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Egg production and individual genetic diversity in lesser kestrels

JOAQUÍN ORTEGO, GUSTAU CALABUIG, PEDRO J. CORDERO and JOSÉ MIGUEL APARICIO
Grupo de Investigación de la Biodiversidad Genética y Cultural, Instituto de Investigación en Recursos Cinegéticos — IREC (CSIC, UCLM, JCCM), Ronda de Toledo s/n, E-13005 Ciudad Real, Spain

Abstract

Fecundity is an important component of individual fitness and has major consequences on population dynamics. Despite this, the influence of individual genetic variability on egg production traits is poorly known. Here, we use two microsatellite-based measures, homozygosity by loci and internal relatedness, to analyse the influence of female genotypic variation at 11 highly variable microsatellite loci on both clutch size and egg volume in a wild population of lesser kestrels (*Falco naumanni*). Genetic diversity was associated with clutch size, with more heterozygous females laying larger clutches, and this effect was statistically independent of other nongenetic variables such as female age and laying date, which were also associated with fecundity in this species. However, egg volume was not affected by female heterozygosity, confirming previous studies from pedigree-based breeding experiments which suggest that this trait is scarcely subjected to inbreeding depression. Finally, we explored whether the association between heterozygosity and clutch size was due to a genome-wide effect (general effect) or to single locus heterozygosity (local effect). Two loci showed a stronger influence but the correlation was not fully explained by these two loci alone, suggesting that a main general effect underlies the association observed. Overall, our results underscore the importance of individual genetic variation for egg production in wild bird populations, a fact that could have important implications for conservation research and provides insights into the study of clutch size evolution and genetic variability maintenance in natural populations.

Keywords: clutch size, egg production, egg size, *Falco naumanni*, genetic variability maintenance, heterozygosity

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Introduction

Mating between closely related parents generally results in progenies with reduced fitness, a fact that has attracted attention since Darwin in several fields of biology and medicine (Wright 1977). This phenomenon, known as inbreeding depression, supposedly occurs because of reduced genome-wide heterozygosity at loci affecting fitness, either increasing the chance of expression of deleterious recessive alleles or reducing the heterozygote advantage for traits that are controlled by genes experiencing some form of balancing selection (Charlesworth & Charlesworth 1987; Falconer & Mackay 1996). However, the recording of deep pedigrees to estimate inbreeding coefficients is a difficult task to do in open natural populations. Given that the effects of inbreeding depression on fitness occur through a reduction of heterozygosity, an alternative measure for genome-wide heterozygosity is the use of multilocus heterozygosity at neutral polymorphic markers. Many recent studies have reported correlations between individual heterozygosity at neutral microsatellite markers and different key components of fitness (e.g. Foerster *et al*. 2003; Bean *et al*. 2004; Hoffman *et al*. 2004; Charpentier *et al*. 2005; Acevedo-Whitehouse *et al*. 2006; see, however, Duarte *et al*. 2003; Hoffman *et al*. 2006). However, the utility of this approach has been questioned on the basis of recent empirical and theoretical studies showing that the small number of neutral markers generally employed to estimate genome-wide heterozygosity only correlates weakly with inbreeding coefficients or does so under a restricted number of circumstances (Balloux

Correspondence: Joaquín Ortego, Fax: +34 926295 451; E-mail: joaquin.ortego@uclm.es

et al. 2004; Slate *et al*. 2004). Thus, it has been suggested that the observed heterozygosity–fitness correlations reported in several studies could have been exaggerated by a number of biases, including publication bias in favour of positive results, or could reflect not a genome-wide effect, the general effect hypothesis (Weir & Cockerham 1973; David 1998), but a linkage disequilibrium between a single or few neutral markers and functional genes under balancing selection (local effect hypothesis) (David 1998; Hansson *et al*. 2001; Hansson & Westerberg 2002; Balloux *et al*. 2004). Hence, it is difficult to generalize about the prevalence of heterozygosity-fitness correlations in natural populations and more studies are necessary to determine their underlying mechanisms (Balloux *et al*. 2004).

