
Electronic appendices are refereed with the text. However, no attempt has been made to impose a uniform editorial style on the electronic appendices.

Electronic Appendix A

Further details of methods.
All birds were maintained on a diet of turkey starter crumbs (BOCM Pauls, UK) administered in a food hopper, which was always kept filled. Each bird was individually colour ringed for identification purposes. Every 10 days there was a probe day to monitor mass (Cuthill et al. 2000), during which birds experienced no food removal. Treatments lasted from 4 June to 23 August 2001 (birds aged 115-130 days at the end of treatments). Juvenile birds were provided with four live tutors housed in an adjacent cage within the same aviary during the period from initial capture until song recording (May 2001 – March 2002).

During song recording individual males were placed in a cage equipped with a nest box, on the morning of the first day of recording (as described by Buchanan et al. 2003). The recordings were made using a Sony DAT Walkman (TDC-D8) and a Sennheiser K6 microphone body, with a Sennheiser (MKE2-60 Gold C) microphone attachment, mounted on top of the nest box. These recordings were transferred to Avisoft SAS-Lab Pro (R. Specht, www.avisoft.de) and this software package was used to identify the different phrase types of individual starlings.
To quantify plasma corticosterone and testosterone levels birds were captured from a cage and a basal blood sample was taken within three minutes of capture. To gain a measure of the stress response birds were then placed in a cloth bag and a further (peak) blood sample taken at 10 minutes after capture (Maddocks et al. 2001). This is known as the capture-handling-restraint method (Wingfield 1994). After the birds were released the aviary was not entered for a period of 2h to allow the other birds to return to baseline levels of stress. Blood samples (100ul) were collected in heparinized capillary tubes after puncture of the brachial vein with a 25 gauge needle, centrifuged and the plasma stored at −20oC. Corticosterone concentrations were measured after extraction of 20ul aliquots of plasma in diethyl ether, by radioimmunoassay (Wingfield, et al. 1992) (anti-corticosterone antiserum code B21-42, Endocrine Sciences, Tarzana, CA; [1,2,6,7-3H]-corticosterone label: Amersham, U.K.). The extraction efficiency of the assay was 80-90%. The assay was run with 50% binding at 1.41 ng/ml, and a detection limit (7.3µl aliquots of plasma) of 0.45 ng/ml. Testosterone concentrations were measured by direct radioimmunoassay (anti-testosterone antiserum code 8680-6004, Biogenesis, U.K.; [125I]-testosterone label code 07-189126, ICN, U.K.) (Parkinson and Follett 1995).

The humoral immune response of individuals within this population was also tested using intraperitoneal injections of sheep red blood cells (SRBC) (Deerenberg et al. 1997), approximately ¾ of the way through the trial period. However, that data from this were not used in this analysis.

Data were analysed using Minitab 13 (Minitab Inc., State College, PA, USA), using a general linear model ANCOVA. Treatment group was added as a fixed factor, with mass at the end of the trial (g), cell-mediated immune response (5h), peak corticosterone levels, number of birds dominated and social rank added as covariates. The effects of mass, hormone levels and immune response on social status were also tested using an ANCOVA. The data were checked for normality and homogeneity of variance.
References


