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Heterozygosity–fitness correlations in a migratory bird: an analysis of inbreeding and single-locus effects

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Abstract

Studies in a multitude of taxa have described a correlation between heterozygosity and fitness and usually conclude that this is evidence for inbreeding depression. Here, we have used multilocus heterozygosity (MLH) estimates from 15 microsatellite markers to show evidence of heterozygosity–fitness correlations (HFCs) in a long-distance migratory bird, the light-bellied Brent goose. We found significant, positive heterozygosity–heterozygosity correlations between random subsets of the markers we employed, and no evidence that a model containing all loci as individual predictors in a multiple regression explained significantly more variation than a model with MLH as a single predictor. Collectively, these results lend support to the hypothesis that the HFCs we have observed are a function of inbreeding depression. However, we do find that fitness correlations are only detectable in years where population-level productivity is high enough for the reproductive asymmetry between high and low heterozygosity individuals to become apparent. We suggest that lack of evidence of heterozygosity–fitness correlations in animal systems may be because heterozygosity is a poor proxy measure of inbreeding, especially when employing low numbers of markers, but alternatively because the asymmetries between individuals of different heterozygosities may only be apparent when environmental effects on fitness are less pronounced.

Keywords: *Branta bernicla hrota*, Brent geese, ecological genetics, internal relatedness, MCMCglmm

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Introduction

Inbreeding depression, where matings between close relatives result in reduced reproductive success when compared to outbred matings, is well documented in animal populations (reviewed in Crnokrak & Roff 1999 and Keller & Waller 2002) and can partially explain observed patterns of reproductive asymmetry among individuals (e.g. Keller 1998; Keller *et al.* 2002; Szulkin & Sheldon 2008). However, measuring inbreeding in wild populations is difficult, as the most accurate estimates of inbreeding are derived from pedigrees (e.g. Hansson *et al.* 2004; Szulkin & Sheldon 2008), which are impractical to obtain for some species (Pemberton 2008; Szulkin *et al.* 2010). Consequently methods have been developed to infer levels of inbreeding in the absence of a pedigree, using genetic markers to calculate the pairwise relatedness of parents (e.g. Wang 2002) or multilocus heterozygosity (MLH) (e.g. Coltman *et al.* 1999; Amos *et al.* 2001; Aparicio *et al.* 2007). A multitude of studies have reported associations between heterozygosity and a

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measurable outcome of fitness such as survival (Acevedo-Whitehouse *et al.* 2003) and reproductive success (Amos *et al.* 2001), so-called heterozygosity–fitness correlations (HFCs, Balloux *et al.* 2004). Inbreeding depression is almost universally invoked as the reason for the observed HFCs in such studies (but see Hansson *et al.* 2004), but in fact there is a great deal of uncertainty as to what HFCs are actually measuring (Hansson & Westerberg 2002; Slate *et al.* 2004; Balloux *et al.* 2004; Szulkin *et al.* 2010). Balloux *et al.* (2004) provide convincing evidence that when using the number of neutral marker loci typical of most studies (i.e. 10–20), the correlation between heterozygosity and true inbreeding should be weak (see also Taylor *et al.* 2010), making it unlikely that all studies reporting HFCs are reporting a true effect of inbreeding. Moreover, Slate *et al.* (2004) showed that the strength of the correlation between heterozygosity and inbreeding is a function of both the mean and the variance of the level of inbreeding, as well as the number of loci used to derive such estimates. In summary, such relationships should be hard to detect if there is a low prevalence of inbreeding in a population and/or a small number of markers are used to investigate inbreeding. Overall *et al.* (2005) provided empirical evidence of the former, showing a weak heterozygosity–inbreeding association as a result of a low prevalence of inbreeding in Soay sheep (*Ovis aries*). In addition, Balloux *et al.* (2004) show through simulations that as the number of markers employed increases (up to 200), the strength of correlation between heterozygosity and inbreeding increases in a commensurate fashion. In reality, very few studies employ such a large number of markers (but see Slate *et al.* 2000). Consequently, many studies reporting significant HFCs, whilst using small numbers of markers, typically report relatively weak relationships between heterozygosity and a fitness metric, with r^2 values of 2–3% (see Balloux *et al.* 2004; Chapman *et al.* 2009). Indeed, there is also the possibility that publication bias has falsely inflated the importance of HFCs in animals (Balloux *et al.* 2004; Chapman *et al.* 2009), as many studies showing a lack of correlation may not be published.