An interesting case study for exploring the association between genetic diversity and fitness in natural populations is maternal allocation to egg production. Although this topic has taken a central role in life-history theory and has become one of the most intensively studied aspects in reproduction, the potential effects of female genetic diversity on egg production have generally been overlooked in wild populations (but see Foerster *et al*. 2003). To identify whether genetic aspects such as heterozygosity operate at the egg-laying stage would have important implications for reproduction evolution, management of endangered species, supportive breeding and commercial egg production in poultry industry. Using egg production to explore heterozygosity–fitness correlations has some additional advantages over other fitness components. First, this fitness trait is mainly under female control (Visser & Lessells 2001), effectively eliminating any influence of confounding factors such as sibling competition and stochastic factors (partial brood predation, unpredictable food abundance changes, etc.) which can alter fitness estimates at latter reproductive stages (e.g. Cordero *et al*. 2004). Second, the amount of effort allocated during the egg-laying stage is now recognized to be very costly in terms of energy, time and nutrients: only some females can produce larger clutches, and producing extra eggs reduces other aspects of female fitness (Heany & Monaghan 1995; Monaghan *et al*. 1995; Monaghan & Nager 1997; Monaghan *et al*. 1998; Visser & Lessells 2001). This makes egg production a good candidate for revealing the effects of genetic diversity in comparison with other less-costly components of fitness (Slate *et al*. 2000). Third, egg production is a very important life-history trait, generally subject to strong directional selection and, thus, inbreeding depression is more likely than in traits less closely linked to fitness (de Rose & Roff 1999).

The lesser kestrel (*Falco naumanni*), a migratory and small size colonial bird of prey, provides a good model system for exploring the relationship between genetic variation and components of fitness. This species suffered a sharp population decline in the middle of the 20th century, with an estimated loss of around 95% of the breeding population in the Iberian Peninsula in only 20 years (reviewed in Bustamante 1997). This population crash would have reduced genetic diversity and forced mating between close relatives, resulting in the expression of deleterious alleles and inbreeding depression (Shields 1993; Frankham *et al*. 2002). How this may affect different components of fitness in lesser kestrels is currently unknown. In this study, we investigate the influence of individual genetic diversity on two parameters related with female fecundity, clutch size and egg volume, after controlling for the effects of some ecological parameters. For this purpose, we employ two heterozygosity-derived measures, heterozygosity by loci (*HL*; Aparicio *et al*. 2006) and internal relatedness (*IR*; Amos *et al*. 2001), allowing us to compare for the first time their relative efficiency using a real database from a wild population. We also explore the underlying mechanism of heterozygosity-fitness correlations, testing whether they reflect a genome-wide effect or a local effect of linkage between marker loci and functional genes. If the effect is only explained by heterozygosity at some particular loci, then genome-wide heterozygosity cannot be considered as the underlying mechanism (e.g. Bean *et al*. 2004; Acevedo-Whitehouse *et al*. 2006).

Materials and methods

Study population and field procedures

The study was conducted in La Mancha, central Spain (600–800 m above sea level), in an area covering approximately 1000 km^2. We studied 22 lesser kestrel colonies clustered in two subpopulations separated by 30 km: 'Villacañas' subpopulation (39°30′N, 3°20′W; 16 colonies) and 'Consuegra' subpopulation (39°35′N, 3°40′W; 6 colonies). From 1991 to date, only one male born in Villacañas has been recaptured attempting, unsuccessfully, to breed in Consuegra, indicating that exchange is rare. The climate is meso-Mediterranean with mean temperatures ranging from 24–26 °C in July to 4–6 °C in January and 300–400 mm of rainfall mainly concentrated in spring and autumn. The area is extensively cultivated with barley (*Hordeum vulgare*), wheat (*Triticum* spp.) and vineyards (*Vitis vinifera*). Other minor habitats include scattered olive groves (*Olea europaea*), some recent pine plantations (*Pinus* spp.) and noncultivated areas covered with pastures.

In our study area, the lesser kestrel mainly breeds in abandoned farmhouses, both under tiled roofs and inside holes in walls. Kestrels normally arrive to this area from mid-February or the beginning of March, depending on the year. Egg laying lasts from the end of April to the first week of June (Aparicio & Bonal 2002). Kestrels are mainly monogamous, and lay only one clutch per season (modal clutch size in our population = 4 eggs; range = 1–6 eggs) with the exception of rare replacement clutches (*c*. 0.5%)

