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Temporal variation of heterozygosity-based assortative mating and related benefits in a lesser kestrel population

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Abstract

Heterozygosity as a target of mate choice has received much attention in recent years and there is growing evidence supporting its role in the evolution of mate preferences. In this study we analyse mating patterns in relation to heterozygosity in a lesser kestrel (*Falco naumanni*) population intensively monitored over six study years (2002–2007). The magnitude of heterozygosity-based assortative mating varied over time, being particularly patent in the last study years (2006, 2007). We have found evidence that this mating pattern entails both direct and indirect-genetic benefits. Clutch size increased with female heterozygosity and more heterozygous males raised a higher number of fledglings particularly in those years when the strength of the heterozygosity-based assortative mating was markedly higher. In the last study year, parent–offspring correlation of heterozygosity was stronger and higher than the expected if individuals would have randomly mated with respect to heterozygosity. Overall, our results offer empirical support to the heterozygous mate hypothesis of sexual selection but suggest that genetic diversity may act as a temporally variable target for mate choice.

Introduction

Understanding mate choice and identifying their underlying mechanisms remains as a key question in evolutionary biology. Mate choice is likely to have evolved through direct and indirect-genetic benefits. Although mating preferences for direct fitness benefits, such as parental care or food provisioning, are relatively easy to understand, explaining mate choice evolution for indirect genetic benefits is much more complicated (Tregenza & Wedell, 2000; Mays & Hill, 2004; Neff & Pitcher, 2008). Mate choice for additive genetic benefits, i.e. the direct inheritance of beneficial alleles by offspring, is likely to result in strong directional selection for individuals carrying the 'good alleles' (Mays & Hill, 2004). This should rapidly deplete the additive genetic variance of the selected trait and eliminate the benefit of the choice, i.e. the 'lek paradox' (Kotiaho *et al.*, 2008). By contrast, sexual selection for non-additive genetic traits such as genetic complementarity and heterozygosity is not expected to deplete the genetic variance and could offer an alternative scenario to resolve the 'lek paradox' (Irwin & Taylor, 2000; Fromhage *et al.*, 2009; Neff & Pitcher, 2009). Particularly, heterozygosity as a target of mate choice has received much attention in recent years, and there is growing theoretical and empirical evidence supporting its potential role in the evolution of mate preferences (e.g. Irwin & Taylor, 2000; Lehmann *et al.*, 2007; Neff & Pitcher, 2009; Fromhage *et al.*, 2009; García-Navas *et al.*, 2009).

Preferences for heterozygous mates can offer both direct and indirect-genetic benefits to the choosier individual (Kempenaers, 2007; e.g. García-Navas *et al.*, 2009). Several studies have found that heterozygosity is linked with individual quality and this can ultimately also increase mate fitness if more heterozygous individuals provide direct benefits like higher parental care and/or fecundity (Seddon *et al.*, 2004; García-Navas *et al.*, 2009). On the other hand, there is growing evidence that under several circumstances heterozygosity is 'heritable' or,

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more properly, that parent–offspring heterozygosities correlate (Mitton *et al.*, 1993; Hoffman *et al.*, 2007; Neff & Pitcher, 2009; Fromhage *et al.*, 2009; García-Navas *et al.*, 2009). However, with the exception of some studies based on the major histocompatibility complex (MHC), there is limited empirical evidence on the function of heterozygosity as a target for mate choice (Kempenaers, 2007). Moreover, the scarce studies on the topic are based on one or a small number of breeding seasons which can result in a biased view on the topic if only positive results are published and/or if heterozygosity operates as a temporally variable target for mate choice (e.g. Kleven & Lifjeld, 2005; García-Navas *et al.*, 2009). The latter may be expected because the magnitude of the heterozygosity-based mate preferences should become stronger when the direct and/or indirect benefits associated with choosing a heterozygous mate increase. In fact, the negative fitness consequences of inbreeding/reduced heterozygosity are not necessarily constant across years and can vary with environmental conditions (Keller, 1998; Coltman *et al.*, 1999; Keller *et al.*, 2002; Keller & Waller, 2002). Thus, temporal changes in the patterns and targets of mate preferences with respect to heterozygosity may be common and this could also explain the coexistence of different mating strategies within a population (Oh & Badyaev, 2006; see also Fromhage *et al.*, 2009).