There are alternative explanations for the prevalence of HFCs in the current literature (reviewed in Hansson & Westerberg 2002). First, rather than being reflective of genome-wide heterozygosity, and thus level of inbreeding (referred to as the 'general effects' hypothesis, Hansson & Westerberg 2002), it may be the case that some of the loci employed are in physical linkage with loci that influence fitness (Slate *et al.* 2004), known as the 'local effects' hypothesis (Hansson & Westerberg 2002). The main criticism of this hypothesis is that it requires that a large proportion of the genome be in linkage disequilibrium to so frequently discover neutral markers demonstrating such 'local effects' (Hansson & Westerberg 2002; Slate *et al.* 2004). In spite of this, there is some evidence suggestive of local effects in wild populations (Hansson *et al.* 2004), but it is vital to acknowledge that absence of evidence for general effects in the presence of HFCs does not immediately support a 'local effects' explanation (Kupper *et al.* 2010). Another recently proposed explanation is the existence of cryptic population structure in the sample, which could present as a HFC if there is systematic variation in mean heterozygosity (e.g. because of genetic drift) across each deme, and demes also vary in fitness, for reasons that are completely unrelated (e.g. systematic variation in habitat/environmental quality across geographic areas), see Slate & Pemberton (2006). For this reason, prior to invoking inbreeding depression as the explanation for HFCs, it is important to rule out the possibility of population stratification either by explicitly testing for heterozygosity differences by site or by providing evidence that the population being studied is, in fact, unstructured (e.g. Harrison *et al.* 2010a).

We establish the presence and form of the relationship between heterozygosity and fitness in a long-distance migratory bird, the light-bellied Brent goose (*Branta bernicla hrota*). We characterize the nature and location of pair formation in this species, as pairing in areas of high kin structure creates a high risk of inbreeding in the absence of kin recognition/avoidance. We also investigate the potential for systematic variation in heterozygosity among geographic regions of their winter home range to investigate the possibility that cryptic population structure has resulted in HFCs being present. We discuss our results in the context of understanding the factors driving fitness asymmetries in wild animal populations.

Methods

Study species

The east Canadian High Arctic population of light-bellied Brent geese (*B. bernicla hrota*) comprises *c.* 40 000 individuals (Fig. 1), which spend the majority of the winter around the coast of Ireland (Inger *et al.* 2006). They stage on the west coast of Iceland for *c.* 1 month between April and May, before migrating into the Canadian Arctic to breed (Gudmundsson *et al.* 1995). Their diet comprises largely marine plants such as the angiosperm *Zostera* spp. as well as green algae *Ulva lactuca*. However, during late winter in Ireland, these resources are less abundant and therefore Brent geese also feed on terrestrial grasses (Inger *et al.* 2006). Brent geese stage initially at Strangford Lough in Northern Ireland (54.47 N, 5.58 W) at the beginning of

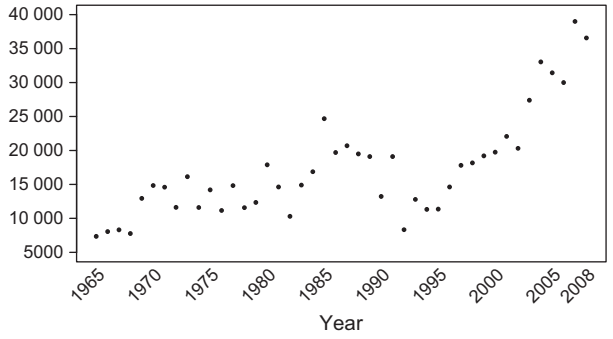

Fig. 1 Population size across years for light-bellied Brent geese. Annual counts are made in October each year and synchronized over 1–2 days in Ireland, Iceland, Britain, Jersey and northern France.

winter to exploit a high abundance of nutrient-dense marine plants (Inger *et al.* 2006), thereafter dispersing to their core wintering areas around the coast of Ireland from November to March, to which they are highly site-faithful between years (Harrison *et al.* 2010a).