that were excluded from this study. During the 2001–2006 breeding seasons, we located nests-sites before the onset of egg laying. Each potential nest was monitored every 6 days from the middle of April to find the first eggs, and then every 2 days until the clutch was finished. Thus, since females usually lay four or five eggs with mean intervals of 2 days, we were able to find the maximum number of unfinished clutches with minimum disturbance of the colonies. Laying date was defined as the date the first egg was laid (Aparicio & Bonal 2002). In a subsample of the monitored nests, we measured maximum length and breadth of all the eggs to the nearest 0.01 mm using a digital caliper. Egg volume was calculated using the equation of Hoyt (1979) (volume = egg length × egg breadth^2 × 0.51). Adults were trapped with a noose carpet or by hand during incubation, measured and individually marked with metallic and coloured plastic rings. Blood samples (100 µL) were obtained by venipuncture of the brachial vein and preserved in ~1200 µL ethanol 96% at –20 °C. To estimate female size, we measured tarsus length and wing length using a caliper and a rule to the nearest 0.01 mm and 1 mm, respectively. We also used pectoral thickness as an estimator of body condition (Aparicio 1997; Aparicio & Cordero 2001). This trait has been used in previous studies as a measure of body condition in several bird species (Bolton *et al*. 1991; Newton 1993), and has been considered a more reliable measure of condition than residuals of body mass on tarsus length (Gosler & Harper 2000). Moreover, it is easy to measure accurately on live birds by using a portable ultrasonic metre, in this case a Krautkrämer USM22F (accuracy 0.1 mm), especially designed to measure animal tissues. We knew the exact age of around a third of individuals that were ringed as fledglings ($n = 78$). For all other females ($n = 159$), we considered that individuals captured for the first time were in their first year if they presented yearling plumage or in their second year if they presented adult plumage (e.g. Aparicio & Cordero 2001; Foerster *et al*. 2003). The relationship between clutch size and age did not differ between females with exact and estimated age (J. M. Aparicio, unpublished data), and so we pooled all data for the analyses. We manipulated and banded lesser kestrels under license from the Spanish institutional authorities (Environmental Agency of the Community of Castilla-La Mancha and the Ringing Office of the Ministry of Environment) and we followed general ethical guidelines for animal welfare and nature conservation.

Table 1 Polymorphism characteristics of microsatellite loci used to type female lesser kestrels. Table shows number of alleles (K), annealing temperature (T), expected heterozygosity (H_E), and observed heterozygosity (H_O) at each locus (based on 237 individuals)

Locus	K	T (°C)	H_E	H_O	Primer origin
Fp5	6	55	0.613	0.612	Nesje *et al*. 2000
Fp13	4	55	0.661	0.532	Nesje *et al*. 2000
Fp31	8	55	0.653	0.654	Nesje *et al*. 2000
Fp46–1	11	55	0.624	0.591	Nesje *et al*. 2000
Fp79–4	31	55	0.938	0.895	Nesje *et al*. 2000
Fp86–2	3	54	0.533	0.328	Nesje *et al*. 2000
Fp89	4	55	0.495	0.464	Nesje *et al*. 2000
Fu1	11	48	0.487	0.485	J. Wetton, unpublished
Fu2	53	54	0.953	0.886	J. Wetton, unpublished
Fn1–11	24	59	0.799	0.781	Ortego *et al*. 2006
Fn2–14	128	56	0.974	0.873	Ortego *et al*. 2006

Molecular analyses

We quantified multilocus heterozygosity in 237 female lesser kestrels across 11 polymorphic microsatellite markers (Table 1). All females were genotyped at all 11 microsatellite markers. We used QIAamp DNA Blood Mini Kits (QIAGEN) to extract and purify genomic DNA from the blood samples. Approximately 5 ng of template DNA was amplified in 10-µL reaction volumes containing 1× reaction buffer (67 mM Tris-HCl, pH 8.3, 16 mM $(NH_4)_2SO_4$, 0.01% Tween-20, Ecostart Reaction Buffer, Ecogen), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.15 µM of each dye-labelled primer (FAM, HEX or NED) and 0.1 U of *Taq* DNA EcoStart Polymerase (Ecogen). All reactions were carried out on a Mastercycler EP gradient S (Eppendorf) thermal cycler. The polymerase chain reaction (PCR) programme used was 9 min denaturing at 95 °C followed by 30 cycles of 30 s at 94 °C, 45 s at the annealing temperature (see Table 1) and 45 s at 72 °C, ending with a 5-min final elongation stage at 72 °C. Amplification products were electrophoresed using an ABI PRISM 310 Genetic Analyser (Applied Biosystems) and genotypes were scored using GENESCAN 3.7 (Applied Biosystems). Tests for deviation from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium between markers were calculated following Weir (1996).

Estimation of heterozygosity

We used two metrics to estimate genetic diversity that may improve on simple heterozygosity: (i) internal relatedness (*IR*), that weights allele sharing by the frequency of the alleles involved (Amos *et al*. 2001); (ii) homozygosity by loci (*HL*), a new measure that weights the contribution of each locus to the homozygosity value depending on their allelic variability (Aparicio *et al*. 2006).

