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Becoming more like your mate: hormonal similarity reduces divorce rates in a wild songbird

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In animals with biparental care, maintaining a pair bond is of adaptive value because it increases reproductive success and reduces costs, such as energy and time, for finding a new mate. Hormones are important mediators of social behaviours as well as parental care, and endocrine mechanisms are therefore likely to be involved in the decision whether to stay with the same mate or separate after a breeding season. Because behavioural compatibility has been shown to increase fitness and hormones have been shown to regulate behavioural traits, here we examined whether the degree of endocrine similarity is also related to reproductive success and pair bond longevity. We used a 3-year study on free-living great tits, *Parus major*, to test whether mates had similar hormone levels during the parental phase. We tested specifically whether the metabolic hormone corticosterone was related to pair bond longevity and reproductive success. Baseline, but not stress-induced, corticosterone concentrations were highly correlated among members of a pair and became more similar among members of pairs that stayed together for multiple years. Pairs that increased their hormonal similarity within a season (from pre-breeding to breeding) had the highest reproductive success. Pairs with more similar baseline corticosterone levels and higher reproductive success were also more likely to remain together after the breeding season. The results of this study suggest that pair bond longevity is related to endocrine similarity and reproductive success, and raise the possibility that hormonal mechanisms may be under sexual selection.

Reproductive success can vary considerably among pairs in a population (Lack, 1964). In biparental species, reproductive success depends not only on individual quality but also on pair-specific characteristics, such as the degree of compatibility and coordination among parents (Hirschenhauser, Möstl, & Kotrschal, 1999; Mariette & Griffith, 2012; Marzluff & Balda, 1988; Spoon, Millam, & Owings, 2006). For example, disassortative mating based on boldness behaviour in guppies, *Poecilia reticulata*, decreases reproductive success (Ariyomo & Watt, 2013). Stable, long-term pair bonds have high reproductive success (Adkins-Regan & Tomaszycki, 2007; Black, 2001; Sánchez-Macouzet, Rodríguez, & Drummond, 2014), whereas unsuccessful pairs can separate, which in turn results in physiological costs and reduced reproductive success (Angelier, Moe, Clement-Chastel, Bech, & Chastel, 2007; Black, 2001; Choudhury, 1995). These costs result from a lack of coordination among new pair members or from the stress associated with forced or natural pair separation (Catry, Ratcliffe, & Furness, 1997; Dhondt & Adriaensen, 1994; Remage-Healey, Adkins-Regan, & Romero, 2003). Physiological mechanisms are likely to mediate decisions on mate selection and pair bond maintenance, as well as costs associated with these decisions.

Hormones are coordinators of reproductive and social behaviours in a wide range of vertebrate species (Adkins-Regan, 2005), and are influenced by the environment and social circumstances, such as the social mate. A study in greylag geese, *Anser anser*, showed that pairs with highly correlated testosterone levels throughout many years were more likely to nest in a given year and had larger clutches and heavier eggs (Hirschenhauser et al., 1999). However, testosterone is associated with courtship behaviour and can be a male-biased trait (Hau, 2007). In contrast, glucocorticoids (corticosterone in birds) are regulated year-round in both sexes with higher levels in birds during the breeding season than at other times of the

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year (Romero, 2002). Corticosterone levels are also directly related to parental effort (Bonier, Moore, & Robertson, 2011; Ouyang, Sharp, Quetting, & Hau, 2013), can predict individual reproductive success (Bonier, Moore, Martin, & Robertson, 2009; Ouyang, Hau, & Bonier, 2011; Patterson, Hahn, Cornelius, & Breuner, 2014) and therefore are candidate mechanisms by which pair bond longevity may be achieved through the modulation of parental behaviour. Evidence from a few studies indicates that pair separation or mating with an unattractive partner increases circulating levels of corticosterone (Angelier et al. 2007; Griffith, Pryke, & Buttemer, 2011; Remage-Healey et al. 2003). Higher levels of faecal corticosterone metabolites (Moreno et al. 2010) and baseline corticosterone (Villavicencio, Apfelbeck, & Goymann, 2014) are also found to be associated with a greater loss of paternity in males. In white-throated sparrows, *Zonotrichia albicollis*, during the parental/nestling phase white-striped morph males have higher levels of baseline corticosterone than tan-striped males (Horton & Holberton, 2010; Swett & Breuner, 2008), and white-striped males also have more extrapair fertilizations and provide less parental care than the tan-striped males (Tuttle, 2003). Moreover, females of both morphs have baseline corticosterone levels comparable to the tan-striped males with which they prefer to mate (Horton & Holberton, 2010). Pair similarity in corticosterone levels could therefore, via behavioural compatibility or sexual selection (van Oers, Drent, Dingemanse, & Kempenaers, 2008), provide a proximate explanation for the variation in levels of extrapair paternity. This idea has been confirmed in parrots, in which a high degree of behavioural compatibility, as defined by an inverse relationship between affiliation and aggression, was found to decrease extrapair paternity and increase pair bond longevity (Spoon, Millam, & Owings, 2007).