Here, we analyse the role of heterozygosity on mating patterns in the lesser kestrel (*Falco naumanni*), a socially monogamous colonial bird of prey. In contrast to previous studies on the topic based on a single or two study years (e.g. Kleven & Lifjeld, 2005; Bonneaud *et al.*, 2006; García-Navas *et al.*, 2009), we analyse mating patterns with respect to heterozygosity over six consecutive breeding seasons. This allows us to study temporal variability on heterozygosity-based mating patterns which may have gone unresolved by short-term studies. In particular, we first analyse the patterns of heterozygosity-based assortative mating during six consecutive breeding seasons. Second, we study the possible benefits associated with this mating pattern considering both the direct benefits derived from the association between heterozygosity and mate quality and the expected indirect genetic benefits resulted from parent–offspring resemblance with respect to heterozygosity. We have found that the occurrence and strength of heterozygosity-based assortative mating varies over time in the study population of lesser kestrels and that the magnitude of such mating pattern correlates with the strength of both the direct and indirect benefits associated with obtaining highly heterozygous mates.

Material 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 (see Ortego *et al.*, 2007a for a detailed description). In our study area, lesser kestrels form colonies located in abandoned farm houses where they nest under tiled roofs and inside holes in walls. The studied colonies are clustered in two subpopulations separated by 30 km: 'Villacañas' subpopulation (39°30′N, 3°20′W; 24 colonies) and 'Consuegra' subpopulation (39°35′N, 3°40′W; 6 colonies) without genetic structure (Ortego *et al.*, 2007a,b). Colony size in the study population ranged between one and 60 breeding pairs (mean ± SE for each study year: 2002: 12.9 ± 18.6; 2003: 9.6 ± 3.2; 2004: 13.6 ± 11.8; 2005: 17.9 ± 14.1; 2006: 15.1 ± 13.9; 2007: 17.0 ± 12.1). Five colonies were monitored all study years (2002–2007 breeding seasons). Other small size colonies were only monitored some study years (5 years = three colonies; 4 years = five colonies; 3 years = three colonies; 2 years = seven colonies; 1 year = seven colonies) generally because they were founded, disappeared and/or re-colonized over the study period.

Kestrels normally arrive in the study area from their winter quarters in Africa in mid-February or the beginning of March, depending on the year. Lesser kestrels are mainly monogamous and extra-pair fertilizations are very rare in our population (0.7% based on 712 typed offspring; Ortego *et al.*, 2008). Egg laying lasts from the end of April to the first week of June (Aparicio & Bonal, 2002). Females lay a single clutch per year (range 1–6 eggs; more frequently 3–5 eggs) with rare replacement clutches (ca. 0.5%). This species shows a high philopatric behaviour and most adult individuals (83%; $n = 235$) return to their previous breeding colony every year. By contrast, mate fidelity is relatively low (22%; $n = 63$). During the study period (2002–2007 breeding seasons) we have monitored 429 breeding attempts. We have captured a total of 619 individuals of which 114 were re-captured in two or more subsequent breeding seasons. Adult lesser kestrels were trapped with a noose carpet or by hand during incubation, measured and individually marked with metallic and coloured plastic rings for further identification. Blood samples (100 μl) were obtained by puncture of the brachial vein and preserved in ~1200 μL ethanol 96% at −20 °C. To estimate individual size we measured tarsus length and wing length using a caliper and a ruler to the nearest 0.01 mm and 1 mm, respectively. We used pectoral thickness as an estimator of body condition (Aparicio, 1997; Aparicio & Cordero, 2001). This trait has been also 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 using a portable ultrasonic meter, in our case a Krautkrämer USM22F (accuracy 0.1 mm), especially designed to measure animal tissues. We knew the exact age of

approximately one-third of individuals that were ringed as fledglings. For all other birds, 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; Ortego *et al.*, 2007a).