Statistical analyses

We analyse the relationship between both *IR* and *HL* and egg-production-related parameters using Generalized Linear Mixed Models (GLMM) implemented with the

GLIMIX macro of SAS (SAS Institute 2004). GLMMs allow analyses of data where the response variable is determined by both random and fixed effects. Clutch size (359 clutches from 237 females) was analysed using a Poisson error structure and log link, whereas egg volume (228 eggs from 49 females) was fitted using a normal distribution of errors and an identity link function. For each of these dependent variables we constructed two separate GLMMs, fitting *IR* or *HL* as explanatory variable together with nongenetic terms (laying date, pectoral thickness, age, and wing and tarsus length) that could potentially influence egg production (Perrins 1970; Kruuk *et al*. 2002; Reid *et al*. 2003). No pair of these independent variables was strongly correlated (all $r < 0.35$) so we initially included all of them in our analyses (Green 1979). Year was also included as a fixed effect into the models to account for possible differences in clutch size/egg size between breeding seasons. Also, by including the interaction between year and *HL* or *IR*, we explored whether the correlations between the studied components of fitness and heterozygosity were particularly strong in certain years, as would be expected if such association vary with ecological conditions (e.g. Keller *et al*. 2002). The identity of colony and female cohort were included as random effects to control for the possible nonindependence of clutch size/egg size within colonies and cohorts, in the manner of a randomized complete block design to avoid pseudoreplication (Krackow & Tkadlec 2001). As several females were monitored across years, we also included female identity nested within colony identity (i.e. higher-level effect; for the rationale of the model, see Singer 1998) as a random effect. Finally, we included nest identity nested within female identity as a random effect in the analysis of egg size to control for possible differences in this parameter among clutches within the same female.

To eliminate the possibility that spurious associations between fitness and multilocus heterozygosity arise from population stratification (Slate *et al*. 2004; Slate & Pemberton 2006, e.g. Bean *et al*. 2004), we used a Bayesian model-based clustering method (STRUCTURE 2.1, Pritchard *et al*. 2000) to assess evidence for genetic subdivision between and within Villacañas and Consuegra subpopulations and assign individuals to these subpopulations based on multilocus genotype data. We ran STRUCTURE assuming correlated allele frequencies and admixture, and conducted five independent runs of $K = 1–5$ to estimate the true number of clusters with 10^6 Markov chain Monte Carlo (MCMC) cycles, following a burn-in period of 100 000 iterations. Although we found no evidence for genetic subdivision (see Results section), we followed the conservative criterion of fitting subpopulation as a categorical effect into the models.

Initially, each GLMM was constructed with all explanatory terms fitted, including first-order interactions and quadratic effects to account for potential nonlinear relationships. Final models were selected following a backward procedure, by progressively eliminating nonsignificant variables ($P < 0.05$). The significance of the remaining variables was tested again until no additional variable reached significance. The result is the minimal most adequate model for explaining the variability in the response variable, where only the significant explanatory variables are retained. Hypotheses were tested using *F*-statistics and all *P* values refer to two-tailed tests.

Finally, we assessed evidence for the contribution of single locus to heterozygosity–fitness associations that might result from linkage between marker loci and genes influencing fitness. For this purpose, we re-analysed our data including into the models the heterozygosity at each marker as a binary variable and *HL* or *IR* calculated without including the locus being considered.

Results

The mean number of alleles per locus was 25.7, and ranged from 3 to 128 (Table 1). After applying sequential Bonferroni corrections to compensate for multiple statistical tests, four loci deviated from HWE (Fp13, Fp86–2, Fu2, Fn2–14). The probability of departure from HWE had no effect on the contribution of each particular locus to the correlation obtained between heterozygosity and clutch size [$F_{(1,9)} = 0.04$, $P = 0.848$; see Results below and Tables 2, 3; see Lieutenant-Gosselin & Bernatchez 2006 for a similar analysis]. There was no evidence of genotypic linkage disequilibrium at any pair of loci and subpopulation (all $P > 0.05$). The measures *HL* and *IR* were highly correlated ($P < 0.0001$; $r = 0.95$). Average female heterozygosity did not differ between Villacañas and Consuegra [ANOVA, *HL*: $F_{(1,237)} = 0.263$, $P = 0.608$; *IR*: $F_{(1,237)} = 0.362$, $P = 0.548$]. Results of our STRUCTURE analyses showed a maximum $\Pr(X \mid K)$ for $K = 1$, indicating no genetic subdivision within the study area (Fig. 1). With $K = 2$, $\Pr(X \mid K)$ was only slightly lower, but the probability of assignment of individuals to clusters was independent of the two localities studied (in all simulations with $K = 2$; $\chi^2_1 < 0.0002$; $P > 0.99$).

