# Evidence of opposing fitness effects of parental heterozygosity and relatedness in a critically endangered marine turtle?

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## Abstract

How individual genetic variability relates to fitness is important in understanding evolution and the processes affecting populations of conservation concern. Heterozygosity–fitness correlations (HFCs) have been widely used to study this link in wild populations, where key parameters that affect both variability and fitness, such as inbreeding, can be difficult to measure. We used estimates of parental heterozygosity and genetic similarity ('relatedness') derived from 32 microsatellite markers to explore the relationship between genetic variability and fitness in a population of the critically endangered hawksbill turtle, ***Eretmochelys imbricata***. We found no effect of maternal MLH (multilocus heterozygosity) on clutch size or egg success rate, and no single-locus effects. However, we found effects of paternal MLH and parental relatedness on egg success rate that interacted in a way that may result in both positive and negative effects of genetic variability. Multicollinearity in these tests was within safe limits, and null simulations suggested that the effect was not an artefact of using paternal genotypes reconstructed from large samples of offspring. Our results could imply a tension between inbreeding and outbreeding depression in this system, which is biologically feasible in turtles: female-biased natal philopatry may elevate inbreeding risk and local adaptation, and both processes may be disrupted by male-biased dispersal. Although this conclusion should be treated with caution due to a lack of significant identity disequilibrium, our study shows the importance of considering both positive and negative effects when assessing how variation in genetic variability affects fitness in wild systems.

## Introduction

How genetic variability relates to individual fitness is a fundamental question in evolutionary biology (Charlesworth & Charlesworth, 1999), with potential implications for a wide range of life history parameters that affect survival and reproductive success (reviewed in Chapman ***et al.***, 2009). It is also an important concept in conservation management, with practical implications for populations of conservation concern that may be facing challenges arising from depleted genetic variation (Crnokrak & Roff, 1999; Keller & Waller, 2002; Gooley ***et al.***, 2017). A common method for studying the relationship between individual genetic variability and fitness has been to test for correlations between individual heterozygosity and fitness parameters, so-called heterozygosity–fitness correlations or HFCs (Hansson & Westerberg, 2002; Chapman

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*et al.*, 2009; Miller & Coltman, 2014). HFCs have been reported in a variety of taxa (Chapman *et al.*, 2009), but the relative roles of the hypothesized explanatory mechanisms behind the correlations remain a topic of debate (e.g. Hansson & Westerberg, 2008; Szulkin *et al.*, 2010). With the widespread use of microsatellite markers for HFC studies, this discussion has focussed on the relative roles of 'general' and 'local' effects (e.g. Hansson & Westerberg, 2002; Rodriguez-Quilon *et al.*, 2015), and the associated question of the link between marker-based heterozygosity and inbreeding coefficients (e.g. Balloux *et al.*, 2004; Berenos *et al.*, 2016; Nietlisbach *et al.*, 2017), although these models are not mutually exclusive. A third mechanism, 'direct effects', was applicable to older study techniques such as allozymes (e.g. David, 1998), but is not usually considered for microsatellites, which are typically treated as neutral and not directly subject to selection (e.g. Li *et al.*, 2002, 2004). Under the 'general effects' model, average heterozygosity across a number of independent loci is used as an estimate of genome-wide heterozygosity, which itself is treated as a proxy for an individual's level of inbreeding. Individuals that are more inbred are predicted to have lower genome-wide heterozygosity and, consequently, lower fitness due to increased expression of deleterious recessive alleles and loss of heterosis ('inbreeding depression'; Charlesworth & Charlesworth, 1987, 1999). However, this interpretation is controversial, as even when calculated from large panels of markers, multilocus heterozygosity (MLH) may be a poor correlate of pedigree inbreeding coefficients (e.g. Balloux *et al.*, 2004). Under the 'local effects' model, a given marker demonstrates an HFC because it is in linkage disequilibrium with a functional locus (Hansson & Westerberg, 2002; Szulkin *et al.*, 2010). This model is less dependent on individual inbreeding levels than general effects, but there is widespread theoretical concern about how to interpret such effects, compounded by statistical challenges in demonstrating them robustly (Szulkin *et al.*, 2010).

Many HFC studies have reported positive linear effects (Chapman *et al.*, 2009; Kéfupper *et al.*, 2010). Such positive effects may have been overrepresented due to publication bias (Coltman & Slate, 2003; bias may be diminishing – see Chapman *et al.*, 2009), biases arising from the properties of the genetic mark- ers used (Kéfupper *et al.*, 2010), and because of a ten- dency for HFC studies to be conducted on small populations that have high inbreeding variance (see Coltman & Slate, 2003; Chapman *et al.*, 2009; Kéfupper *et al.*, 2010). The other end of the heterozygosity spec- trum – negative HFCs – has received less attention (but see Szulkin & David, 2011). Negative multilocus HFCs may represent outbreeding depression, where population admixture breaks up coadapted gene com- plexes or disrupts local adaptation (Templeton *et al.*, 1986; Waser, 1993). This phenomenon has been observed in captive populations (Lacy *et al.*, 1993), but its importance among wild populations is less clear (Marshall & Spalton, 2000; Szulkin & David, 2011). Somewhere between deleterious inbreeding and deleterious outbreeding, there should be an optimal level of outcrossing that maximizes fitness. This has been well demonstrated in plants (Waser & Price, 1989, 1994; Willi & van Buskirk, 2005). Studies that have demonstrated both inbreeding and outbreeding depression on animals are few (e.g. Marshall & Spalton, 2000; Neff, 2004; Escobar *et al.*, 2008; see also Edmands, 2007), but have highlighted important processes. For example, inbreeding and outbreeding may act on the same trait, or simultaneously on different traits, and directions of HFC may differ between age classes and sexes (e.g. Marshall & Spalton, 2000; Esco- bar *et al.*, 2008; Olano-Marin *et al.*, 2011b).

An individual's genetic variability may affect the fitness of its offspring, for example, through differential fertilization success (e.g. Bretman *et al.*, 2009; Fitzpatrick & Evans, 2009), egg hatchability (e.g. Keller, 1998; Cordero *et al.*, 2004) or because variability correlates with the quality of parental care (e.g. Richardson *et al.*, 2004; Brouwer *et al.*, 2007). The genetic similarity/relatedness (henceforth 'relatedness') of the parents – which is directly related to offspring genetic variability – may also be important (e.g. Bensch *et al.*, 1994; Van de Casteele *et al.*, 2003). Thus, the success of a given breeding event could be determined by the heterozygosity of each parent, but also by the average heterozygosity of the offspring that they produce as a result of their relatedness (Cordero *et al.*, 2004).

HFC studies are biased towards species that mature quickly and have short lifespans, towards mammals and birds, and against species of high fecundity (Chapman *et al.*, 2009). However, it is important for the development of any theory in evolutionary biology that it be tested against a range of life-history backgrounds. Here, we test for correlations between individual genetic variability and fitness in a population of the critically endangered hawksbill turtle (*Eretmochelys imbricata*; family Cheloniidae), a long-lived, slow-to- mature, migratory reptile. We focus on two parameters that relate directly to marine turtle fitness: the number of eggs in a clutch and the proportional success of those eggs. For clutch size, we test for correlations with maternal heterozygosity, whereas for egg success we also test for correlations with paternal heterozygosity and parental relatedness. We also compare multilocus (general effect) and single-locus (local effect) models. Our study population in the Republic of Seychelles is one of the world's most important populations of hawksbill turtles, but has declined substantially as the islands were colonized by humans 200+ years ago (Mortimer, 1984, 2004). Assessing the link between genetic variability and fitness in this population may help conservation managers better understand the

processes driving variation in reproductive output in this species.

## Materials and methods

### Field methods

Sampling of adult female and hatchling turtles was conducted on Cousine Island, Republic of Seychelles (04°21′S, 55°38′E), over two nesting seasons (Sep-Apr) spanning 2007-2009. Laying females and nest locations were identified by patrolling Cousine's 1 km beach hourly between 6 am and 6 pm (hawksbills in Seychelles nest almost exclusively by day; Diamond, 1976). Observed females were measured (curved carapace length), tagged (unique titanium tags issued by Seychelles Island Foundation), and sampled (6-mm sterile biopsy from trailing edge of foreflipper). Cousine's conservation managers relocate the majority (> 95%) of clutches to 'erosion-safe' beach zones and line nest chambers with nets to limit predation from *Oocypode* crabs (Hitchins *et al.*, 2004). Eggs were counted during this process, which was undertaken within 12 h of laying (later disturbance can increase egg mortality; Parmenter, 1980). Because protective nets prevent hatchlings escaping, nests were checked daily for signs of activity from 10 days prior to the projected hatching date. Upon release, live hatchlings were counted, and tissue samples (2-mm biopsy) were taken from the marginal scute of 20 randomly chosen hatchlings per clutch (all hatchlings if fewer than 20 emerged). The number of unhatched eggs and dead hatchlings remaining in the nest was counted, with unhatched eggs assigned as either 'developed' (embryo in evidence) or 'undeveloped' (no evident embryo).