These studies raise the question whether a greater similarity in hormone levels, as a cause or result of behavioural similarity, provides benefits to members of a pair. Therefore, we used a multiyear study to examine the relationship between endocrine similarity in corticosterone concentrations, pair bond status and reproductive success in a wild population of great tits, *Parus major*, to answer the following questions. (1) Is pair similarity in corticosterone levels related to pair bond duration? (2) Is corticosterone similarity related to reproductive success? (3) Is extrapair fertilization related to hormonal similarity, and potentially associated with pair bond longevity?

METHODS

Study Site and Standard Protocols

This study was carried out between March 2009 and July 2011 in Möggingen, southern Germany (47°N, 8°E, see Ouyang, Quetting, & Hau, 2012 for a detailed description of the study site and standard protocols). Our breeding population was established in February of 2009, and in 2009 the vast majority of breeders were first-year birds. Great tits are socially monogamous cavity nesters with biparental care, in which both sexes provide food to the nestlings (Lack, 1964). Nests were monitored regularly so that the date of the first egg and the date of hatch (as day 1) could be determined. Nestlings were then monitored every 5 days until fledging and the total number of fledglings was recorded for each nest.

We videorecorded feeding rates for one 2 h period between 0800 and 1200 hours (i.e. noon) in 2009 and 2010 when the adults were feeding their 12-day-old young. The number of local recruits from each nest was also determined as the number of offspring present in the study population the next breeding season.

For 2 weeks in March of 2009 and 2010 (about 2 weeks before the first egg was laid in the population, hereafter termed 'pre-breeding'), adults were captured in mist nets at feeding stations. All adults were marked with a numbered aluminium ring and Darvic bands with a unique colour combination for individual identification. In May–June of 2009 and 2010 (hereafter termed 'breeding'), both members of a breeding pair were captured on the same day in their nestbox between 0800 and 1200 hours with a manually triggered metal trap that closed the entrance hole after birds entered it to feed their 8- or 9-day-old chicks. A blood sample (80–120 μl) was taken within 3 min of capture (mean ± SD; 2009: 1.8 ± 1.0 min, $N = 89$; 2010: 1.9 ± 0.9 min, $N = 149$) for later determination of hormone concentrations. Birds were then placed in a cloth bag for a standard capture and restraint procedure (Ouyang, Hau, et al., 2011; Wingfield et al. 1982) and another blood sample (<50 μl) was taken at 30 min post capture to determine stress-induced corticosterone concentrations. Blood samples were immediately stored on ice and centrifuged (822 × *g*, 10 min) within 4 h; the plasma was then removed and stored at −80 °C. The remaining red blood cells were stored in Queen's lysis buffer for later DNA extraction. While the adults were being held in bags for blood sampling, we weighed all chicks to the nearest 0.1 g while keeping them on warming pads, took a small blood sample (10 μl) and then returned them immediately to the nest. We released the adults after taking the second blood sample and measuring body mass (nearest 0.1 g) and tarsus length (nearest 0.1 mm). Adult age was scored by plumage appearance as a first-year breeder or older (Jenni & Winkler 1994), or from previous banding records.

Hormone Analysis

Plasma corticosterone concentrations were determined using an enzyme immunoassay kit (Cat. No. 901-097; 80-0045, Assay Designs, Ann Arbor, MI, U.S.A.), following a double diethyl ether extraction of 5 μl plasma sample aliquots following Ouyang, Hau, et al. (2011). Samples, along with a blank buffer and two separate, stripped-chicken plasma standards (at 20 ng/ml) were then redissolved in assay buffer at a 1:80 dilution and reconstituted overnight. The next day, 100 μl of each sample (in duplicate) were added randomly to individual wells on an assay plate. For comparative purposes, we added 10% to the final concentrations to account for recoveries (average extraction efficiency after double diethyl ether extraction: 89.7 ± 6.1%; Ouyang, Hau, et al., 2011). The average intraplate coefficient of variation was 10.7% (two replicate standards per plate), and the interplate coefficient of variation was 5.1% ($N = 29$ plates).

Extrapair Paternity

We obtained extrapair paternity data for 338 offspring from 57 nests (total $N = 453$) in 2010. DNA for the adults was extracted using a DNA isolation robot (Qiagen, Venlo, The Netherlands). Paternity analysis was performed for all nestlings that were present at 8–9 days after hatching. Genomic DNA from both the nestlings and the parents were isolated using the PureGene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, U.S.A.), and a polymerase chain reaction (PCR) was carried out using the Multiplex PCR kit (Qiagen). Five microsatellite regions (PmaTAGAn71, PmaGAn27, PmaTGAn33, PmaC25, PmaD105; Kawano, 2003; Saladin, Bonfils, Binz, & Richner, 2003) were amplified. PCR products were run on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.) with a molecular size standard (GeneScan-500 LIZ, Applied Biosystems). Sizes of the amplification products were determined using commercial software (GeneMapper 4.0, Applied Biosystems), and parentage was assigned using CERVUS 3.0 (Kalinowski, Taper, & Marshall, 2007; Marshall, Slate, Kruuk, & Pemberton, 1998). Critical values were calculated by using the following parameters in CERVUS: 10 000 cycles, 98% of loci typed, error rate 0.001%, 120