General heterozygosity effects on egg-production parameters

Clutch size was negatively associated with *HL* (Table 2; Fig. 2) and *IR* [$F_{(1,354)} = 9.02$, $P = 0.003$; Fig. 2], indicating that more homozygous females lay smaller clutches. As found in other bird species, clutch size increased and then declined significantly with female age (Table 2, e.g. Kruuk *et al*. 2002; Reid *et al*. 2003). Predictably, clutch size was negatively associated with laying date (Table 2; Perrins 1970). However, neither wing [$F_{(1,353)} = 0.01$, $P = 0.932$] and tarsus length [$F_{(1,353)} = 1.36$, $P = 0.245$] nor pectoral thickness

Table 2 GLMM for clutch size (Poisson error and log link function) and egg volume (normal error and identity link function) in lesser kestrels in relation to *HL* and nongenetic terms (laying date, female pectoral thickness, age, wing and tarsus length, year and subpopulation). Only variables included in the models are indicated

	Estimate	± S.E.	d.f.	*F*	*P*
(a) Clutch size					
Intercept	2.232	0.125			
HL	−0.237	0.075	1, 354	10.03	0.002
Laying date	−0.007	0.001	1, 354	70.46	<0.001
Female age	0.093	0.027	1, 354	12.26	<0.001
Female age^2	−0.011	0.004	1, 354	9.38	0.002
Covariance parameter estimates:					
	Estimate ± S.E.	*Z*	*P*		
Female identity	0	—	—		
Colony identity	0.001 ± 0.001	0.91	0.180		
Female cohort	0	—	—		
(b) Egg volume					
Intercept	10344.00	5565.00			
Laying date	−59.54	18.43	1, 225	10.44	0.001
Tarsus length	331.18	144.00	1, 225	5.29	0.022
Covariance parameter estimates:					
	Estimate ± S.E.	*Z*	*P*		
Female identity	1 206 074 ± 273 959	4.40	< 0.001		
Nest identity	0	—	—		
Colony identity	0	—	—		
Female cohort	0	—	—		

Table 3 Test for the effects of single locus heterozygosity and general (all loci, measured as *HL* and *IR* excluding the locus being considered) heterozygosity on clutch size in lesser kestrels. Table shows *P* values

	HL		*IR*	
Locus	Single locus	General	Single locus	General
Fp5	0.021	0.010	0.020	0.014
Fp13	0.442	0.002	0.470	0.004
Fp31	0.998	< 0.001	0.945	0.001
Fp46–1	0.205	0.004	0.222	0.006
Fp79–4	0.637	0.002	0.656	0.003
Fp86–2	0.416	0.002	0.409	0.005
Fp89	0.625	0.001	0.658	0.002
Fu1	0.020	0.012	0.019	0.020
Fu2	0.322	< 0.001	0.264	0.001
Fn1–11	0.133	0.017	0.122	0.020
Fn2–14	0.185	0.018	0.150	0.031

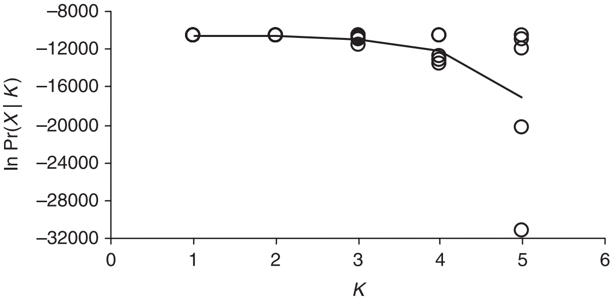

Fig. 1 Results of Bayesian clustering analysis for 237 female lesser kestrels. For each number of population clusters tested (*K*), Pr(*X* | *K*) is the probability of the data.

[$F_{(1,353)} = 1.57$, $P = 0.212$] influenced clutch size. Clutch size did not differ between subpopulations [$F_{(1,353)} = 0.73$, $P = 0.394$] and the interaction between *HL* or *IR* and subpopulation was not significant [*HL*: $F_{(1,353)} = 0.72$, $P = 0.396$; *IR*: $F_{(1,353)} = 0.82$, $P = 0.365$], indicating that the association between genetic diversity and clutch size was consistent and similar in both subpopulations. Clutch size did not differ between years [$F_{(5,349)} = 1.60$, $P = 0.158$] and the interaction between *HL* or *IR* and year was not significant [*HL*: $F_{(5,349)} = 1.55$, $P = 0.174$; *IR*: $F_{(5,349)} = 0.58$, $P = 0.715$], indicating that the correlation between heterozygosity and clutch size was consistent across the years of study. The relationship between *HL* or *IR* and clutch size was also highly significant when each metric was included alone into the models [*HL*: $F_{(1,357)} = 11.79$, $P = 0.0007$; *IR*: $F_{(1,357)} = 11.20$, $P = 0.0009$], so that the effect was not due to interactions among independent variables.