### Molecular techniques

Samples were genotyped at 32 microsatellite loci (details in Phillips *et al.*, 2013). Female genotypes were only used in downstream analyses if ≥ 29 of 32 loci amplified. For each hatchling, its genotype for a given multiplex was not used downstream if more than four loci from a multiplex (10–11 loci) failed to amplify, and its whole genotype was removed if two multiplexes were discounted or if more than ten loci failed in total.

### Parentage analysis and reconstruction of paternal genotypes

We used COLONY 2.0.4 (Wang & Santure, 2009; Jones & Wang, 2010) to identify clusters of offspring that shared a father, and to reconstruct the genotypes of these males. COLONY parameters were as in Phillips *et al.* (2013). We henceforth use 'family' to refer to a clutch or group of clutches produced by a single female in a given year. COLONY reconstructs genotypes on a locus-by-locus basis and provides a confidence value for each reconstruction. As in Phillips *et al.* (2013), when assembling paternal multilocus genotypes, we only incorporated single-locus genotypes with confidence ≥ 0.90, and only used multilocus genotypes in downstream analyses if they contained ≥ 29 of 32 loci and were reconstructed from ≥ 10 offspring. All three of these thresholds bear on the fact that paternal genotypes are easier to reconstruct if the male is heterozygous or is dissimilar from the female. Alternative thresholds might have been chosen: increasing the required minimum numbers of offspring or reconstructed loci reduces reconstruction bias, and increasing per-locus confidence *increases* bias (because heterozygous/dissimilar genotypes hit the threshold more easily; Appendix S1). However, we opted for consistency with the previous work, given that simulations undertaken for the present study showed that the chosen thresh- olds did not problematically elevate type I error risk for the analyses we conducted (see below). Note that any conclusions arising from paternal genotype data are only applicable to families that pass the minimum offspring criterion.

All loci satisfied assumptions of Hardy–Weinberg and linkage equilibria (GENEPOP v4.1; Raymond & Rousset, 1995) and had null allele frequencies < 0.1 (CERVUS v3.0.3; Marshall *et al.*, 1998). We calculated the $g_2$ measure of identity disequilibrium in the program RMES (David *et al.*, 2007; Oct 2009 version), as recommended by Szulkin *et al.* (2010). Significant identity disequilibrium means that locus states (heterozygous or homozygous) correlate within individuals, which suggests that heterozygosity across the marker panel correlates with individual inbreeding (Szulkin *et al.*, 2010).

### Genetic predictors

For each genotyped adult, we calculated multilocus heterozygosity (MLH) as standardized heterozygosity (SH), which gives all loci equal weighting but corrects for missing genotypes (Coltman *et al.*, 1999). To aid future reviews and meta-analyses, we also performed all multilocus analyses with two alternative metrics: 'internal relatedness' (Amos *et al.*, 2001) and 'homozygosity by loci' (Aparicio *et al.*, 2006). The results did not differ substantively from those using SH (Appendix S2). Data on single-locus heterozygosity (SLH) for each individual were coded as a series of 0′s (homozygote loci) and 1′s (heterozygote loci) and were then standardized by marker variability to reduce bias when estimating single-locus partial regression slopes (see Szulkin *et al.*, 2010). Missing single-locus genotypes were replaced with the population-level expected heterozygosity for the respective locus (Szulkin *et al.*, 2010).

Genetic similarity between all observed pairings for which we were able to reconstruct paternal genotypes (henceforth 'multilocus parental relatedness') was

quantified using the relatedness metric of Queller & Goodnight (1989) in COANCESTRY v1.0.1 (Wang, 2011), based on allele frequencies taken from the COLONY output. As a measure of single-locus parental similarity/relatedness, we calculated the proportion of a pair's offspring expected to be homozygous at a given locus. Missing values were replaced with the population expected homozygosity for the respective locus.

### Fitness response variables

All HFC analyses were conducted in R (v3.0 or later; R Development Core Team, 2008), using linear mixed models in the package 'lme4' (Bates *et al.*, 2014) and tests of regression slope significance in the package 'lmerTest' (Kuznetsova *et al.*, 2013). We tested whether maternal heterozygosity predicted clutch size, with female identity as a random effect (errors were near-enough normally distributed for us to favour Gaussian over Poisson for a more intuitive interpretation). We included every nest for which we knew the female's genotype and controlled for a relationship between maternal body size and clutch size (Appendix S3). There was no significant correlation between maternal heterozygosity and body size ($N$ = 70, $P$ = 0.86; note that testudine growth is indeterminate).

We tested whether maternal heterozygosity, paternal heterozygosity and parental relatedness predicted the proportion of eggs in a clutch from which surviving hatchlings emerged ('emergence success', logit-transformed), with lay date and incubation duration included as control variables and pair identity as a random effect. Use of alternative egg success metrics (fertilization success and hatching success) produced near-identical interpretations (Appendix S4). We excluded clutches where no egg produced a visible embryo, as either A) these lacked paternal genotype data, or B) an unexplained total clutch failure among a female's otherwise 'normal' clutches implies an exter- nal factor overwhelming other processes. We also excluded multiple-paternity families, as we cannot know the contributions of each male to the proportion of eggs that fail. However, we re-ran the maternal HFC analysis of emergence success with all nests included. Multiple and single paternity families did not have sig- nificantly different emergence success (linear mixed model: difference = 0.35 0.24 (SE); likelihood ratio test: d.f. = 6,7, $P$ = 0.14).

We used a corrected Akaike Information Criterion (AICc; Akaike, 1974; Hurvich & Tsai, 1989) model ranking approach, implemented in the R package 'MuMIn' (Bartón, 2013), to compare 13 multilocus models of interest for egg success. These models included main linear effects, quadratic effects and pair- wise linear interactions for the three multilocus predic- tors, with up to three predictors allowed into a model (see Table 2). A model was considered the nominal 'best' if it was ≥ 2.00 AICc units clear of the next best model. Otherwise, we considered the models comprising the top two units of AICc collectively. For each model, we calculated marginal $R^2$ (Nakagawa & Schielzeth, 2013) to indicate the amount of variance explained by the model's fixed effects, and the Akaike weight, a measure of the model's explanatory power relative to other models. Although paternal MLH and parental relatedness were correlated in our data set (Pearson's $r$ = 0.297), multicollinearity was well within safe limits after all continuous predictors were zero-centred (max. variance inflation factor = 1.17; max. kappa = 2.51; concern would begin at values of 2.5 and 10.0, respectively). However, as a safeguard, any significant effects involving paternal MLH or multilocus parental relatedness were re-tested using A) bootstrapping that held each of the two parameters constant while resampling the other, and B) delete-one jackknifing for each family (Appendix S5).

We initially included an interaction term between genetic variability and study season when testing multilocus predictors, but found no support either for this or for any main effect of season (data not shown).

### Single-locus effects

To test for effects of heterozygosity associated with specific loci, we used likelihood ratio tests to compare models with a multilocus metric fitted as a linear expression against respective models with all 32 single loci fitted simultaneously as covariates (Kopper *et al.*, 2010; Szulkin *et al.*, 2010). Only if this test is significant should a single-locus model be examined for loci with partial regression slopes significantly different from zero (Szulkin *et al.*, 2010). AICc is unsuitable for this test because the majority of loci are expected to be of low explanatory power and to swamp the metric. We performed these analyses for both egg number and egg success, with separate analyses for maternal heterozygosity, paternal heterozygosity and parental relatedness on the latter. Because our sample size relative to the number of loci placed the maximum single-locus models in danger of overfitting, we interpreted any significant likelihood ratio $P$-values relative to those derived from simulated null data sets (see below).