Sample collection

Birds were captured at multiple sites in Ireland and Iceland using cannon nets, and in the Canadian Arctic breeding grounds by herding into v-nets (during breeding the adults are moulting and thus flightless). Birds were fitted with unique colour leg rings, aged as adult or juvenile by plumage, and sexed by cloacal examination. Blood samples were taken and stored in absolute ethanol in 1.5-mL screw-top, o-ring sealed microfuge tubes.

Pair formation

Detailed information regarding methods of field observations of birds can be found in Inger *et al.* (2010). To assign location of pair formation, we extracted all data from individuals whose breeding status was observed to change over the course of the annual cycle from singleton (unpaired) to associated (paired). To assign singletons, we used only birds that had been explicitly labelled as unassociated in the database, which can be assessed unambiguously in the field. To assess the change in status, we used only records for birds that were observed three times or more as associated after the initial change in status, to avoid errors caused by mis-assigning birds as paired when they are in fact still singletons. For each case, we recorded the location of the change in status, based on the following categories: Early Winter (EW), any pairing occurring in Strangford Lough in September–October; Core Winter (CW), pairing during the core period of winter; Winter (W), pairing that occurred at some point in winter but the individual was observed as a singleton during EW and paired in CW; Staging (S), pairing during Icelandic staging; Winter/Staging (WS), individual was observed as singleton during winter and paired during S; Staging/Winter (SW), individual was observed as a singleton during staging in year t and associated in winter in year $t + 1$; Unassigned (between Winter year t and Winter year $t + 1$). We calculated Bayesian 95% credible intervals for the proportions of pairings at each location using the methods of McCarthy (2007) to reflect the degree of precision of the estimates given the sample size of pairings occurring at each stage.

Heterozygosity

We used data from 1108 individuals genotyped using the 15 microsatellite loci detailed in Harrison *et al.* (2010b) to calculate heterozygosity estimates using the R package 'Rhh' (Alho *et al.* 2010) in R v2.12.2 (R Development Core Team 2010). We calculated both internal relatedness (IR, Amos *et al.* 2001) and homozygosity by loci (HL, Aparicio *et al.* 2007) to test the sensitivity of the models to the choice of heterozygosity measure. We also calculated a heterozygosity–heterozygosity correlation (Balloux *et al.* 2004) using the 'hh' function, which repeatedly and randomly divides the loci in half and calculates the correlation between them. If neutral markers such as microsatellites carry information about genome-wide levels of heterozygosity, then comparing two random subsets of such markers should yield a positive, significant correlation (Balloux *et al.* 2004; Alho *et al.* 2010). We ran 100 randomizations of the markers for both the IR and the heterozygosity by loci (HL) estimates of heterozygosity.

Reproductive success

We used the IBGRG database to assign number of offspring produced by the 1108 birds for which we had information on heterozygosity. For each annual cycle (October–September), we took the maximum number of juveniles recorded for each bird as their estimate of reproductive success. When assigning birds as nonbreeders (zero offspring), we considered only birds that had been explicitly recorded as being associated to avoid bias caused by including singletons that have no breeding partner. The data for number of breeders and nonbreeders for each annual cycle are summarized in Table 1. We used the package 'MCMCglmm' (Hadfield 2010) to fit a Bayesian mixed-effects model with a Poisson error structure and a log-link to look for an effect of heterozygosity on number of juveniles produced ($n = 1025$ records comprising 506 unique breeding pairs/individuals). We specified both heterozygosity

Table 1 Sample sizes by annual cycle for light-bellied Brent geese (October–September). *n* is total number of individuals. 'Breeders' and 'Non-breeders' refer to the relative frequency of birds that did and did not reproduce, respectively

Annual cycle	*n*	Breeders	Nonbreeders
2005/2006	164	26	138
2006/2007	222	15	207
2007/2008	382	145	237
2008/2009	257	124	133

measure (IR) and year as main effects as well as their interaction. IR was standardized prior to analysis to make regression coefficients for the effect of heterozygosity directly comparable across studies (Shielzeth 2010). The mean of IR for our data set was 0.027 with a standard deviation of 0.14. We report these data so that our estimates of the effect of standardized model can easily be converted back to the original scale (Shielzeth 2010).