We found no significant effect of *HL* [$F_{(1,224)} = 0.10$, $P = 0.758$] or *IR* [$F_{(1,224)} = 0.04$, $P = 0.841$] on egg volume,

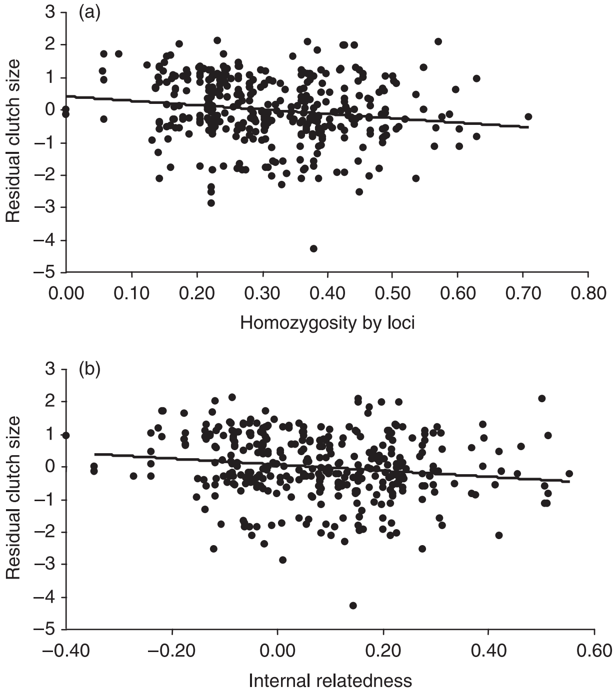

Fig. 2 Relationship between (a) homozygosity by loci and (b) internal relatedness of female lesser kestrels and clutch size. Clutch size is expressed as statistical residuals obtained after controlling for other influencing variables (see Table 2).

which was only influenced by laying date and female size estimated as tarsus length (Table 2). Egg volume did not differ between Villacañas and Consuegra subpopulation [$F_{(1,224)} = 0.62$, $P = 0.433$] and the interaction between *HL* or *IR* and subpopulation was also nonsignificant in this model [*HL*: $F_{(1,224)} = 0.32$, $P = 0.572$; *IR*: $F_{(1,224)} = 0.28$, $P = 0.756$]. Egg volume did not differ between years [$F_{(5,220)} = 0.32$, $P = 0.866$] and the interaction between *HL* or *IR* and year was not significant [*HL*: $F_{(5,220)} = 0.48$, $P = 0.795$; *IR*: $F_{(5,220)} = 0.62$, $P = 0.684$]. Female pectoral thickness [$F_{(1,224)} = 0.01$, $P = 0.905$], wing length [$F_{(1,224)} = 0.87$, $P = 0.353$] and female age [$F_{(1,224)} = 1.49$, $P = 0.224$] did not influence egg volume. We also analysed whether heterozygosity influences both laying date and tarsus length (normal distribution of errors and an identity link function in both cases) to explore possible indirect effects of genetic diversity on egg volume. After controlling for random effects (colony identity, female identity, female cohort, and year), we found no significant effect of *HL* or *IR* on laying date [*HL*: $F_{(1,357)} = 0.92$, $P = 0.337$; *IR*: $F_{(1,357)} = 0.71$, $P = 0.399$] and tarsus length [*HL*: $F_{(1,357)} = 0.43$, $P = 0.513$; *IR*: $F_{(1,357)} = 0.71$, $P = 0.399$]. Quadratic terms, with the exception of female age in clutch size model, and interactions between independent variables were not significant in any analysis ($P > 0.1$ in all cases).

Local heterozygosity effects

We explored whether a particular locus significantly influenced the effect of heterozygosity on clutch size. After removing any single locus from the calculation of *HL* and *IR*, the relationship between both measures of heterozygosity and clutch size remained always significant (Table 3). Of the 11 markers used, only two markers (Fp5 and Fu1) revealed an individual significant association with clutch size (Table 3). In both cases, females laying smaller clutches had lower heterozygosity. The subpopulation–locus interaction was not significant [Fp5: $F_{(1,348)} = 0.28$, $P = 0.598$; Fu1: $F_{(1,348)} = 2.42$, $P = 0.121$]. However, after applying Bonferroni for multiple tests no single locus effect remained significant. In any case, given that these two loci appeared to contribute disproportionately towards the observed effect, we ran the model again after removing them from the calculation of the heterozygosity index. The *P* values obtained for *HL* and *IR* were still lower than 0.1 [*HL*: $F_{(1,354)} = 3.64$, $P = 0.057$; *IR*: $F_{(1,354)} = 3.38$, $P = 0.067$]. The exclusion of these two loci has reduced our microsatellite panel by 18%. Thus, the lower significance of this analysis could be due to a reduction in the panel of markers rather than strictly to local effects, because the expected correlation between genome-wide heterozygosity and multilocus heterozygosity is highly dependent on the number of markers employed (Balloux *et al*. 2004; Aparicio *et al*. 2007).