### Simulations

To test whether inferences based on paternal genotypes were biased by the reconstruction process, we used the observed allele frequencies to generate 1000 null data sets with family sizes and locations of genotyping failures exactly matching the observed data, and with random genotyping errors (per-locus rates as estimated above) applied to females and hatchlings (details in Appendix S6). We ran each null data set through our analysis pipeline (parentage analysis, genotype

reconstruction and statistical analyses), and compared the observed values of key summary metrics (e.g. $\Delta AIC_c$, $R^2$ and coefficient slopes) to their simulated distributions.

## Results

We genotyped 95 adult females and 2455 hatchlings. After excluding 15 clutches attributed to 10 unsampled females, this was reduced to a sample of 142 clutches produced by 70 genotyped females (mean clutches per female = 2.0±1.2 (SD); max. = 5). All runs of COLONY converged on the same parentage assignments. Of the 84 males inferred as contributing paternity, we were able to reconstruct 64 genotypes that met our confidence criteria. Eight of our genotyped families showed multiple paternity. Consistent with Phillips *et al.* (2013), no cases were observed where the paternity changed between a given female's clutches within a season. One male sired offspring in both years of the study, but no males fertilized more than one female per year. Our final sample sizes were 140 clutches in 69 families for the clutch size HFC test, and 111 clutches in 56 families for egg success HFCs. Of the 55 male genotypes used in the egg success analyses, 49 were complete (i.e. 32/32 loci) and only one was missing > 1 locus (29/32). After excluding the missing loci, all males had a harmonic mean per-locus reconstruction confidence ≥ 0.995, 32 of 55 males had confidence ≥ 0.99 for all accepted loci, only one male had > 3 loci with confidence < 0.99 (min = 0.98, no missing loci), and only 5 of 1 752 accepted loci had 0.90 ≤ confidence < 0.95.

### Identity disequilibrium

We found no evidence for overall identity disequilibrium among our loci (RMES, 10 000 iterations: $g_2$= 0.000, SD = 0.002, $P$ = 0.366), suggesting that our markers may better reflect their local genomic environment than individual inbreeding values.

### Clutch size HFC

No maternal heterozygosity term (linear MLH, quadratic MLH or SLH) was a significant predictor of clutch size (Table 1).

### Egg success HFCs

For egg success (emergence), the 'best' model was the interaction between paternal MLH and parental relatedness (Tables 2 and 3). Relative to the null model, this interaction resulted in an $AIC_c$ improvement of 7.67, and relative to the second-best model of 4.19. The second-best model had the same two main predictors but without the interaction (Tables 2 and 3), and this was the only other model to improve significantly upon the null ($\Delta AIC_c$ = 3.48). In terms of marginal $R^2$, the main effects-only model improved upon the null by 7.9%, with the interaction bringing an additional 4.8% (Table 2).

The interaction between paternal MLH and parental relatedness means that the effective slope of paternal MLH against fitness is negative at low values of relatedness, and becomes positive above a critical relatedness value approximately 0.75 SDs above mean relatedness

**Table 1** Model fit statistics for maternal heterozygosity as a predictor of clutch size.

| Focal model | Tested against | *P* | d.f. |
|---|---|---|---|
| Body size | Null model | <0.001 | 3,4 |
| Body size + MLH | Body size | 0.833 | 4,5 |
| Body size + MLH$^2$ | Body size + MLH | 0.296 | 5,6 |
| Body size + SLH | Body size + MLH | 0.081* | 5,36 |

MLH, multilocus heterozygosity; SLH, single-locus heterozygosity; body size, curved carapace length.
All models are linear mixed models fitted by maximum likelihood, with female identity as a random effect, and are compared using likelihood ratio tests.
All analyses were conducted on 140 clutches laid by 69 females.
*Not significant, and thus not corrected for overfitting.

**Table 2** Multilocus heterozygosity (MLH) and parental relatedness models of proportional egg success (emergence), assessed by corrected Akaike Information Criterion ($AIC_c$).

| Model | $AIC_c$ | Akaike weight | Marginal $R^2$ |
|---|---|---|---|
| pat.MLH 9 par.rel | **—7.67** | 75.2 | +12.7 |
| pat.MLH + par.rel | **—3.48** | 9.3 | +7.9 |
| par.rel | —1.35 | 3.2 | +3.9 |
| par.rel$^2$ | —1.23 | 3.0 | +5.7 |
| pat.MLH + mat.MLH + par.rel | —1.12 | 2.9 | +7.8 |
| *Null* | 223.49 | 1.6 | 20.4 |
| pat.MLH | +0.37 | 1.4 | +1.8 |
| mat.MLH + par.rel | +0.94 | 1.0 | +3.9 |
| pat.MLH$^2$ | +1.72 | 0.7 | +2.9 |
| mat.MLH | +2.28 | 0.5 | +0.0 |
| pat.MLH + mat.MLH | +2.69 | 0.4 | +1.8 |
| mat.MLH 9 par.rel | +3.30 | 0.3 | +3.9 |
| mat.MLH$^2$ | +3.42 | 0.3 | +0.7 |
| pat.MLH 9 mat.MLH | +4.29 | 0.2 | +2.6 |

pat.MLH, paternal MLH; mat.MLH, maternal MLH; par.rel, multilocus parental relatedness.
We assess 13 models relative to a 'null' model that controls for clutch lay date and clutch incubation duration.
All models are linear mixed models with parent pair identity as a random effect.
$AIC_c$ values in boldface improve upon the null model by ≥two units; values in underlined comprise the top two units of the $AIC_c$ ranking.
'+' indicates multipredictor models with main effects only.
'9' indicates two-predictor models with main effects and interaction term.

Table 3 Regression slopes for multilocus predictor models of emergence success that improved significantly upon the null model (Table 2).

| | pat.MLH 9 par.rel | | | pat.MLH + par.rel | | |
|---|---|---|---|---|---|---|
| Model term | Slope | SE | *t* | Slope | SE | *t* |
| Intercept | 0.54 | 0.07 | 7.24*** | 0.51 | 0.08 | 6.60*** |
| Lay date | —5.95 9 $10^{-03}$ | 1.76 9 $10^{-03}$ | 3.38** | —5.98 9 $10^{-03}$ | 1.85 9 $10^{-03}$ | 3.23** |
| Lay date$^2$ | —1.21 9 $10^{-04}$ | 4.29 9 $10^{-05}$ | 2.81** | —1.31 9 $10^{-04}$ | 4.42 9 $10^{-05}$ | 2.96** |
| Incubation duration | —5.88 9 $10^{-02}$ | 1.54 9 $10^{-02}$ | 3.83*** | —5.93 9 $10^{-02}$ | 1.59 9 $10^{-02}$ | 3.73*** |
| Paternal MLH | —1.29 | 0.58 | 2.21* | —1.37 | 0.63 | 2.19* |
| Parental relatedness | —1.45 | 0.60 | 2.41* | —1.83 | 0.63 | 2.90** |
| pat.MLH 9 par.rel | 17.11 | 6.49 | 2.64** | – | – | – |
| Between-subjects SD | < 0.01 | | | 0.16 | | |
| Within-subjects SD | 0.59 | | | 0.59 | | |

Tests of regression slope significance were performed in the 'lmerTest' R package.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

(SD = 0.10; Fig. 1). Alternatively, the interaction can be read as parental relatedness having a negative slope when paternal MLH is low and a positive slope when paternal MLH is high (Fig. 2) (see Appendix S7 for quality control plots of model residuals). This model remained the significant best when bootstrapping for paternal MLH (DAICc $P$ = 0.003; interaction coefficient slope $P$ = 0.016; Appendix S5) or parental relatedness (DAICc $P$ = 0.006; interaction coefficient slope $P$ = 0.027; Appendix S5), and was robust to delete-one jack-knifing of families (DAICc improvement: mean = 7.52, SD = 1.12, range = 4.45 – 11.07). The
model was also significant when compared to our null simulations, with the observed DAICc, marginal $R^2$ and coefficient slope having $P$-values of 0.018, 0.018 and 0.012, respectively (Appendix S6). These $P$-values were within 0.001 of those obtained if the null data sets were analysed with the true, complete parental genotypes, indicating a negligible change in type I error risk from the small, nonsignificant biases (all within 0.1 SDs of zero – see Appendix S6) in these parameters that arise from paternal genotype reconstruction.

For maternal heterozygosity, the single-locus model of emergence success did not improve upon the linear multilocus model, even without comparison to the simulated null data sets (d.f. = 7, 38, $P$ = 0.216; $P$ = 0.893 vs. 1 000 null data sets). Single-locus models of paternal heterozygosity and parental relatedness at first seemed to improve significantly upon their respective linear multilocus models ($P$ = 0.009 and 0.001, respectively; d.f. = 7,38), but were not significant when compared to the null data sets ($P$ = 0.506 and 0.127).