We often had heterozygosity data from both members of a breeding pair and so we specified a random intercept term for breeding pair. Because of the low frequency of successful reproduction in the first 2 years (Table 1), we subsetted the data to the last 2 years and ran the same model ($n = 639$ records comprising 435 unique breeding pairs) on the restricted data set, on the basis that with such low variance in productivity in 2 years, asymmetries among individuals would be hard to detect. We suppressed the global intercept to prevent parameter estimates being expressed as the difference from the first year of data (2005/2006). Raw data of reproductive success by year are presented in Fig 2.

Single-locus effects

To test for the possibility that local, rather than general, effects were driving the observed HFCs, we ran a multiple regression following Szulkin *et al.* (2010) where each locus ($n = 15$) was included as an individual predictor and coded as 0 or 1 for homozygous/heterozygous, respectively. Missing genotypes were replaced with the mean heterozygosity for that locus (Szulkin *et al.* 2010; see also Chapman & Sheldon 2011). If this model explains more variation than a basic model where MLH is included as a single predictor, then this lends support to the local effects hypothesis. We used only the last 2 years of data for this mode to avoid overparameterizing the model by having to specify year and its interactions with the 15 separate locus predictors. We then compare this model to its equivalent where only MLH is fitted as a main effect. To calculate the F ratio, we followed the formula outlined in Szulkin *et al.* (2010) and used the posterior mode of the deviance calculated for each model in our calculations.

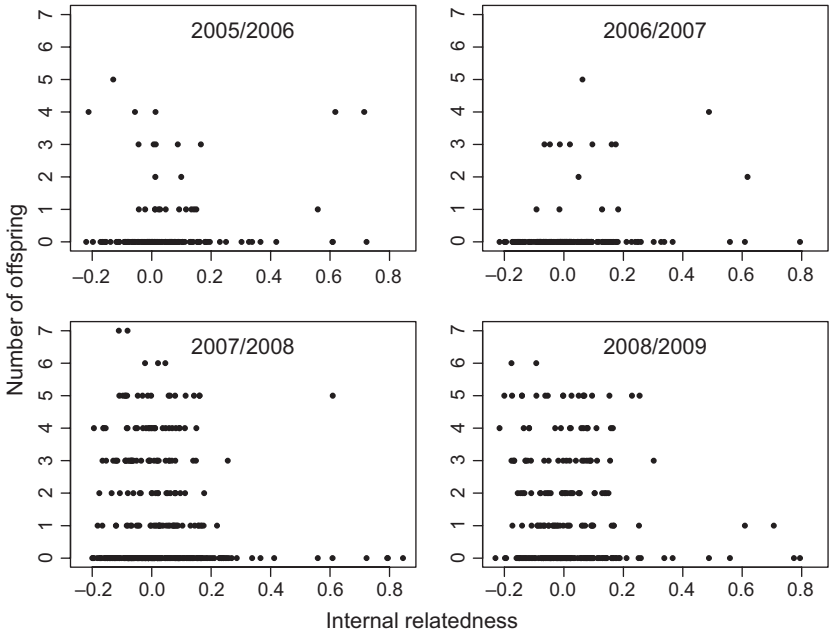

Fig. 2 Relationship between multilocus heterozygosity (measured as internal relatedness) and reproductive success for four successive years in light-bellied Brent geese. A significant heterozygosity–fitness correlation was found only for the latter 2 years of the data set (2007/2008 and 2008/2009, Table 2), when population-level variance in breeding success was high enough for the asymmetry between more and less heterozygous individuals to be detectable (Table 1).