Discussion

Here, we show that egg production, measured as clutch size, correlates with female heterozygosity, an effect that was statistically independent of other effects (age and laying date), which have been previously found to affect different fecundity-related parameters. After multiple test correction, two loci (Fp5 and Fu1) contributed marginally towards the observed effect. Thus, these two typed loci may be in linkage disequilibrium with coding genes that experience some form of balancing selection and affect egg production. However, the association between clutch size and multilocus heterozygosity was not fully explained by these two loci, suggesting that genome-wide heterozygosity may be involved and local effects are not the only underlying mechanism (e.g. Hoffman *et al*. 2004; Acevedo-Whitehouse *et al*. 2005; Da Silva *et al*. 2006). In our study population, some factors could have generated enough inbreeding variance which may be reflected by heterozygosity measured at neutral markers (Balloux *et al*. 2004; Slate *et al*. 2004). Lesser kestrels form relatively small colonies (generally less than 20 pairs) and the species is highly philopatric, with most surviving adults returning to the same colony and young generally settling in their natal colony or close to it (Serrano & Tella 2003; J. M. Aparicio, unpublished data). This philopatric behaviour together with the apparently lack of active inbreeding avoidance reported in some studies on the species ecology (Negro *et al*. 1997; Serrano *et al*. 2003) may lead to crosses between close relatives. Thus, although monogamy is the preponderant mating system in lesser kestrels (Negro *et al*. 1996) and, hence, inbreeding events are not likely to be exceptionally severe or frequent in this species (see Balloux *et al*. 2004), the study population would present certain variance in inbreeding coefficients. On the other hand, although colonies are arranged in discrete geographical areas forming subpopulations with low exchange of individuals, long-distance disperses arrive occasionally to the local colonies from several kilometres away (up to 300 km, J. M. Aparicio, unpublished data; see also Serrano *et al*. 2003) what could result in crosses between individuals from genetically diverse origins, promoting certain degree of genetic variation in our population (*HL* ranged from 0 to 0.71) and thus the possibility of detecting genetic-diversity fitness correlations (Da Silva *et al*. 2006).

The genetics of the fitness trait being considered is likely to have an important influence on the detection of heterozygosity–fitness correlations. In birds, as well as in other organism groups, egg production is known to be a result of many genes acting on several biochemical processes, which in turn control a range of anatomical and physiological traits (Crawford 1990; Jones 2004). This polygenic nature of egg production increases the chance that one or some neutral typed loci are in close linkage with genes influencing this trait (i.e. local effect), but also enhances the expected correlation between multilocus heterozygosity measured at neutral markers and the heterozygosity of the pool of genes controlling this fitness component under identity disequilibrium (i.e. general effect). This last expectation should occur because random effects due to mendelian segregation do not depend on the number of typed marker loci only, but also on the number of coding loci effectively involved in the correlation (Aparicio *et al*. 2007). Thus, the fact that egg production was a polygenic trait could have favoured detecting the observed association between heterozygosity and clutch size.

Most evidence from detrimental effects of reduced genetic diversity on different egg production traits come from pedigree-based breeding experiments in poultry research (Crawford 1990; but see also Haikola 2003; Perrot-Minnot *et al*. 2004; for other organism groups). However, laboratory, captive or domestic populations and wild populations are usually very different in terms of environmental constraints and inbreeding histories. To our knowledge, only one previous study has found a positive effect of female multilocus heterozygosity on clutch size in a wild population (Foerster *et al*. 2003). Thus, our results suggest that egg-production traits might also be sensitive to reduced genetic diversity in wild populations. Field studies based on parental relatedness, estimated either from pedigree data (Greenwood *et al*. 1978; Rowley *et al*. 1986; Gibbs & Grant 1989; Kruuk *et al*. 2002) or using genotypic information (Bensch *et al*. 1994; Kempenaers *et al*. 1996; Van de Casteele *et al*. 2003; Hansson 2004), failed to find any effect of genetic similarity between mates on clutch size. However, these negative results could be expected even if maternal genotype (i.e. the inbreeding status/genetic diversity of breeding females) determines clutch size regardless of paternal genotype (e.g. Kempenaers *et al*. 1996; Haikola 2003; Cordero *et al*. 2004). Given that larger clutches generally result in higher lifetime parental fitness (Charmantier *et al*. 2006), the relationship found here may provide additional empirical evidence supporting the link between inbreeding and lower productivity in small endangered populations and species inhabiting insular environments (e.g. Thibault *et al*. 1992; Westemeier *et al*. 1998; Mackintosh & Briskie 2005) which generally show reduced genetic diversity in comparison with continuous or large and open populations (e.g. Aguilar *et al*. 2004). The negative effect of reduced genetic diversity on fecundity contributes to explain how inbreeding may facilitate extinction in small populations, because lower productivity in genetically depauperated populations would lead a subsequent population size reduction and deplete genetic variability even more, following a positive feedback loop towards the complete population extinction. In our study system, the effects of individual genetic diversity on clutch size may be particularly severe as lesser kestrels suffered a recent population bottleneck in their Iberian distribution range (Bustamante 1997). A pattern like this would be expected in any other populations that have suffered a similar reduction in size, suggesting that the maintenance of genetic diversity may be critical to allow the persistence of small and endangered populations.