No interpretive differences arose from expanding the maternal heterozygosity analyses of egg success to include six families (14 clutches) showing multiple paternity and six families (six clutches) producing too few offspring for paternal genotyping (data not shown).

## Discussion

In this study of the relationship between genetic variability and fitness in hawksbill turtles, we found no correlation between maternal heterozygosity and either clutch size or egg success. In contrast, we found support for effects of multilocus paternal heterozygosity and multilocus parental relatedness on egg success, but interacting with each other in a way that suggests that both positive and negative HFCs may be present in our study. This result needs treating with caution given that identity disequilibrium ($g_2$) was not significantly greater than zero. However, the magnitude of improvement of fit due to the interaction (DAICc = 4.19 vs second-best model, with the models only differing in presence/absence of the interaction term), the fact that the second-best model is itself a substantial improvement on the third-best model (DAICc = 2.13), the 'safe' multicollinearity diagnostics, and the results from our simulations all give us confidence that this result is unlikely to be an artefact of our analysis pipeline and is thus worth further discussion. We did not find support for single-locus effects, but without our simulations we would likely have committed a type I error in this analysis due to overfitting.

The 'best' model of egg success featured an interaction between paternal MLH and parental relatedness. Under this model, at low to intermediate levels of paternal MLH, the effect of relatedness conforms to a traditional inbreeding interpretation, with increasing relatedness correlating with decreasing fitness. However, when paternal MLH is high, the effect of relatedness disappears and may even reverse. To think about it the other way around, low paternal MLH only has a negative effect when parental relatedness is high (i.e. when inbreeding is implied). At low and intermediate parental relatedness (outbreeding), high paternal MLH (i.e. a father that is likely outbred himself) appears to

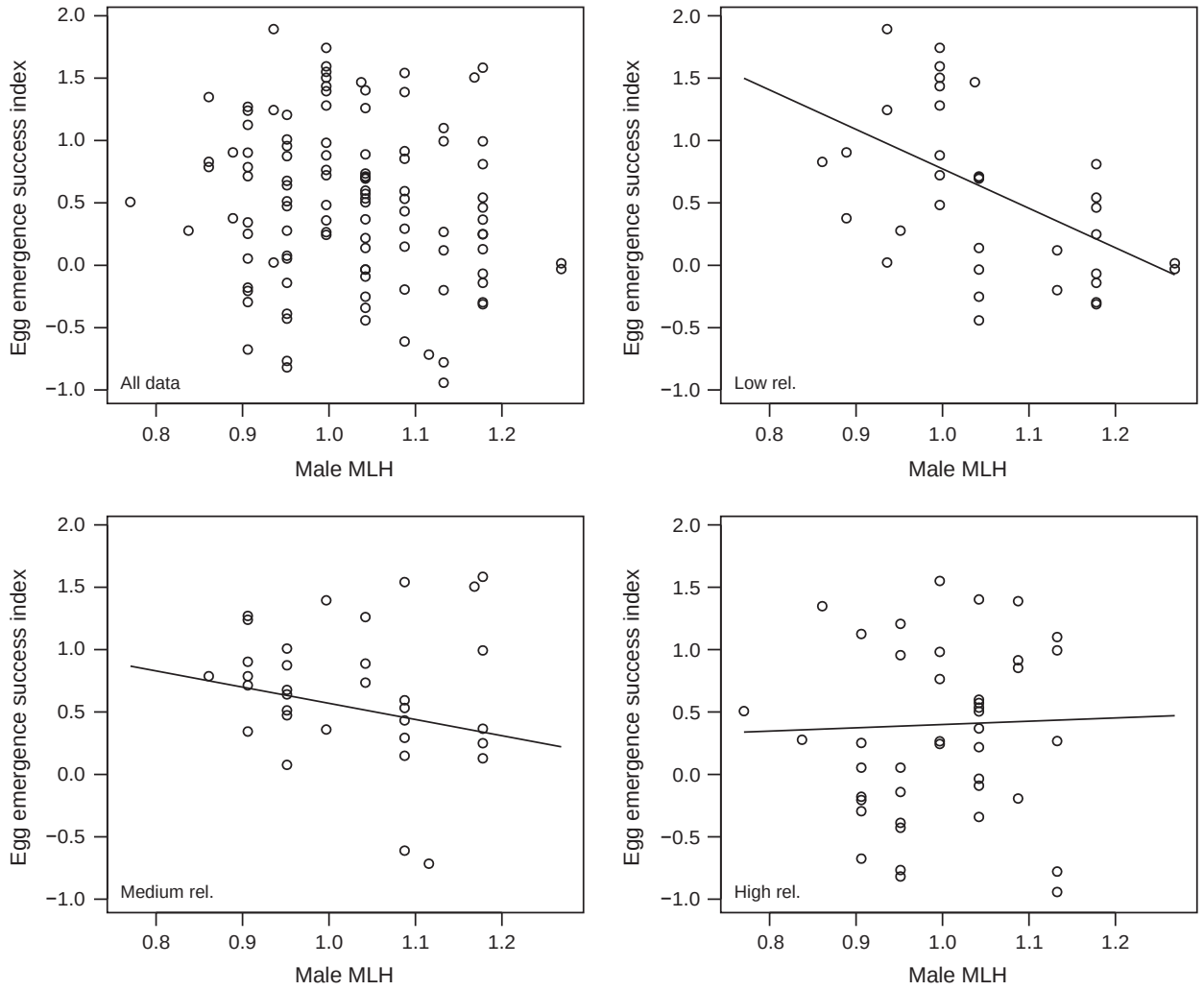


Fig. 1 Effect of interaction between paternal multilocus heterozygosity (MLH) and multilocus parental relatedness on proportional egg success (emergence; logit-transformed), focusing on paternal MLH. See Tables 2-3 for model fit assessments, and Appendix S7 for quality control plots of residuals. All plots show data *after* controlling for significant effects of clutch lay date and incubation duration. Top-left: all data points. Top-right: families with 'low' parental relatedness (lower third of relatedness data; relatedness ≤—0.03). Bottom-left: families with 'medium' parental relatedness (mid-third of relatedness data; —0.03 < relatedness ≤ 0.04). Bottom-right: families with 'high' parental relatedness (upper third of relatedness data; relatedness > 0.04). All lines are fitted at mean relatedness for the focal subset (—0.10, 0.01 and 0.10, respectively).

exert a negative effect on fitness. One possible explanation is that if parents are unrelated (dissimilar), and are thus already producing offspring of high heterozygosity, the addition of extra variability from a particularly heterozygous father may be deleterious (outbreeding depression). If the parents are related (similar), the fact that the father is particularly heterozygous will limit how often offspring will inherit the same allele at each locus from both parents, meaning that offspring will be more heterozygous and, in theory, suffer less from inbreeding depression. How this might apply in practice, and the long-term consequences, will be affected by the heritability of heterozygosity (Mitton *et al.*, 1993; Nietlisbach *et al.*, 2016) and the relationship between heterozygosity and relatedness (Roberts *et al.*, 2006), but modelling this is beyond the scope of this study.