Classifying loci as functional or neutral

To assess the potential for the loci employed in this study to be functional, we followed the guidelines of Olano-Marin *et al.* (2011) by assessing homology of the microsatellite sequences to avian expressed-sequence tags (ESTs). We performed a BLAST search of all sequences against the EST databases of both chicken (*Gallus gallus*) and zebra finch (*Taeniopygia guttata*) using the NCBI blast suite (http://blast.ncbi.nlm.nih.gov/). For each organism, we queried only the EST database for that species and optimized for both 'somewhat similar sequences (blastn)' and 'highly similar sequences (megablast)' in the programme selection. Details of genomic location of these loci in both zebra finch and chicken can be found in Harrison *et al.* (2010b), fig. 1.

Estimating the correlation between heterozygosity and fitness using a multiresponse model

We fitted reproductive success and heterozygosity as a multivariate response using MCMCglmm. We fitted separate models for the first 2 years of data (n = 386), last 2 years of data (n = 639) and finally a model using data from all years (n = 1025). This allowed us to estimate the form of the variance/covariance matrix between the two variables, and consequently the correlation between them. We extracted the posterior correlation between terms in the variance/covariance matrix (see Data S1 material for code, Supporting information).

Heterozygosity differences by site

Of the 549 individuals in our sample, we censored the data to those individuals for which we had information on consistent site use (n = 457). Birds were assigned to that site only if they had been recorded there three times within the 'Core Winter' period of November to March. Of these, we chose only those where we had at least 10 representatives from each site (n = 415 across eight sites) to ensure there were sufficient data for calculation of a reliable mean for each site. We fitted an ANOVA in MCMCglmm with heterozygosity (IR) as the response, with site as the predictor. We suppressed the intercept so that each site had its own mean calculated, rather than the parameter estimate being expressed as the difference from the first site's mean.

MCMCglmm models and diagnostics

Model code for all MCMCglmm Models and priors can be found in Data S1 (Supporting information). All MCMCglmm models were run for 200 000 iterations after a burnin of 20 000 and a thinning interval of 200 to yield a final sample of 1000 iterations per chain. Autocorrelation for the MCMC chains of both fixed effects and variance of all models was assessed using the 'autocorr' function in R. Autocorrelation between successive stored iterations for all chains was low (all values <0.1). Convergence of chains was assessed and calculated using the Gelman–Rubin diagnostic statistic (Gelman & Rubin 1992) in the coda package (Plummer *et al.* 2010) by running all models in duplicate with overdispersed starting values and comparing the mixing properties of chains between identical models. All models showed adequate convergence (multivariate potential scale reduction factors all <1.05).

Univariate priors. For all models, we specified uninformative inverse Gamma priors (Hadfield 2010) with the mean of the univariate inverse Wishart distribution set to 1 and a degree-of-belief parameter (ν) set to 0.002 for both random effects (group and unit level) variance components. MCMCglmm fits a unit-level variance component to allow the extra-Poisson variation in the data to be modelled as additive overdispersion on the link scale (Nakagawa & Shielzeth 2010). As such, an estimate of zero implies the data follow a standard Poisson distribution. To test the sensitivity of the analyses to choice of priors, we reran all models with ν set to both 2 and 0 and found that posterior estimates for all models were insensitive to choice of prior. Details of priors for this model can be found in Data S1 (Supporting information).

Multivariate priors. For the multiresponse model, we specified an uninformative inverse Wishart prior, parameterized with a 2×2 matrix and $\nu = 2$ (as there are two response variables) for both the group- and the unit-level (R and G) random effects. We also specified an empty (zero) variance/covariance matrix. Details of priors for this model can be found in Data S1 (Supporting information).

