**Online Resource 1**

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A typical common kestrel nest in a typical nest-box in the study population.The photo shows the usual lightness found inside the nest boxes designed for this species. Some grass is added by researchers to prevent the eggs from rolling on the floor.

**Online Resource 2**

EGGSHELL PIGMENT IDENTIFICATION AND MEASUREMENTS

Eggshells and half cotton pieces were extracted (and esterified) by 15 ml absolute methanol (LiChrosolv, gradient grade for chromatography, Merck, Darmstadt, Germany) containing 5% concentrated sulphuric acid at room temperature in the dark under N2 for 24 hours. Extracts were decanted and 10 ml chloroform (Merck; chloroform GR, ISO) and 10 ml distilled water were added, then shaken. The lower (chloroform) phase was collected, and the higher (water) phase was again extracted with chloroform (chloroform phases from both extractions were collected). These phases were washed with 5 ml 10% NaCl, followed by distilled water until the washing was neutral. Extracts were evaporated to dryness and reconstituted in 0.5 ml chloroform with an internal standard (5,10,15,20-tetra(4-pyridyl)-21H,23H-porphine, Aldrich, Sigma-Aldrich, St. Louis, MO, USA; 0.01 mg/ml). Standards for quantification (protoporphyrin IX and biliverdin, MP Biomedicals, LLC, Eschwege, Germany) were treated by the same procedure.

Pigments were determined and quantified by reversed-phase high-performance chromatography using an Agilent 1100 LC system (Agilent, Palo Alto, CA, USA) consisting of a degasser, binary pump, auto-sampler, thermostat column compartment, and multi-wavelength detector and coupled to an ion-trap mass spectrometer (Agilent LC-MSD Trap XCT-Ultra; Agilent, Palo Alto, CA, USA). Chromatographic separation was conducted in a Gemini 5u C18 110A column (250 x 2.0 mm I.D., Phenomenex, Torrence, CA, USA). The sample (10 µl) was injected into the column and eluted using a linear gradient (A = water with 0.1% formic acid, and B = acetonitrile with 0.085% formic acid), flow rate 0.35 ml/min and temperature 55°C. The gradient started at A/B 80:20 reaching 10:90 rations after 15 min and reaching 100% B after 5 minutes. For the next 10 min the elution was isocratic. Elution was monitored by absorbance at 410 nm. Atmospheric pressure ionization-electrospray ionization (API-ESI) positive mode ion-trap mass spectrometry at MRM (multiple reaction monitoring) mode was used when precursor ions were 619 (internal standard), 611 (biliverdin IXα) and 591 (protoporphyrin IX).

Operating conditions of MS were as follows: drying gas (N2), 12 l/min; drying gas temperature, 350°C; nebulizer pressure, 30 psi (207 kPa). Elution was also monitored by absorbance at 410 nm.

EGGSHELL PROTEIN IDENTIFICATION AND MEASUREMENTS

Sixteen (8 used for wiping eggs and 8 control) half cotton pieces were incubated in 200 l of sample reduced buffer (10 min, 105°C), and 20 l of this solution was loaded per line. Sample reduced buffer contained: 60% of H2O; 10% of 0.5 M/l of Tris/HCl (pH 6.8); 8% of glycerine; 16% of SDS solution in H2O (10%); 4% of 2--mercaptoethanol; 2% of bromphenol blue solution (0.05%). All chemicals were bought from Sigma (St. Louis, MO, USA).

One-dimensional polyacrylamide gel electrophoresis (SDS-PAGE) using homogeneous 12 % acrylamide resolving gel of 1 mm thickness essentially followed the methods of Laemmli (1970). Gels were run on Mini-Protean Tetra Cell system at 200 V until the bromophenol blue reached the end of the gel (ca 45 min). The gels were then stained with Bio-Safe Coomassie Blue G250 Stain. After staining, the gels were washed with water, scanned with GS-800 Calibrated Densitometer, and processed by software for image analysis (Quantity One®, Bio-Rad, Hercules, CA, USA).