In our analyses of reproductive success in relation to genetic variability in the hawksbill turtle, the two top multilocus models imply the presence of positive and negative HFCs in this system, a process that could exert a stabilizing influence on population genetic variability (Neff, 2004). Indeed, if the genetic predictors are interpreted in a classic inbreeding context, our result could be read (cautiously) as a tension between inbreeding and outbreeding depression, with an optimum level of

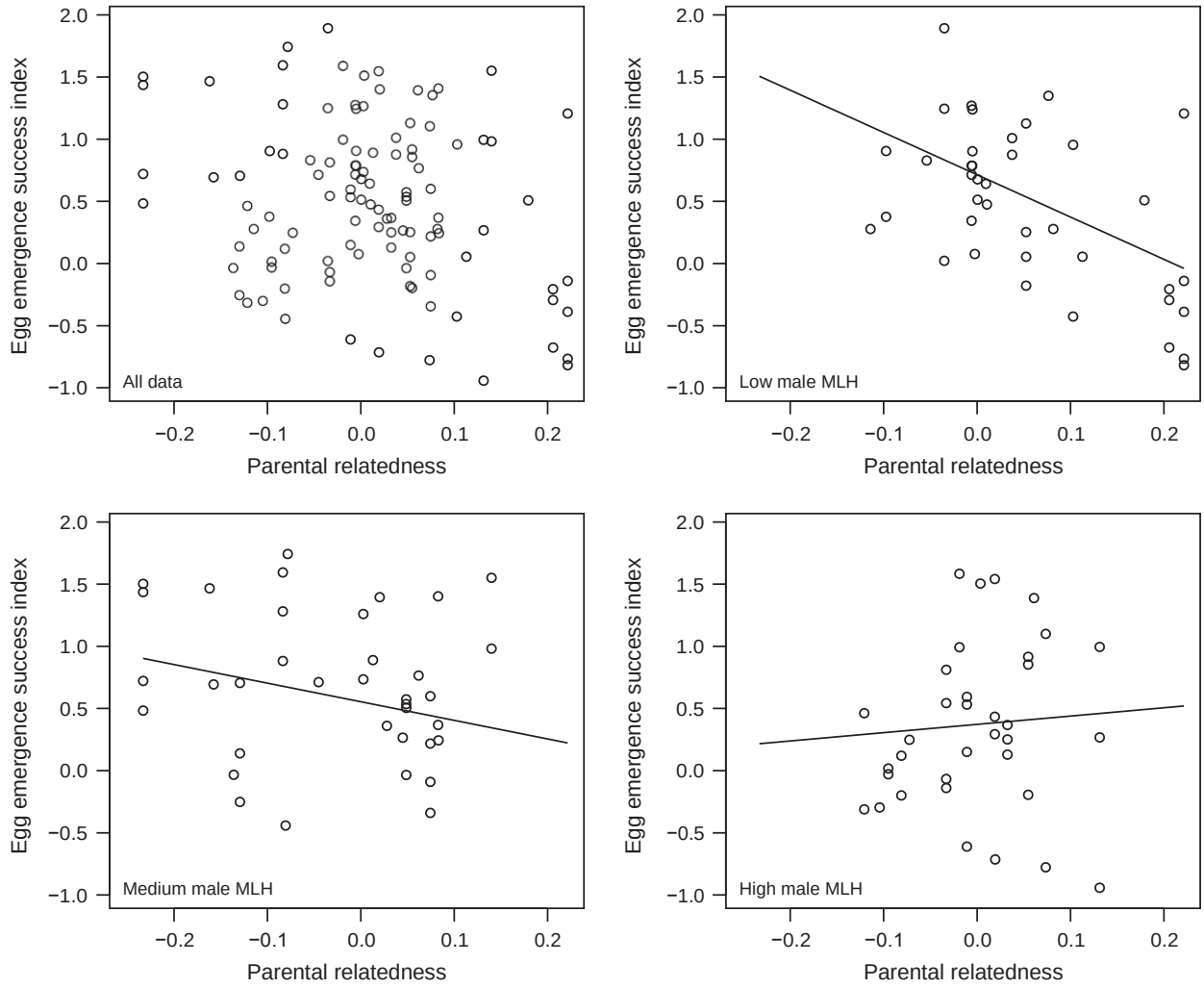


Fig. 2 Effect of interaction between paternal multilocus heterozygosity (MLH) and multilocus parental relatedness on proportional egg success (emergence; logit-transformed), focusing on parental relatedness. See Tables 2-3 for model fit assessments, and Appendix S7 for quality control plots of residuals. All plots show data ***after*** controlling for significant effects of clutch lay date and incubation duration. Top-left: all data points. Top-right: families with 'low' male paternal MLH (lower third of paternal MLH data; MLH ≤ 0.95). Bottom-left: families with 'medium' parental relatedness (mid-third of relatedness data; 0.95 <relatedness ≤ 1.04). Bottom-right: families with 'high' parental relatedness (upper third of relatedness data; relatedness > 1.04). All lines are fitted at mean relatedness for the focal subset (0.91, 1.02 and 1.15, respectively).

outcrossing. Studies inferring both inbreeding and outbreeding depression acting on the same fitness trait in the same system are few (e.g. Waser & Price, 1989, 1994; Marshall & Spalton, 2000; Neff, 2004), although further examples of them operating on different traits in the same population exist (Olano-Marin ***et al.***, 2011a,b). The collective implication of these studies is that some form of population structure (e.g. caused by isolation-by-distance, philopatry or founder effects) facilitates both local adaptation and elevated inbreeding risk and that there is a trade-off between reducing inbreeding and avoiding outbreeding depression. Could such a process operate in marine turtles? Potentially, yes. Female green turtles nesting on Ascension Island show fine-scale local adaptation despite evidence for strong male-biased gene flow between nesting beaches (Lee ***et al.***, 2007; Weber ***et al.***, 2012). More generally, studies across marine turtle species have emphasized that females show natal philopatry but that males are more dispersive (reviewed in Bowen & Karl, 2007; see also Lee, 2008; Komoroske ***et al.***, 2017), which could potentially give rise to an inbreeding–outbreeding tension analogous to that seen in some plants (Waser & Price, 1989, 1994). Fine-scale sex-biased natal philopatry has also been implicated in HFCs in blue tits (***Cyanistes caeruleus***), where positive and negative HFCs have

been observed in the same population (Olano-Marin *et al.*, 2011b; see also Szulkin & David, 2011). In our system, there is no regional structuring of nesting beaches, but pairwise genetic relatedness is higher among females nesting on Cousine than among the males that fertilize them, indicating that females are more philopatric than males (Phillips *et al.*, 2014) and creating the potential for a dispersal–adaptation trade-off. Unfortunately, we do not have comparable data from other sites in Seychelles that would allow us to test the validity, spatial scale and strength of such an inference.

We found no HFCs associated with maternal genotypes. Examples of differences between sexes in HFCs and effects of inbreeding are common, with effects more likely to be associated with females than with males (reviewed in Olano-Marin *et al.*, 2011b). These effects can range from early-life survival of females (Coulson *et al.*, 1999; Olano-Marin *et al.*, 2011b) to maternally transmitted effects on the next generation (Brouwer *et al.*, 2007; Bebbington *et al.*, 2016). In our case, the absence of any maternal HFC may be a conse- quence of female breeding strategy. Marine turtle females are capital breeders, meaning they accumulate an energy reserve ('capital') with which to produce and provision offspring. This is a slow and variable process (most marine turtle species nest once every 2-5 years; Miller, 1997) and is known to be affected by prevailing environmental conditions (e.g. Wood & Wood, 1980; Limpus & Nicholls, 1988). Correlations between maternal heterozygosity and reproductive metrics may therefore be difficult to detect in marine turtles without controlling for variance in rates of capital accumulation (Broderick *et al.*, 2001). However, with sufficient long-term data, it might be possible to test the hypothesis that heterozygosity affects efficiency at accumulating energy capital, and thereby remigration frequency and reproductive success, or to use biomarkers of stress such as telomeres (Plot *et al.*, 2012; Bebbington *et al.*, 2016).

There is considerable debate in the HFC literature as to how well MLH represents individual inbreeding status (Balloux *et al.*, 2004; Szulkin *et al.*, 2010; Berenos *et al.*, 2016; Nietlisbach *et al.*, 2017), and all HFC studies are thus urged to assess the utility of MLH as an inbreeding proxy through a test for identity disequilibrium (Szulkin *et al.*, 2010; Miller & Coltman, 2014). We did not find significant identity disequilibrium in our study, suggesting that, despite a reasonable panel of markers, MLH in our case is not a good proxy for inbreeding. Without identity disequilibrium, the effects associated with MLH are difficult to explain (Chapman & Sheldon, 2011; Miller & Coltman, 2014). However, for several reasons, we are wary of dismissing the MLH–inbreeding link as an explanation for the patterns observed. First, multilocus HFCs underpinned by inbreeding can reach significance before identity disequilibrium is significant (Szulkin *et al.*, 2010). Second, several authors have argued that HFCs, both multi- and single locus, are more likely to be detected when conserved markers are used, and marine turtle microsatellite loci are extremely conserved (discussed further below). Third, empirical support from meta-analyses for the premise that HFC effect sizes should be larger with greater inbreeding variance is mixed. Miller & Coltman (2014) report a significant correlation between $g_2$ and HFC effect sizes, but Chapman *et al.* (2009), using a coarser metric but much larger sample size, did not find a relationship between inbreeding variance and HFC effect size. Finally, our sampled population, although large and genetically well mixed, is not homogeneous, and shows some evidence of female natal philopatry (Phillips *et al.*, 2014). In large populations, any kind of structure creates a greater potential for inbreeding than does full mixing (Olano-Marin *et al.*, 2011a,b; see also Szulkin *et al.*, 2010). We have therefore discussed MLH in its traditional inbreeding interpretation, but with caution.