Results

Heterozygosity–fitness correlations

MultiLocus heterozygosity. We found that increased heterozygosity had a significant positive effect on number of juveniles produced in 2 of 4 years of our data set (IR*Year interactions for 2007/2008 and 2008/2009, Table 2). Heterozygosity was also significant as a main effect when running a separate model using only the latter 2 years of the data set (standardized IR, mean = −0.2, 95% CI = −0.36 to −0.04 N.B. The parame-

Table 2 Parameter estimates from a Bayesian mixed-effects model fitted using MCMCglmm investigating the interaction between heterozygosity (IR) and year. Estimates in bold are significant (95% credible intervals do not cross zero). All estimates are on the log scale. The predictor IR was standardized prior to analysis to make coefficient estimates comparable across studies (Shielzeth 2010). 'Pair' variance is the variance estimate of the group-level (categorical) random effect

Variable	Mean	95% CI
IR	0.244	−0.056 to 0.56
2005/2006	−2.447	−2.93 to −1.9
2006/2007	−3.271	−3.86 to −2.7
2007/2008	−0.918	−1.18 to −0.67
2008/2009	−0.638	−0.9 to −0.38
IR*2006/2007	0.261	−0.22 to 0.74
IR*2007/2008	**−0.459**	**−0.82 to −0.05**
IR*2008/2009	**−0.453**	**−0.83 to −0.056**
Pair variance	1.09	0.53 to 1.63
Additive over dispersion	1.28	0.8 to 1.82

IR, internal relatedness.

ter estimate for IR is negative because less heterozygous individuals have a higher IR score). There was no evidence for heterozygosity by year interaction (mean = −0.02, 95% CI = −0.29 to 0.39) or year effect (mean = 0.22, 95% CI = −0.03 to 0.49), suggesting the effect of heterozygosity was similar across the 2 years.

Correlation between heterozygosity and fitness from the multiresponse model. Using the latter 2 years of the data set (n = 639), the correlation between heterozygosity and fitness was estimated to be −0.27 with a 95% credible interval of −0.1 to −0.45. (2 d.p.; a negative sign because a higher IR score means individuals are less heterozygous). This estimate is consistent with the mixed-effects regression, which predicts a negative effect of decreased heterozygosity on reproductive success (Table 2). Estimates of the posterior mode of the variance/covariance matrix can be found in Table S1 (Supporting information). Using all 4 years of data (n = 1025), the correlation was estimated as −0.16 (95% CI −0.34 to 0.05), and finally using only the first 2 years of data (n = 386), the correlation was estimated as 0.15 (95% CI −0.03 to 0.3). The fact that both sets of credible intervals cross zero is again consistent with the mixed-effects regression, showing that significant effects are not present when pooling all data across years.

Single-locus effects. Following the guidelines of Szulkin *et al.* (2010), when comparing a multiple regression model containing all individual loci as predictors vs. a model containing only MLH as the sole predictor, model comparison revealed no significant difference between the two models ($F_{1,14}$ = 0.82, P = 0.38, n = 639). Therefore, we find no support for the hypothesis that the single-locus multiple regression explains significantly more variation than the MLH regression.

Table 3 Heterozygosity–heterozygosity correlations (Balloux *et al.* 2004) for 1108 individuals using 15 microsatellite loci. Both IR and HL measures show positive, significant correlations after 100 randomizations (95% quantiles do not cross zero)

Heterozygosity estimate	Mean r	95% Quantile
IR	0.3	0.26–0.34
HL	0.29	0.23–0.33

IR, internal relatedness; HL, heterozygosity by loci.

Functional vs. neutral loci. When the BLAST search was optimized for 'highly similar sequences', we found no homologies to ESTs for either chicken or zebra finch. Only one locus (Bbh043) showed limited homology to an EST sequence when the BLAST search was optimized for 'somewhat similar sequences', the chicken ChEST362b10 cDNA clone (Accession Number BU359727.1) located on the W chromosome. However, our previous work has shown no pattern of sex linkage of this marker that would be consistent with presence on the W chromosome (Harrison *et al.* 2010b), and query coverage was only 46%, suggesting this homology is spurious. Therefore, with obvious caveats regarding the power of this approach, we suggest that none of the other loci employed in this study showed homology to ESTs in either the zebra finch or the chicken genomes, which following the example of Olano-Marin *et al.* (2011) suggests that all of the loci we employ here can be considered 'neutral'.

Heterozygosity–heterozygosity correlations

We found a positive and significant correlation between randomly assigned subsets of loci following the method of Balloux *et al.* (2004) (Table 3). The correlations were robust to the choice of heterozygosity measure used. Mean correlation coefficient for the IR estimate of heterozygosity after 100 random draws was 0.3, leading to an r^2 of 9%. These estimates are consistent with the expected r^2 for HHC given the modest number of markers employed (15) (Balloux *et al.* 2004).