Genotyping and genetic diversity estimates

We genotyped 619 adult lesser kestrels across 12 highly polymorphic microsatellite markers: Fp5, Fp13, Fp31, Fp46-1, Fp79-4, Fp86-2, Fp89 (Nesje *et al.*, 2000), Fu1, Fu2 (J. H. Wetton, unpublished data), Age5 (Topinka & May, 2005), Fn1-11, and Fn2-14 (Ortego *et al.*, 2007c; see Ortego *et al.*, 2007a for microsatellite details). All individuals were genotyped at all these 12 microsatellite markers. We used QIAamp DNA Blood Mini Kits (Qiagen, Hilden, Germany) to extract and purify genomic DNA from the blood samples. Approximately 5 ng of template DNA was amplified in 10-μL reaction volumes containing 1X reaction buffer (67 mM Tris–HCl, pH 8.3, 16 mM $(NH_4)_2SO_4$, 0.01% Tween-20, EcoStart Reaction Buffer; Ecogen, Madrid, Spain), 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 EpgradientS (Eppendorf, Hamburg, Germany) thermal cycler. The 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 (Ortego *et al.*, 2007c) and 45 s at 72 °C, ending with a 5 min final elongation stage at 72 °C. Amplification products were electrophoresed using an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and genotypes were scored using GeneMapper 3.7 (Applied Biosystems). We used two metrics to estimate individual genetic diversity: (i) uncorrected heterozygosity (H_O), calculated as the proportion of loci at which an individual is heterozygous; (ii) homozygosity by loci (*HL*), a microsatellite derived measure that improves heterozygosity estimates in open populations by weighting the contribution of each locus to the homozygosity value depending on their allelic variability (Aparicio *et al.*, 2006). *HL* is calculated as follows: $HL = (\Sigma E_h)/(\Sigma E_h + \Sigma E_j)$, where E_h and E_j are the expected heterozygosities of the loci that an individual bears in homozygosis (h) and in heterozygosis (j), respectively (Aparicio *et al.*, 2006). H_O and *HL* were calculated using CERNICALIN, an excel spreadsheet available on request (Aparicio *et al.*, 2006).

Heterozygosity-based assortative mating pattern

For each study year (2002–2007 breeding seasons), we compared heterozygosity between social mates using two-tailed Pearson's correlation analyses. We used a linear regression to analyse whether the strength of the association between mate heterozygosities (estimated using correlation coefficients, r) has changed over the 6-year study period. Correlations coefficients (r) obtained for each breeding season were analysed as the dependent variable whereas year was included as a continuous explanatory variable (covariate). The precision of the obtained correlation coefficients could be different because sample sizes (i.e. the number of analysed mating pairs) used for their estimation varied between years. So, we used sample size to give observations different weights in a weighted least-squares analysis. For each study year, we also explored the possibility of assortative mating with respect to other variables which may affect mating decisions in lesser kestrels. In particular, we compared age, size (tarsus and wing length) and pectoral thickness between social mates. Finally, we used linear regressions to analyse the association between these variables and heterozygosity in SPSS 7.5.

Heterozygosity and mate quality: direct benefits

In each study year, we analysed the effects of individual genetic diversity on different parameters which may ultimately have important consequences on mate reproductive performance. We analysed the effects of male and female heterozygosity on clutch size and number of fledged young. For this purpose we used multiple linear regression analyses in SPSS 7.5, including in all analyses laying date as additional covariate to control for the expected decline of breeding performance as breeding season advances (Perrins, 1970).

Parent–offspring correlations in heterozygosity: indirect benefits

We analysed for each study year the association between expected offspring heterozygosity and both maternal and paternal heterozygosities. For this purpose, we used parental genotype data to calculate the expected offspring heterozygosity using an EXCEL spreadsheet. For each breeding season, we analysed the association between estimated average offspring heterozygosity and both maternal and paternal heterozygosity using multiple linear regression analyses in SPSS 7.5. We also used the observed genotypes to calculate the heritability of heterozygosity under a hypothetical random mating in our population. For this purpose we designed an EXCEL macro to perform Monte Carlo simulations and generate the distribution of expected correlations between parent–offspring heterozygosities in our data set assuming that mating is random with respect to heterozygosity. We performed 1000 iterations simulating a number of mating pairs equal to that available for each study year to create a distribution of expected correlations coefficients (r) between parent–offspring heterozygosities. Then, we compared this distribution with the actual values observed in each study year.