The observed association between heterozygosity and clutch size may also have important evolutionary implications and give insight to explain the maintenance of genetic variability for clutch size. Many long-term studies found that clutch size presents relatively high additive genetic variance and so high heritability (review in Boag & van Noordwijk 1987) in populations where this trait is apparently under strong and positive directional selection (e.g. Boyce & Perrins 1987). It is puzzling that both high heritability values and high selection differentials can co-exist in the same population (e.g. Charmantier *et al*. 2006). Price & Liou (1989) suggested that the persistence of a selection differential could be due to individual differences in female nutritional state, which would cause positive phenotypic correlations among life-history traits such as clutch size and number of surviving young. Nevertheless, the maintenance of genetic variance requires a high mutation rate. Alternatively, one possible mechanism to explain this phenomenon is that some coding loci influencing egg production exhibit overdominance. Under the overdominance hypothesis, there will be a stable equilibrium that allows the constancy of genotypic values and the maintenance of genetic polymorphism (e.g. Kojima 1959). The positive correlation between heterozygosity and egg production would reveal the existence of coding loci operating under overdominance. Furthermore, given that our results suggest that the observed association reflects a genome-wide effect rather than a local effect, fecundity-mediated selection against more homozygous individuals might also be an important mechanism contributing to maintain the high levels of overall genetic variability generally observed in natural populations. Thus, heterozygote advantage for fecundity-related traits would contribute to maintaining not only polymorphism in genes tightly involved in egg production, but also across the whole genome.

Finally, we found no association between female heterozygosity and egg volume. This is in accordance with some pedigree-based breeding experiments which suggest that egg size shows low heterosis/inbreeding depression (Crawford 1990; Gavora *et al*. 1996). Egg volume was associated with female size and laying date, but heterozygosity did not affect any of these parameters, suggesting that indirect heterozygosity-mediated effects do not influence this trait in lesser kestrels. This and previous studies have found that egg size correlates with female size and shows scarce flexibility within individuals (Christians 2002), suggesting that egg volume may be a morphological related trait. De Rose & Roff (1999) demonstrated that inbreeding depression is lower in morphological traits in comparison with life-history traits more closely linked to fitness, which could explain why egg size is scarcely affected by individual heterozygosity. In any case, this does not rule out the possibility that more heterozygous mothers can invest more in their eggs in other ways not analysed here such as egg composition that might affect components of offspring fitness (Schwabl 1993; Williams 1994).

Several microsatellite-derived measurements have been proposed to estimate genetic diversity, but which is the most effective method for quantifying such variation is not clear (Tsitrone *et al*. 2001; Slate & Pemberton 2002; Aparicio *et al*. 2006). A recent evaluation of *IR* index suggest that this estimate of genetic diversity may suffer certain problems in open populations because it underestimates heterozygosity of individuals carrying rare alleles, such that descendants of immigrants paired with natives bearing novel or rare alleles are considered more homozygous than descendants of native parents (Aparicio *et al*. 2006). *HL* could be an alternative as it avoids such problems giving more weight to more informative loci in relation with their allelic variability instead of weighting by allele frequencies. Our data seems to support this last prediction, since *HL* correlated with clutch size slightly better than *IR* did. Arrival of individuals carrying novel alleles has not been assessed in our study population, but the detection of occasional long-distance dispersers in this species suggest that alleles of lower frequencies carried by offspring from matings between locals and immigrants could determine the relative efficiency of both indexes in the study population.

In conclusion, we found that female genetic characteristics affect significantly egg production in a wild bird population, extending the range of fitness-related traits correlated with multilocus heterozygosity at neutral markers in open populations. The relative importance of genome-wide vs. local effects on heterozygosity–fitness correlations needs further research, although our results suggest a main general effect influencing the association we found.

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J.O. and G.C. are studying genetic aspects of mating and dispersal and the importance of genetic variation on different components of fitness as PhD students at the University of Castilla-La Mancha. Now, J.M.A. is also interested in the relationship between heterozygosity and fitness in natural populations. P.J.C. is interested in the role of environment and genetic variation on evolution.