Heterozygosity differences by site

We found no significant differences in mean heterozygosity among sites (all pairs of 95% Credible Intervals overlap, Table S2, Supporting information). Three sites

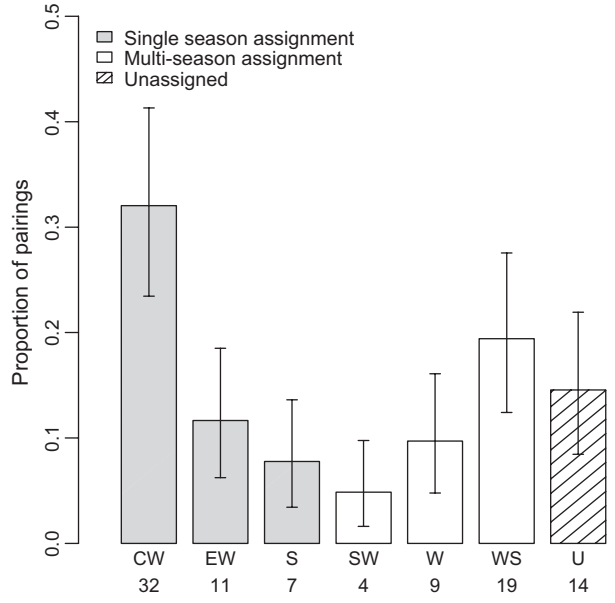

Fig. 3 Histogram of location of pair formation in 96 breeding pairs of Light-bellied Brent geese (*Branta bernicla hrota*). All assignments, expressed as proportions of the total (96), including multiseason assignments where we could not be certain of location. Location codes: EW, Early Winter; S, Staging; W, During Early or Core Winter; CW, Core Winter; WS, Between Winter and Staging; SW, Between Staging year t and Winter year $t + 1$ (possible breeding ground pairing); U, Unassigned (between Winter year t and Winter year $t + 1$. Bars are 95% credible intervals, calculated following the methods of McCarthy (2007). Raw counts of number of pairings are presented below location codes.

(Dundrum, Dungarvan and Tralee) were estimated to have nonzero means (credible intervals do not cross zero) whilst the remaining sites (Carlingford, Strangford, Dublin, Sligo and Wexford) had means that could not be distinguished from zero.

Pair formation

Of the 96 records of pair formation, 33% were found to occur during the 'Core Winter' period in Ireland (typically November–March), whilst 11% occurred during the 'Early Winter' period, where the majority of the population mix in Strangford Lough, Northern Ireland, at the beginning of the season (Inger 2006). A further 7% were identified on the Icelandic staging grounds (Fig. 3). As birds were observed as singletons in one season and associated in the next, 33% could not be assigned a definitive single season location. Of these, four were observed to have occurred between Staging in May and the following winter and thus could potentially represent pairing on the breeding ground. Approximately 14% were 'Unassigned' as the pairing could have occurred in any of the three major seasons (Winter, Staging or Breeding). All proportions are shown with 95% credible intervals following the methods of McCarthy (2007).

Discussion

The HFCs that we observed were only detectable in years with sufficient variation in productivity to result in an asymmetry among individuals of different heterozygosity. This has important implications for the investigation of HFCs, as studies that thus far have failed to detect their presence may simply have chosen periods in which variance in reproductive success among individuals was insufficient to produce a signal. The amount of variation explained by HFCs is often low (Balloux *et al.*2004), and therefore if population-level productivity is influenced by substantial temporal variation in other parameters (such as environmental variation e.g. Dickey *et al.* 2008), detectable variation in such productivity may only occur at times where such conditions are conducive to breeding. However, we caution that the interpretation of such a pattern depends entirely on the fitness trait being measured, as Keller 1998 found that inbred individuals suffered lower survival during severe weather events. Such interactions between environment and inbreeding can thus take several forms (Armbruster & Reed 2005), but detection of the relationship between inbreeding and fitness (survival or productivity) may rely on either favourable (this study) or unfavourable (Keller 1998) environmental conditions, which will not be present at all sampling points.