Identification of proteins: Electrophoretic bands were destained by incubation in 100 µl of 100 mM ammonium bicarbonate/acetonitrile (1:1, v/v) for 1 hour. After destaining, the gel pieces were dehydrated by acetonitrile dried in a vacuum centrifuge and proteins were reduced and alkylated by DTT and iodoacetamide. Briefly, incubation was made with 10 mM DTT in 100 mM ammonium bicarbonate for 1 hour at 56 °C; after cooling to room temperature, the DTT solution was replaced by roughly the same volume of 55 mM iodoacetamide in 100 mM ammonium bicarbonate, and the gels were incubated at ambient temperature for 45 min in the dark. Then the gel pieces were washed with 100 µl of 100 mM ammonium bicarbonate, and dehydrated by addition 500 µl of acetonitrile. Subsequently, the liquid phase was removed and the gel pieces were dried in a vacuum centrifuge. Gel bands were digested in a 50 µl of digestion buffer containing trypsin (30 µg/ml) in 50 mM ammonium bicarbonate. After 1 hour in a cold, the gel pieces were placed to air circulation termostat and incubated overnight at 37 °C. The resulting tryptic peptides were extracted with sonication (15 min) by twice changes of 150 µl of extraction buffer (5% formic acid, 30% acetonitrile in water).

Proteins/peptides were identified by nano-HPLC/MS system. The nano-HPLC apparatus used was a Proxeon Easy-nLC (Proxeon, Odense, Denmark). Separation was done on NS-AC-11-C18 Biosphere C18 column (particle size: 5µm, pore size: 12 nm, length: 150 mm, inner diameter: 75 µm), and precolumn NS-MP-10 Biosphere C18 (particle size: 5 µm, pore size: 12 nm, length: 20 mm, inner diameter: 100 µm ), both from NanoSeparations (Nieuwkoop, Netherlands). Flow rate was 0.25 µl, and the column was held at ambient temperature (25 °C).

Separation of peptides was achieved via a linear gradient between mobile phase A (water) and B (acetonitrile), both contained 0.1 % (v/v) formic acid. Separation was started by running the system with the 5 % mobile phase B, followed by gradient elution to 30 % B at 70 min. The next step was gradient elution to 50 % B in 10 min., then gradient to 100 % in 8 min. Finally, the column was eluted with 100 % B for 2 min. Equlibration before the next run was achieved by washing the column with 5 % mobile phase B for 10 min. It was coupled to QTOF (quadrupole – time of flight) mass spectrometer with ultrahigh resolution (UHR-TOF) maXis (Bruker daltonics, Bremen, Germany) by nanoelectrosprayer. The maXis spectrometer has the resolution at least 40 000 FWHM and precision of mass determination at least 1 ppm. The reference ion used (internal mass lock) was the monocharged ion m/z 1221.9906 of C24H19F36N3O6P3.The LC-MS/MS instruments were controlled by software HyStar 3.2 and micrOTOF-control 3.0. The data were collected and manipulated by the software ProteinScape 2.0 and DataAnalysis 4.0 (all from Bruker Daltonics, Bremen, Germany).

Proteins were identified by correlation of tandem mass spectra to the IPI chicken (http://www.ebi.ac.uk/IPI/IPIhelp.html) and SwissProt (http://www.uniprot.org/) databases, using the MASCOT searching engine (www.matrixscience.com). The parameter of enzyme was chosen as trypsin. One missed cleavage was allowed, and an initial peptide mass tolerance of ±10.0 ppm was used for MS and ±0.05 Da for MS/MS analysis. Second round search used error tolerant search for elucidation of possible changes in amino acid composition of peptides and the last search used semitrypsin cleavage. Cysteines were assumed to be carbamidomethylated, proline and lysine to be hydroxylated, and methionine was allowed to be oxidated. All these possible modifications were set to be variable. Monoisotopic peptide charge was set 1+, 2+ and 3+. The Peptide Decoy option was selected during the data search process to remove false-positive results. At least two unique peptides were required to match for each protein. Only significant hits (score ≥80), as defined by the MASCOT probability analysis (P<0.05) were accepted. Proteins with one unique peptide were also accepted if their score was higher than 80 and the individual score for the unique peptide was ≥60.

**References**

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)*, **227**, 680–685.

