, with no statistical inference, or reflect differences in song production rather than physical aggression in simulated territorial intrusions (Collins & Houtman, 1999; Horton et al., 2014; Kopachena & Falls, 1993a; Lowther, 1962). Moreover, the behavioural differences found in breeding-induced captive birds in Houtman and Falls (1994) and Tuttle (1993) could be associated with differences in stress response to captivity rather than differences in aggression levels per se. In our opinion, the claim that white morphs are more aggressive needs to be re-evaluated. We believe that there is a need to increase the scope of the behavioural experiments measuring other aggression-related traits apart from song, as well as to take into consideration stress levels when performing experiments in captivity.

The use of wild animals as models in molecular ecology and behavioural genomics is successfully expanding (Bengston et al., 2018; Kocher et al., 2018; Kukekova et al., 2018). In our opinion, the flourishing of these new research lines should be sustained, not substituted, by hypothesis-driven traditional behavioural ecology research. In our study species, having a more complete understanding of the behavioural differences between the morphs and their consistency across seasons is crucial to determine the role of the chromosomal rearrangement, and the genes it contains, in the characterization and maintenance of behavioural polymorphisms.

Conflict of Interest

None.

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Appendix

Table A1
Repeated measures ANOVA results for the logarithm of the number of hops

	SS	df	F	P
Between subjects				
Intercept	1612.002	1	451.584	<0.0001
Morph	1.170	1	0.328	0.5704
Sex	0.779	1	0.218	0.6432
Morph∗sex	0.563	1	0.158	0.6936
Error	132.078	37		
Within subjects				
Treatment	2.946	1	8.654	0.0056
Treatment∗morph	0.689	1	2.025	0.1631
Treatment∗sex	0.081	1	0.239	0.6282
Treatment∗morph∗sex	0.006	1	0.017	0.8983
Error	12.595	37		

Table A3
Generalized linear models results for the probability to approach the feeder and to catch the worm

	Parameter	SE	Wald λ	P
Feeder Y/N				
Intercept	0.4977	0.2648	3.5334	0.0601
Morph (white)	0.0267	0.2606	0.0105	0.9183
Sex (female)	-0.2385	0.2529	0.8895	0.3456
Object (present)	0.3225	0.2409	1.7923	0.1807
Worm Y/N				
Intercept	1.2182	0.3001	16.4733	<0.0001
Morph (white)	0.0291	0.2999	0.0094	0.9228
Sex (female)	-0.2191	0.2870	0.5825	0.4453
Object (present)	0.1439	0.2761	0.2717	0.6021

Table A2
Repeated measures ANOVA results for the percentage of time spent close to the feeder

	SS	df	F	P
Between subjects				
Intercept	125367.9	1	60.606	<0.0001
Morph	18.2	1	0.009	0.9237
Sex	1537.4	1	0.743	0.3770
Morph∗sex	142.1	1	0.069	0.7948
Error	76536.8	37		
Within subjects				
Treatment	1111.9	1	2.984	0.0486
Treatment∗morph	10679.2	1	28.659	<0.0001
Treatment∗sex	5384.6	1	14.450	0.0003
Treatment∗morph∗sex	3011.1	1	8.081	0.0089
Error	13787.3	37		

P values have been estimated by 10 000 Monte Carlo simulations.

Table A4
Repeated measures ANOVA results for the body mass of individuals at capture and on release days

	SS	df	F	P
Between subjects				
Intercept	851.34	1	149.6654	<0.0001
Days captive	0.00	1	0.0002	0.9877
Morph	8.41	1	1.4786	0.2329
Sex	54.93	1	9.6568	0.0039
Morph∗sex	8.89	1	1.5622	0.2204
Error	182.03	32		
Within subjects				
Treatment	22.08	1	10.7448	0.0025
Treatment∗days captive	36.73	1	17.8744	0.0002
Treatment∗morph	8.07	1	3.9277	0.0561
Treatment∗sex	10.59	1	5.1547	0.0301
Treatment∗morph∗sex	0.40	1	0.1955	0.6613
Error	65.76	32		