Several authors have argued for the importance of marker type in HFC studies. MLH measured using microsatellites located in expressed or otherwise conserved regions may be more likely to yield HFCs than MLH using anonymous/nonconserved loci by virtue of, on average, being closer to polymorphic loci under selection (Kü pper *et al.*, 2010; Olano-Marin *et al.*, 2011a,b; Szulkin & David, 2011; Ferrer *et al.*, 2015, 2016). The effect is statistically still a 'general' one, as it is the net, cumulative effect of multiple small effects (Szulkin & David, 2011). Interestingly, the majority of marine turtle microsatellite loci characterized to date show a remarkably high degree of conservation. Indeed, almost all primers designed in the family Cheloniidae, in which the extant species started diverging approximately 63 MYA (Naro-Maciel *et al.*, 2008), amplify across multiple family members, and some even amplify in other testudine families (e.g. Shamblin *et al.*, 2007; Lin *et al.*, 2008). Of the 32 loci used in our study, 18 were first characterized in species other than the hawksbill (Phillips *et al.*, 2013). Thus, our finding of significant multilocus HFCs may not be out of keeping with prevailing HFC theory, given the highly conserved nature of our markers. However, marine turtle sequence evolution is known to be particularly slow (FitzSimmons *et al.*, 1995), potentially due to long gen- eration times and low metabolic rates in these species (Avise *et al.*, 1992; Scott *et al.*, 2012). This may limit the comparability between our 'conserved' markers and those explicitly chosen for being conserved in taxa with faster substitution rates (e.g. Kü pper *et al.*, 2010; Olano-Marin *et al.*, 2011a,b).

Although multiple paternity is widespread in marine turtles (reviewed in Tedeschi *et al.*, 2015), we are aware of no published study that has demonstrated a benefit to females from multiple fertilizations (e.g. Lee & Hays, 2004; Wright *et al.*, 2013) or that females bias paternity on genetic grounds (Phillips *et al.*, 2013). Our study

may thus have implications for marine turtle mating systems, as it shows that, hypothetically, a female's choice of mate can affect the success of her clutches. However, it would only help explain mate choice, rather than multiple mating ***sensu lato***, unless post-copulatory mechanisms bias paternity to the 'best' male (Parker, 1970; Eberhard, 1996). Moreover, it is difficult to see how a female could assess and utilize the effect we describe here, especially if the population is widely dispersed at the time of mating, as argued by Phillips ***et al.*** (2013).

A conceptual follow-up to our study would be to examine whether the effects we observe extend to candidate loci known to exhibit HFCs, such as immune genes (e.g. major histocompatibility complex, Piertney & Oliver, 2006; Toll-like receptors, Grueber ***et al.***, 2012). It would also be informative to extend the study into additional years, as HFCs can vary in strength between breeding seasons, being stronger in 'bad' years that expose deleterious genotypes (e.g. Brouwer ***et al.***, 2007; Harrison ***et al.***, 2011; Annavi ***et al.***, 2014). More generally, how well MLH reflects individual inbreeding could be better tested by using much larger marker sets, such as next-generation sequencing approaches that generate thousands of markers across the genome (e.g. restriction site-associated DNA, Hoffman ***et al.***, 2014; single-nucleotide polymorphisms, Berenos ***et al.***, 2016).

To summarize, our results emphasize the importance of looking for fitness effects of both low and high levels of genetic variability within a system, even on the same fitness trait. Such studies are relatively few, but play an important role in understanding how genetic variation is maintained in wild populations and how this might affect individual fitness. Our study is also of value to the HFC literature because of the distinct characteristics of this species – a long-lived, slow-to-mature, fecund reptile – which are all traits that are underrepresented by the species examined in HFC studies (Chapman ***et al.***, 2009). From a conservation perspective, our results suggest that both inbreeding and outbreeding may affect fitness in marine turtles, at least in the Seychelles population of hawksbill turtles. Whether these effects have been altered by the substantial population declines caused by two centuries of overhunting is impossible to say from our study, but their mutual presence may highlight an important balance that could be disturbed by anthropogenic processes.

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## Author contributions

KPP assisted in fieldwork, performed the laboratory work, analysed the data and wrote the first draft of the manuscript. KGJ coordinated and led the fieldwork. THJ assisted with interpreting the results and writing the manuscript. DSR designed the study, managed the overall project, assisted with writing the manuscript, and won the UEA 2013 shotput.

## Data accessibility

All data and analysis scripts have been uploaded to the Dryad repository: https://doi.org/10.5061/dryad.6697t.

## References

Akaike, H. 1974. A new look at the statistical model identification. ***IEEE Trans. Autom. Control*** **19**: 716–723.

Amos, W., Wilmer, J.W., Fullard, K., Burg, T.M., Croxall, J.P., Bloch, D. ***et al.*** 2001. The influence of parental relatedness on reproductive success. ***Proc. R. Soc. B.*** **268**: 2021–2027.

Annavi, G., Newman, C., Buesching, C.D., Macdonald, D.W., Burke, T. & Dugdale, H.L. 2014. Heterozygosity-fitness correlations in a wild mammal population: accounting for parental and environmental effects. ***Ecol. Evol.*** **4**: 2594–2609.

Aparicio, J.M., Ortego, J. & Cordero, P.J. 2006. What should we weigh to estimate heterozygosity, alleles or loci? ***Mol. Ecol.*** **15**: 4659–4665.

Avise, J.C., Bowen, B.W., Lamb, T., Meylan, A.B. & Bermingham, E. 1992. Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the Testudines. ***Mol. Biol. Evol.*** **9**: 457–473.

Balloux, F., Amos, W. & Coulson, T. 2004. Does heterozygosity estimate inbreeding in real populations? ***Mol. Ecol.*** **13**: 3021–3031.

Bartón, K. 2013. MuMIn: Multi-model inference. R package version 1.9.5. http://CRAN.R-project.org/package=MuMIn.

Bates, D., Maechler, M., Bolker, B. & Walker, S. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7. http://CRAN.R-project.org/package=lme4.

Bebbington, K., Spurgin, L.G., Fairfield, E.A., Dugdale, H.L., Komdeur, J., Burke, T. ***et al.*** 2016. Telomere length reveals