Results

Heterozygosity-based assortative mating pattern

The measures H_O and *HL* were highly correlated ($r = -0.974$, $P < 0.001$) and for this reason statistical analyses are only presented for H_O analyses. *HL* data provided analogous results and are available from the authors. Heterozygosity was positively correlated between social mates during 2007 ($r = 0.335$, $P = 0.006$, $n = 67$) and 2006 ($r = 0.181$, $P = 0.041$, $n = 94$) breeding seasons, but no significant correlation was found in the previous years (2005: $r = 0.101$, $P = 0.323$, $n = 97$; 2004: $r = -0.031$, $P = 0.738$, $n = 118$; 2003: $r = -0.129$, $P = 0.466$, $n = 34$; 2002: $r = 0.040$, $P = 0.869$, $n = 19$). The strength of the correlation (estimated by the correlation coefficients, r) between mate heterozygosities has increased over the 6 years of study (WLS linear regression analysis; $r = 0.913$, $P = 0.011$, $n = 6$). We analysed assortative mating within colonies only considering those colonies with five or more genotyped pairs. No correlation was significant in any colony and year (all $P > 0.1$), probably because sample sizes (mean ± SE = 10.52 ± 5.16 pairs per colony; range = 5–21 pairs per colony) are relatively small when each colony is analysed separately. However, we found that correlation coefficients were significantly different from zero in 2006 (student t-test: $t = 4.703$, $P = 0.009$, $n = 5$ colonies) and 2007 (student t-test: $t = 2.906$, $P = 0.034$, $n = 6$ colonies) but not in 2005 ($t = 0.224$, $P = 0.831$, $n = 6$ colonies) and 2004 ($t = -0.031$, $P = 0.976$, $n = 10$ colonies) years. The number of colonies with five or more analysed pairs in previous years was too small as to be adequately analysed (2003: $n = 2$; 2002: $n = 2$). A general linear model weighted for the number of analysed individuals within each colony and controlling for colony identity (included as random effect; $F_{1,\ 29} = 1.41$, $P = 0.248$) showed that correlations coefficients of intra-pair male and female heterozygosities correlations have progressively increased over the study years (year included as covariate; $F_{1,\ 29} = 11.18$, $P = 0.003$). However, we found no significant interaction between colony identity and study year, indicating that the observed temporal pattern was similar among the studied colonies ($F_{10,\ 19} = 1.41$, $P = 0.249$). We explored the possibility that the observed correlation of intra-pair male and female heterozygosities is a consequence of differences in genetic diversity between the study colonies which may have resulted in a passive heterozygosity-assortative mating independent of individual preferences (*sensu* Ferrer & Penteriani, 2003; see García-Navas *et al.*, 2009). We found that individual genetic diversity did not differ between colonies in any study year (one-way ANOVAS, 2007: $F_{4,\ 107} = 0.98$; $P = 0.422$; 2006: $F_{6,\ 149} = 1.07$; $P = 0.384$; 2005: $F_{1,\ 172} = 0.62$; $P = 0.813$; 2004: $F_{12,\ 209} = 0.62$; $P = 0.820$; 2003: $F_{6,\ 56} = 1.72$; $P = 0.134$; 2002: $F_{3,\ 27} = 0.847$; $P = 0.480$). The age structure neither differed among the studied colonies in any year ($P > 0.1$ in all cases). Pair-wise F_{ST} values calculated using the program FSTAT 2.9.3.2 (Goudet, 2001) showed a complete absence of genetic differentiation among the studied colonies in all study years (all $P > 0.1$ in all cases).

Size (tarsus and wing length) and pectoral thickness were not correlated between social mates in any study year (all $P > 0.1$). However, individuals showed an age-based assortative mating in some study years (2007: $r = 0.309$, $n = 67$, $P = 0.011$; 2006: $r = 0.131$, $n = 94$, $P = 0.207$; 2005: $r = 0.361$, $n = 97$, $P < 0.001$; 2004: $r = 0.367$, $n = 118$, $P < 0.001$; 2003: $r = 0.249$, $n = 34$, $P = 0.149$; 2002: $r = -0.119$, $n = 19$, $P = 0.616$). Linear regression analyses revealed that age, individual size and pectoral thickness were not associated with heterozygosity in any year and sex (all $P > 0.1$).