We found no evidence that a model containing all loci as individual predictors performed better than the simple model containing only a MLH measure. Moreover, we observed a positive, significant heterozygosity–heterozygosity correlation between random sets of our markers ($r = 0.3$, $r^2 = 9\%$), which is consistent with the idea that neutral marker heterozygosity is representative of genome-wide heterozygosity, and therefore inbreeding (Balloux *et al.* 2004; Slate *et al.* 2004) in light-bellied Brent geese. Moreover, the degree of correlation we observed was consistent with the expected value derived from the simulations of Balloux *et al.* (2004) using a similar number of markers. Following Slate *et al.* (2004), these data suggest that the mean and variance of inbreeding in light-bellied Brent geese must be relatively high, allowing us to detect such a signal with a modest number of markers. Our previous work has shown that adult Brent geese show a high degree of site fidelity and that this fidelity is culturally inherited from parents during the first year of life (Harrison *et al.* 2010a). Because family lineages will consistently show fidelity to the same sites, there is likely signifi-

cant kin structure within discrete areas on both the wintering and the staging grounds (Harrison *et al.* 2010a). As such, our data on pair formation provide complementary support to the general effects hypothesis, as we have shown that a large proportion of pairings occur during 'Core Winter' where this kin structure operates, and could thus give rise to consanguineous matings.

Whilst it has been suggested that cryptic population stratification could drive HFCs even in the absence of both local and general effects (Slate & Pemberton 2006), we have previously shown that there is no genetic structure in the light-bellied Brent goose (Harrison *et al.* 2010a). Moreover, we found no evidence of any differences in mean heterozygosity on a site-by-site basis (see Table S2, Supporting information) as would be predicted by this hypothesis. We therefore discount the population structure hypothesis as being highly unlikely to be the driver of the HFCs observed in this study. Finally, using a multivariate response model, we estimated the correlation between IR and reproductive success to be −0.27 (95% CI −0.1 to −0.45) using the latter 2 years of the data set only. Consistent with the mixed-effects regression, repeating this analysis with the entire 4 year data set (mean 0.16; 95% CI −0.34 to 0.05), or using only the first 2 years (mean 0.15; 95% CI −0.04 to 0.3), showed no significant correlation (credible intervals cross zero), suggesting that the effect of heterozygosity is not consistent across years. We suggest estimates of this nature should be calculated routinely for studies involving HFCs, as they are of high utility in showing the magnitude of effect of differences in heterozygosity among individuals. Perhaps more importantly, correlation coefficients derived this way are directly comparable across studies (Shielzeth 2010), which will prove invaluable should one wish to compare the strength of evidence for HFCs across multiple studies, species and taxa.

Conclusion

We have demonstrated a relationship between heterozygosity and fitness in a highly vagile wild vertebrate species, most likely driven by the fact that a large proportion of pairings occur where there is a high risk of inbreeding. Estimates of correlations among random subsets of loci (Balloux *et al.* 2004; Alho *et al.* 2010) and knowledge of the likelihood of the occurrence of inbreeding make such conclusions about HFCs far more robust than evidence of HFCs being presented in isolation. We suggest that even in the presence of such evidence, it is important to explore alternative hypotheses regarding what could be driving observed patterns (e.g. Slate & Pemberton 2006). Moreover, studies investigating HFCs should ideally seek to do so across multiple generations, as we have shown that such effects can be undetectable when population-level variance in reproductive success is low.

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Data accessibility

DNA sequences: GenBank Accession Numbers FN691780–FN691904 and FN812687–FN812698) (see Harrison *et al.* 2010b). Microsatellite data deposited at Dryad: doi:10.5061/dryad.52dk8.

Supporting information

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

Table S1 Variance/covariance matrix from a multi-response model fitted in order to estimate the correlation between heterozygosity and number of juveniles.

Table S2 Mean heterozygosity by site for 8 major sites on the Irish wintering grounds.

Data S1 Model code for all models run using MCMCglmm.

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