cumulative individual and transgenerational inbreeding effects in a passerine bird. *Mol. Ecol.* 25: 2949–2960.
Bensch, S., Hasselquist, D. & Vonschantz, T. 1994. Genetic similarity between parents predicts hatching failure - non-incestuous inbreeding in the great reed warbler. *Evolution* 48: 317–326.
Berenos, C., Ellis, P.A., Pilkington, J.G. & Pemberton, J.M. 2016. Genomic analysis reveals depression due to both individual and maternal inbreeding in a free-living mammal population. *Mol. Ecol.* 25: 3152–3168.
Bowen, B.W. & Karl, S.A. 2007. Population genetics and phylogeography of sea turtles. *Mol. Ecol.* 16: 4886–4907.
Bretman, A., Newcombe, D. & Tregenza, T. 2009. Promiscuous females avoid inbreeding by controlling sperm storage. *Mol. Ecol.* 18: 3340–3345.
Broderick, A.C., Godley, B.J. & Hays, G.C. 2001. Trophic status drives interannual variability in nesting numbers of marine turtles. *Proc. R. Soc. B* 268: 1481–1487.
Brouwer, L., Komdeur, J. & Richardson, D.S. 2007. Heterozygosity-fitness correlations in a bottlenecked island species: a case study on the Seychelles warbler. *Mol. Ecol.* 16: 3134–3144.
Chapman, J.R. & Sheldon, B.C. 2011. Heterozygosity is unrelated to adult fitness measures in a large, noninbred population of great tits (*Parus major*). *J. Evol. Biol.* 24: 1715–1726.
Chapman, J.R., Nakagawa, S., Coltman, D.W., Slate, J. & Sheldon, B.C. 2009. A quantitative review of heterozygosity-fitness correlations in animal populations. *Mol. Ecol.* 18: 2746–2765.
Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Evol. Syst.* 18: 237–268.
Charlesworth, B. & Charlesworth, D. 1999. The genetic basis of inbreeding depression. *Genet. Res.* 74: 329–340.
Coltman, D.W. & Slate, J. 2003. Microsatellite measures of inbreeding: A meta-analysis. *Evolution* 57: 971–983.
Coltman, D.W., Pilkington, J.G., Smith, J.A. & Pemberton, J.M. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution* 53: 1259–1267.
Cordero, P.J., Aparico, J.M. & Veiga, J.P. 2004. Parental genetic characteristics and hatching success in the spotless starling, *Sturnus unicolor*. *Anim. Behav.* 67: 637–642.
Coulson, T., Albon, S., Slate, J. & Pemberton, J. 1999. Microsatellite loci reveal sex-dependent responses to inbreeding and outbreeding in red deer calves. *Evolution* 53: 1951–1960.
Crnokrak, P. & Roff, D.A. 1999. Inbreeding depression in the wild. *Heredity* 83: 260–270.
David, P. 1998. Heterozygosity-fitness correlations: new perspectives on old problems. *Heredity* 80: 531–537.
David, P., Pujol, B., Viard, F., Castella, V. & Goudet, J. 2007. Reliable selfing rate estimates from imperfect population genetic data. *Mol. Ecol.* 16: 2474–2487.
Diamond, A.W. 1976. Breeding biology and conservation of hawksbill turtles, *Eretmochelys imbricata* L., on Cousin Island Seychelles. *Biol. Conserv.* 9: 199–215.
Eberhard, W.G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton University Press, Princeton.
Edmands, S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Mol. Ecol.* 16: 463–475.
Escobar, J.S., Nicot, A. & David, P. 2008. The different sources of variation in inbreeding depression, heterosis and outbreeding depression in a metapopulation of *Physa acuta*. *Genetics* 180: 1593–1608.
Ferrer, E.S., Garcia-Navas, V., Bueno-Enciso, J., Sanz, J.J. & Ortego, J. 2015. Multiple sexual ornaments signal heterozygosity in male blue tits. *Biol. J. Linn. Soc.* 115: 362–375.
Ferrer, E.S., Garcia-Navas, V., Sanz, J.J. & Ortego, J. 2016. The strength of the association between heterozygosity and probability of interannual local recruitment increases with environmental harshness in blue tits. *Ecol. Evol.* 6: 8857–8869.
Fitzpatrick, J.L. & Evans, J.P. 2009. Reduced heterozygosity impairs sperm quality in endangered mammals. *Biol. Lett.* 5: 320–323.
FitzSimmons, N.N., Moritz, C. & Moore, S.S. 1995. Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. *Mol. Biol. Evol.* 12: 432–440.
Gooley, R., Hogg, C.J., Belov, K. & Grueber, C.E. 2017. No evidence of inbreeding depression in a Tasmanian devil insurance population despite significant variation in inbreeding. *Sci. Rep.* 7: 1830.
Grueber, C.E., Wallis, G.P., King, T.M. & Jamieson, I.G. 2012. Variation at innate immunity Toll-like Receptor Genes in a bottlenecked population of a New Zealand robin. *PLoS ONE* 7: e45011.
Hansson, B. & Westerberg, L. 2002. On the correlation between heterozygosity and fitness in natural populations. *Mol. Ecol.* 11: 2467–2474.
Hansson, B. & Westerberg, L. 2008. Heterozygosity-fitness correlations within inbreeding classes: local or genome-wide effects? *Conserv. Genet.* 9: 73–83.
Harrison, X.A., Bearhop, S., Inger, R., Colhoun, K., Gudmundsson, G.A., Hodgson, D. *et al.* 2011. Heterozygosity-fitness correlations in a migratory bird: an analysis of inbreeding and single-locus effects. *Mol. Ecol.* 20: 4786–4795. Hitchins, P.M., Bourquin, O. & Hitchins, S. 2004. Nesting suc- cess of hawksbill turtles (*Eretmochelys imbricata*) on Cousine Island Seychelles. *J. Zool.* 264: 383–389.
Hoffman, J.I., Simpson, F., David, P., Rijks, J.M., Kuiken, T., Thorne, M.A.S. *et al.* 2014. High-throughput sequencing reveals inbreeding depression in a natural population. *PNAS* 111: 3775–3780.
Hurvich, C.M. & Tsai, C.L. 1989. Regression and time-series model selection in small samples. *Biometrika* 76: 297–307.
Jones, O.R. & Wang, J. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. *Mol. Ecol. Resour.* 10: 551–555.
Keller, L.F. 1998. Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). *Evolution* 52: 240–250.
Keller, L.F. & Waller, D.M. 2002. Inbreeding effects in wild populations. *Trends Ecol. Evol.* 17: 230–241.
Komoroske, L.M., Jensen, M.P., Stewart, K., Shamblin, B.M. & Dutton, P.H. 2017. Advances in the application of genetics in marine turtle biology & conservation. *Front. Mar. Sci.* 4: 156.
Küpper, C., Kosztolanyi, A., Augustin, J., Dawson, D.A., Burke, T. & Székely, T. 2010. Heterozygosity-fitness correlations of conserved microsatellite markers in Kentish plovers *Charadrius alexandrinus*. *Mol. Ecol.* 19: 5172–5185.
Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. 2013. lmerTest: Tests for random and fixed effects for linear mixed

effect models (lmer objects of lme4 package). R package version 1.2-1. http://CRAN.R-project.org/package=lmerTest.

Lacy, R.C., Petric, A. & Warneke, M. 1993. Inbreeding and outbreeding in captive populations of wild animal species. In: *The Natural History of Inbreeding and Outbreeding* (N.W. Thornhill, ed), pp. 352–374. University of Chicago Press, Chicago.

Lee, P.L.M. 2008. Molecular ecology of marine turtles: new approaches and future directions. *J. Exp. Mar. Biol. Ecol.* **356**: 25–42.

Lee, P.L.M. & Hays, G.C. 2004. Polyandry in a marine turtle: females make the best of a bad job. *PNAS* **101**: 6530–6535.

Lee, P.L.M., Luschi, P. & Hays, G.C. 2007. Detecting female precise natal philopatry in green turtles using assignment methods. *Mol. Ecol.* **16**: 61–74.

Li, Y.C., Korol, A.B., Fahima, T., Beiles, A. & Nevo, E. 2002. Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. *Mol. Ecol.* **11**: 2453–2465.

Li, Y.C., Korol, A.B., Fahima, T. & Nevo, E. 2004. Microsatellites within genes: structure, function, and evolution. *Mol. Biol. Evol.* **21**: 991–1007.

Limpus, C.J. & Nicholls, N. 1988. The Southern Oscillation regulatestheannualnumbersofgreenturtles(*Cheloniamydas*) breedingaroundnorthernAustralia.*Aust.WildlifeRes.*15:157–161.

Lin, G., Chang, A., Yap, H.W. & Yue, G.H. 2008. Characterization and cross-species amplification of microsatellites from the endangered Hawksbill turtle (*Eretmochelys imbricate*). *Conserv. Genet.* **9**: 1071–1073.

Marshall, T.C. & Spalton, J.A. 2000. Simultaneous inbreeding and outbreeding depression in reintroduced Arabian oryx. *Anim. Conserv.* **3**: 241–248.

Marshall, T.C., Slate, J., Kruuk, L.E.B. & Pemberton, J.M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* **7**: 639–655.

Miller, J.D. 1997. Reproduction in Sea Turtles. In: *The Biology of Sea Turtles*, vol. 1 (P.L. Lutz, J.A. Musick, eds), pp. 51–82. CRC Press, Boca Raton, FL.

Miller, J.M. & Coltman, D.W. 2014. Assessment of identity disequilibrium and its relation to empirical heterozygosity fitness correlations: a meta-analysis. *Mol. Ecol.* **23**: 1899–1909.

Mitton, J.B., Schuster, W.S.F., Cothran, E.G. & Defries, J.C. 1993. Correlation between the individual heterozygosity of parents and their offspring. *Heredity* **71**: 59–63.

Mortimer, J.A. 1984. *Marine turtles in the Republic of the Seychelles: status and management: report on project 1809, 1981-1984*, pp. 80. IUCN/WWF, Gland, Switzerland.

Mortimer, J.A. 2004. Seychelles Marine Ecosystem Management Project (GEF-SEYMEMP): Turtle Component. Final Report.

Nakagawa, S. & Schielzeth, H. 2013. A general and simple method for obtaining $R^2$ from generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**: 133–142.

Naro-Maciel, E., Le, M., FitzSimmons, N.N. & Amato, G. 2008. Evolutionary relationships of marine turtles: a molecular phylogeny based on nuclear and mitochondrial genes. *Mol. Phylogenet. Evol.* **49**: 659–662.