Heterozygosity and mate quality: direct benefits

Clutch size and number of fledged chicks did not differ among the studied colonies in any year (all $P > 0.1$). When each year was analysed separately, clutch size was positively associated with female heterozygosity during the 2007 breeding season but not in the previous years. The standardized coefficients (β) for the linear regressions between clutch size and female heterozygosity showed positive values across the six study breeding seasons (sign test, $P = 0.031$, $n = 6$ years; Table 1). The standardized coefficients (β) for the linear regressions between clutch size and female heterozygosity tended to be positively associated with the correlations coefficients (r) of intra-pair heterozygosity correlations in each year (WLS linear regression analysis; $r = 0.750$, $P = 0.086$, $n = 6$ years). However, clutch size was not associated with male heterozygosity in any breeding season (Table 1) and the obtained standardized coefficients (β) did not show positive values across all the study years (sign test, $P = 0.688$, $n = 6$ years; Table 1). In males, standardized coefficients (β) were neither associated with the correlations coefficients (r) of intra-pair heterozygosity correlations in each year (WLS linear regression analysis; $r = 0.551$, $P = 0.258$, $n = 6$ years).

The number of fledged chicks was positively associated with male heterozygosity in 2006 and 2007 breeding seasons, but we found no significant effect in previous years (Table 1). The standardized coefficients (β) did not show positive values across all the study years (sign test, $P = 0.688$, $n = 6$ years; Table 1). Further, the standardized coefficients (β) were positively associated with the correlations coefficients (r) of intra-pair heterozygosity correlations in each year (WLS linear regression analysis; $r = 0.863$, $P = 0.027$, $n = 6$ years).

Female heterozygosity was not associated with the number of fledglings in any breeding season (Table 1) and the obtained standardized coefficients (β) did not show positive values across all the study years (sign test, $P = 1.00$, $n = 6$ years; Table 1). Standardized coefficients

Table 1 Correlations between heterozygosity and clutch size/number of fledglings for each study year.

	Clutch size							Number of fledglings						
	Males			Females				Males			Females			
Year	β	t	P	β	t	P	n	β	t	P	β	t	P	n
2002	0.11	0.50	0.625	0.04	0.18	0.858	17	0.20	0.87	0.399	−0.20	−0.76	0.460	19
2003	−0.09	−0.47	0.640	0.15	0.86	0.399	31	−0.11	−0.55	0.585	0.09	0.45	0.658	26
2004	−0.01	1.14	0.888	0.14	1.64	0.103	114	−0.03	−0.26	0.797	−0.17	−1.49	0.141	66
2005	0.05	0.46	0.647	0.09	0.90	0.368	88	0.12	0.98	0.330	0.02	0.15	0.880	65
2006	0.05	1.07	0.285	0.15	1.48	0.142	85	0.40	3.50	0.001*	0.12	0.98	0.332	56
2007	0.02	0.17	0.865	0.63	2.37	0.021*	56	0.31	2.17	0.037†	−0.10	0.66	0.516	36

Table shows standardized coefficients (β), statistics (t-student), P-values and sample size (n). Significant after sequential Bonferroni corrections: †$P < 0.1$, *$P < 0.05$.

(β) were not associated with the correlations coefficients (r) of intra-pair heterozygosity correlations in each year (WLS linear regression analysis; $r = 0.212$, $P = 0.687$, $n = 6$ years).

The strength of the correlations between heterozygosity and clutch size (WLS linear regression analyses; males: $r = 0.330$, $P = 0.523$, $n = 6$ years; females: $r = 0.672$, $P = 0.143$, $n = 6$ years) and number of fledglings (WLS linear regression analyses; males: $r = 0.672$, $P = 0.143$, $n = 6$ years; females: $r = 0.332$, $P = 0.520$, $n = 6$ years) was not associated with study year in any sex.