Neff, B.D. 2004. Stabilizing selection on genomic divergence in a wild fish population. *PNAS* **101**: 2381–2385.

Nietlisbach, P., Keller, L.F. & Postma, E. 2016. Genetic variance components and heritability of multiallelic heterozygosity under inbreeding. *Heredity* **116**: 1–11.

Nietlisbach, P., Keller, L.F., Camenisch, G., Guillaume, F., Arcese, P., Reid, J.M. *et al.* 2017. Pedigree-based inbreeding coefficient explains more variation in fitness than heterozygosity at 160 microsatellites in a wild bird population. *Proc. R. Soc. B.* **284**. https://doi.org/10.1098/rspb.2016.2763.

Olano-Marin, J., Mueller, J.C. & Kempenaers, B. 2011a. Correlations between heterozygosity and reproductive success in the blue tit (*Cyanistes caeruleus*): an analysis of inbreeding and single locus effects. *Evolution* **65**: 3175–3194.

Olano-Marin, J., Mueller, J.C. & Kempenaers, B. 2011b. Heterozygosity and survival in blue tits (Cyanistes caeruleus): contrasting effects of presumably functional and neutral loci. *Mol. Ecol.* **20**: 4028–4041.

Parker, G.A. 1970. Sperm competition and its evolutionary consequences in insects. *Biol. Rev. Camb. Philos. Soc.* **45**: 525–567.

Parmenter, C.J. 1980. Incubation of the eggs of the green sea turtle, *Chelonia mydas*, in Torres Strait, Australia – the effect of movement on hatchability. *Aust. Wildlife Res.* **7**: 487–491.

Phillips, K.P., Jorgensen, T.H., Jolliffe, K.G., Jolliffe, S.-M., Henwood, J. & Richardson, D.S. 2013. Reconstructing paternal genotypes to infer patterns of sperm storage and sexual selection in the hawksbill turtle. *Mol. Ecol.* **22**: 2301–2312.

Phillips, K.P., Mortimer, J.A., Jolliffe, K.G., Jorgensen, T.H. & Richardson, D.S. 2014. Molecular techniques reveal cryptic life history and demographic processes of a critically endangered marine turtle. *J. Exp. Mar. Biol. Ecol.* **455**: 29–37.

Pierney, S.B. & Oliver, M.K. 2006. The evolutionary ecology of the major histocompatibility complex. *Heredity* **96**: 7–21.

Plot, V., Criscuolo, F., Zahn, S. & Georges, J.-Y. 2012. Telomeres, age and reproduction in a long-lived reptile. *PLoS ONE* **7**: e40855.

Queller, D.C. & Goodnight, K.F. 1989. Estimating relatedness using genetic markers. *Evolution* **43**: 258–275.

R Development Core Team 2008. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Raymond, M. & Rousset, F. 1995. GENEPOP (Version-1.2) - population-genetics software for exact tests and ecumenicism. *J. Hered.* **86**: 248–249.

Richardson, D.S., Komdeur, J. & Burke, T. 2004. Inbreeding in the Seychelles warbler: environment-dependent maternal effects. *Evolution* **58**: 2037–2048.

Roberts, S.C., Hale, M.L. & Petrie, M. 2006. Correlations between heterozygosity and measures of genetic similarity: implications for understanding mate choice. *J. Evol. Biol.* **19**: 558–569.

Rodriguez-Quilon, I., Santos-del-Blanco, L., Grivet, D., Jaramillo-Correa, J.P., Majada, J., Vendramin, G.G. *et al.* 2015. Local effects drive heterozygosity-fitness correlations in an outcrossing long-lived tree. *Proc. R. Soc. B* **282**: 20152230.

Scott, R., Marsh, R. & Hays, G.C. 2012. Life in the really slow lane: loggerhead sea turtles mature late relative to other reptiles. *Funct. Ecol.* **26**: 227–235.

Shamblin, B.M., Faircloth, B.C., Dodd, M., Wood-Jones, A., Castleberry, S.B., Carroll, J.P. *et al.* 2007. Tetranucleotide microsatellites from the loggerhead sea turtle (*Caretta caretta*). *Mol. Ecol. Notes* **7**: 784–787.

Szulkin, M. & David, P. 2011. Negative heterozygosity-fitness correlations observed with microsatellites located in functional areas of the genome. *Mol. Ecol.* 20: 3949–3952.
Szulkin, M., Bierne, N. & David, P. 2010. Heterozygosity-fitness correlations: a time for reappraisal. *Evolution* 64: 1202–1217.
Tedeschi, J., Mitchell, N., Berry, O., Whiting, S., Meekan, M. & Kennington, W. 2015. Reconstructed paternal genotypes reveal variable rates of multiple paternity at three rookeries of loggerhead sea turtles (*Caretta caretta*) in Western Australia. *Aust. J. Zool.* 62: 454–462.
Templeton, A.R., Hemmer, H., Mace, G., Seal, U.S., Shields, W.M. & Woodruff, D.S. 1986. Local adaptation, coadaptation, and population boundaries. *Zoo. Biology.* 5: 115–125.
Van de Casteele, T., Galbusera, P., Schenck, T. & Matthysen, E. 2003. Seasonal and lifetime reproductive consequences of inbreeding in the great tit *Parus major*. *Behav. Ecol.* 14: 165–174.
Wang, J.L. 2011. COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Mol. Ecol. Resour.* 11: 141–145.
Wang, J. & Santure, A.W. 2009. Parentage and sibship inference from multilocus genotype data under polygamy. *Genetics* 181: 1579–1594.
Waser, N.M. 1993. Population structure, optimal outbreeding, and assortative mating in angiosperms. In: *The Natural History of Inbreeding and Outbreeding* (N.W. Thornhill, ed), pp. 173–199. University of Chicago Press, Chicago.
Waser, N.M. & Price, M.V. 1989. Optimal outcrossing in *Ipomopsis aggregata* - seed set and offspring fitness. *Evolution* 43: 1097–1109.
Waser, N.M. & Price, M.V. 1994. Crossing-distance effects in *Deplhinium nelsonii* - outbreeding and inbreeding depression in progeny fitness. *Evolution* 48: 842–852.
Weber, S.B., Broderick, A.C., Groothuis, T.G.G., Ellick, J., Godley, B.J. & Blount, J.D. 2012. Fine-scale thermal adaptation in a green turtle nesting population. *Proc. R. Soc. B* 279: 1077–1084.
Willi, Y. & van Buskirk, J. 2005. Genomic compatibility occurs over a wide range of parental genetic similarity in an outcrossing plant. *Proc. R. Soc. B* 272: 1333–1338.
Wood, J.R. & Wood, F.E. 1980. Reproductive biology of captive green sea turtles *Chelonia mydas*. *Am. Zool.* 20: 499–505.
Wright, L.I., Fuller, W.J., Godley, B.J., McGowan, A., Tregenza, T. & Broderick, A.C. 2013. No benefits of polyandry to female green turtles. *Behav. Ecol.* 24: 1022–1029.

## Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
**Appendix S1** Exploration of parameters used for reconstructing male hawksbill turtle microsatellite genotypes from hatchling genotypes; Table S1.1 and Figs S1.1-S1.12.
**Appendix S2** Tables S2.1-S2.4: re-runs of main multilocus analyses using two alternative MLH metrics ('homozygosity by loci' and 'internal relatedness').
**Appendix S3** Fig. S3.1: relationship between clutch size (number of eggs laid) and female body size.
**Appendix S4** Tables S4.1-S4.2 and Fig. S4.1: re-runs of analyses of multilocus effects on egg success using two alternative fitness metrics in addition to that used in the main document (emergence success; proportion of eggs producing a hatchling that leaves the nest): 'fertilization success' (proportion of eggs producing a visible embryo) and 'hatching success' (proportion of eggs that hatch).
**Appendix S5** Exploration of how the correlation between paternal multilocus heterozygosity and parental relatedness may affect our analyses, including bootstrap tests of both parameters; Figs S5.1-S5.4.
**Appendix S6** Description of simulations used to generate null data sets against which to compare our observed effects on egg emergence success; Table S6.1.
**Appendix S7** Figs S7.1-S7.2: quality-control plots of model residuals for best ('interaction') model of genetic predictors of egg emergence success.

Data deposited at Dryad: doi: https://doi.org/10.5061/dryad.6697t.