Parent–offspring correlations in heterozygosity: indirect benefits

Offspring heterozygosity was positively associated with maternal and paternal heterozygosity in 2007 breeding season but not in the previous years (Table 2). The obtained standardized coefficients (β) for the linear regressions between offspring heterozygosity and parental heterozygosities showed positive values across the six study breeding seasons (sign tests, mother: $P = 0.031$, $n = 6$ years; father: $P = 0.031$, $n = 6$ years; Table 2). Monte Carlo simulations revealed that the observed parent–offspring correlations in heterozygosity were significantly higher than the expected under a hypothetical random mating in 2007 breeding season, but not in the previous years (Table 3). By contrast, in 2003 breeding season the correlation coefficient actually observed between father–offspring correlation in heterozygosity were even significantly lower than those expected under random mating (Table 3). For each sex, we calculated the difference of 'heritability' of heterozygosity between random mating and the 'heritability' actually observed in the population for each study year using the formula: $(r_{observed})/(r_{random})$, where r is the correlation coefficient obtained in each case. In males, the difference between observed and expected heritability tended to be positively associated with the correlation coefficients (r) of intra-pair heterozygosity correlations for each year (WLS linear regression analysis; $r = 0.786$, $P = 0.064$, $n = 6$ years). In females, the difference between observed and expected heritability was positively associated with the correlation coefficients (r) of intra-pair heterozygosity correlations for each year (WLS linear regression analysis; $r = 0.846$, $P = 0.034$, $n = 6$ years). When we averaged values for males and females in each year, the association with the correlation coefficients (r) of intra-pair heterozygosity correlations for each year was also significant (WLS linear regression analysis; $r = 0.866$, $P = 0.026$, $n = 6$ years). Thus, the

Table 2 Parent–offspring correlations of heterozygosity for each study year.

	Father			Mother			Model	
Year	β	t	P	β	t	P	r	n
2002	0.028	0.13	0.900	0.447	2.06	0.055	0.451	19
2003	0.076	0.43	0.668	0.135	0.77	0.447	0.150	34
2004	0.179	1.96	0.052	0.123	1.35	0.181	0.214	118
2005	0.123	1.23	0.223	0.191	1.90	0.061	0.234	97
2006	0.168	1.63	0.106	0.151	1.46	0.147	0.242	94
2007	0.365	3.31	0.002*	0.314	2.85	0.006*	0.556	67

Table shows standardized coefficients (β), statistics (t-student), P-values, model correlation coefficients (r) and sample size (n). Significant after sequential Bonferroni corrections: *$P < 0.05$.

Table 3 Parent–offspring correlations in heterozygosity observed in our population (*r* observed) and under random mating (*r* random) with respect to heterozygosity (obtained performing 1000 iterations).

Year	*n* pairs	*r* observed	*r* random (mean ± S.E.)	*P* observed-random
Father				
2002	19	0.066	0.298 ± 0.185	0.108
2003	34	0.066	0.212 ± 0.137	0.017*
2004	118	0.175	0.224 ± 0.076	0.246
2005	97	0.136	0.241 ± 0.087	0.115
006	94	0.191	0.224 ± 0.087	0.338
2007	67	0.470	0.268 ± 0.102	0.019*
Mother				
2002	19	0.450	0.322 ± 0.189	0.206
2003	34	0.130	0.160 ± 0.147	0.284
2004	118	0.117	0.152 ± 0.082	0.086
2005	97	0.199	0.161 ± 0.092	0.346
2006	94	0.177	0.169 ± 0.090	0.459
2007	67	0.436	0.185 ± 0.105	0.005*

Significant after sequential Bonferroni corrections: $*P < 0.05$.

heritability of heterozygosity has progressively increased more than expected under random mating with the strength of the correlation of within pair male and female heterozygosities. However, the difference between observed and expected heritability was not associated with study year in males ($r = 0.803$, $P = 0.054$, $n = 6$ years), females ($r = 0.669$, $P = 0.146$, $n = 6$ years) or when averaged values for males and females were considered ($r = 0.772$, $P = 0.072$, $n = 6$ years).

Discussion

We have found that the magnitude of heterozygosity-based assortative mating has progressively changed over a 6-year study period in our population of lesser kestrels. This mating pattern was stronger in the last two study years, being particularly patent in the 2007 breeding season. These findings together with a previous MHC- and recent microsatellite-based studies add empirical evidence for heterozygosity-based assortative mating (Bonneaud *et al.*, 2006; García-Navas *et al.*, 2009). With the exception of age, other variables such as individual size or physical condition did not correlate between social mates. None of these variables were associated with individual genetic diversity in any study year, suggesting that the observed heterozygosity-based assortative mating is not mediated by a third association between heterozygosity and any of these parameters potentially related with individual quality (García-Navas *et al.*, 2009). Thus, individuals could be able of assessing heterozygosity directly or other unmeasured phenotypic traits (e.g. secondary sexual characters) may be reflecting individual genetic diversity as has been previously reported in other bird species (e.g. Aparicio *et al.*, 2001; Foerster *et al.*, 2003; García-Navas *et al.*, 2009; see also below).

Individuals can obtain direct benefits from mating with more heterozygous individuals if genetic diversity is associated with mate quality and this ultimately results in increased own fitness (Brown, 1997; Kempenaers, 2007; García-Navas *et al.*, 2009). Accordingly, we have found a positive association between female heterozygosity and clutch size over the 6-year study period, an effect which was stronger in the last study year (see also Ortego *et al.*, 2007a). Particularly in the last study years (2006, 2007), females also obtained important benefits by preferring heterozygous males as social partners because genetically more diverse males raised a higher number of fledglings in those years. However, we found no effect of female heterozygosity on the number of fledged chicks, probably due to the lower parental investment of females during the chick rearing period (G. Calabuig, unpublished data; e.g. García-Navas *et al.*, 2009). Overall, the strength of the heterozygosity-based assortative mating observed tended to increase with the strength of the correlations between heterozygosity and the studied fitness related traits. Thus, both males and females seemed to mate with more heterozygous individuals in the last study years just when the direct benefits related with such choice were particularly high. Another possibility to explain the observed pattern is that the joint action of a relatively heterozygous pair have resulted in a breeding performance higher than that obtained if a parent alone is highly heterozygous. Thus, when males and females prefer more heterozygous individuals, the relationship between heterozygosity and variables related to breeding performance would be particularly high.

The influence of heterozygosity on mating preferences can be also mediated by the 'heritability' of heterozygosity, i.e. when heterozygous parents produce a greater proportion of heterozygous offspring than do homozygous parents (Mitton, 1993; see also Reid *et al.*, 2006; Roberts *et al.*, 2006). In lesser kestrels increased genetic diversity in offspring will have important consequences on different components of progeny fitness such as increased parasite resistance (Ortego *et al.*, 2007d) and future reproduction (Ortego *et al.*, 2007a; present study). Parent–offspring correlation in heterozygosity was particularly high in 2007 breeding season, just the year when the heterozygosity-based assortative mating was strongest. In that breeding season the 'heritability' of heterozygosity was much higher than the expected if individuals would have mated randomly with respect to heterozygosity and the expected fitness differences associated with offspring heterozygosity increased with the strength of the heterozygosity-based assortative mating over the 6-year study period. Thus, individuals obtained important indirect genetic benefits by mating with genetically diverse individuals particularly when the heterozygosity-based assortative mating was stronger.

To the best of our knowledge, this is the first empirical study showing that parent–offspring resemblance in heterozygosity varies across years depending on the magnitude of a heterozygosity-based mating pattern (see also Reid *et al.*, 2006). Our results also offer empirical support to previous theoretical studies analysing the factors influencing parent–offspring correlations in heterozygosity and open an interesting question to be addressed in the future (Fromhage *et al.*, 2009; Neff & Pitcher, 2009). This has important evolutionary implications as temporal dynamics of the strength of parent–offspring correlations in heterozygosity could alter heritability estimates for additive genetic traits and/or overestimate the magnitude of the effects of reduced heterozygosity if part of those negative effects can be attributed to reduced genetic diversity of parents (Reid *et al.*, 2006; Szulkin & Sheldon, 2006).

Overall, we have found that heterozygosity seems to act as a temporally variable target for mate choice in the study population of lesser kestrels, suggesting that this species may base their mating preferences on partner's heterozygosity depending on the magnitude of the related direct and indirect benefits associated with such choice. The way by which individuals assess individual genetic diversity and the potential association between heterozygosity and secondary sexual characters in lesser kestrels remains as subject for future research and can help to resolve the ultimate mechanism underlying mate choice (e.g. Aparicio *et al.*, 2001; Foerster *et al.*, 2003; García-Navas et al., 2009). This study provides empirical evidence that mate preferences have an important non-additive genetic component which may help to resolve the lek paradox from the perspective of heterozygous mate hypothesis.

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