**Online Resource 3**

List of proteins found in cotton samples used in the experiment (wiped and control treatments). M.S. = Mascot Score; N.U.P. = No. Unique peptides (all); S.C. = Sequence coverage (%).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Acession** | **Protein** | **M.S.** | **N.U.P.** | **S.C.** | **Sequence** |
| K1H1\_MOUSE | Keratin, type I cuticular Ha1 OS=Mus musculus GN=Krt31 PE=2 SV=2 | 656 | 10 (12) | 32 | K.ETMQFLNDR.L |
|  |  |  |  |  | R.ENAELECR.I |
|  |  |  |  |  | R.LVVQIDNAK.L |
|  |  |  |  |  | R.QLVESDINGLR.R |
|  |  |  |  |  | R.ILDELTLCK.S |
|  |  |  |  |  | K.SDLEAQVESLK.E |
|  |  |  |  |  | R.LNVEVDAAPTVDLNR.V |
|  |  |  |  |  | R.NSLENTLTESEAR.Y |
|  |  |  |  |  | R.YSSQLSQVQCLITNVESQLGEIR.A |
|  |  |  |  |  | R.LECEINTYR.G |
| K2C6C\_HUMAN | Keratin, type II cytoskeletal 6C OS=Homo sapiens GN=KRT6C PE=1 SV=3 | 617 | 7 (13) | 22 | R.SGFSSISVSR.S |
|  |  |  |  |  | K.WTLLQEQGTK.T |
|  |  |  |  |  | R.QLDSIVGER.G |
|  |  |  |  |  | R.TAAENEFVTLK.K |
|  |  |  |  |  | K.ADTLTDEINFLR.A |
|  |  |  |  |  | K.QEIAEINR.M |
|  |  |  |  |  | R.AIGGGLSSVGGGSSTIK.Y |
| KT33A\_MOUSE | Keratin, type I cuticular Ha3-I OS=Mus musculus GN=Krt33a PE=2 SV=1 | 608 | 3 (12) | 33 | K.LASDDFR.T |
|  |  |  |  |  | K.YETELSLR.Q |
|  |  |  |  |  | K.QNHEQEVNTLR.C |
| KRT34\_MOUSE | Keratin, type I cuticular Ha4 OS=Mus musculus GN=Krt34 PE=2 SV=1 | 602 | 1 (12) | 34 | K.SDLEAQVESLR.E |
| K2C5\_HUMAN | Keratin, type II cytoskeletal 5 OS=Homo sapiens GN=KRT5 PE=1 SV=3 | 576 | 4 (11) | 17 | R.VSLAGACGVGGYGSR.S |
|  |  |  |  |  | R.ISISTSGGSFR.N |
|  |  |  |  |  | K.YEELQQTAGR.H |
|  |  |  |  |  | K.LAELEEALQK.A |
| K1C14\_HUMAN | Keratin, type I cytoskeletal 14 OS=Homo sapiens GN=KRT14 PE=1 SV=4 | 454 | 6 (10) | 21 | K.GSCGIGGGIGGGSSR.I |
|  |  |  |  |  | K.YETELNLR.M |
|  |  |  |  |  | R.VLDELTLAR.A |
|  |  |  |  |  | R.EVATNSELVQSGK.S |
|  |  |  |  |  | K.SEISELR.R |
|  |  |  |  |  | K.ASLENSLEETK.G |
| K1C16\_HUMAN | Keratin, type I cytoskeletal 16 OS=Homo sapiens GN=KRT16 PE=1 SV=4 | 394 | 2 (9) | 19 | K.EELAYLR.K |
|  |  |  |  |  | K.EVASNSELVQSSR.S |
| KRT81\_MOUSE | Keratin, type II cuticular Hb1 (Fragment) OS=Mus musculus GN=Krt81 PE=2 SV=1 | 388 | 8 (9) | 19 | -.NLEIDPNAQCVK.H |
|  |  |  |  |  | K.FAAFIDK.V |
|  |  |  |  |  | R.EAECVEADSGR.L |
|  |  |  |  |  | R.ATAENEFVALK.K |
|  |  |  |  |  | K.DVDCAYLR.K |
|  |  |  |  |  | R.AEAESWYR.T |
|  |  |  |  |  | R.LTAEIENAK.C |
|  |  |  |  |  | R.LLEGEEQR.L |
| KRT83\_HUMAN | Keratin, type II cuticular Hb3 OS=Homo sapiens GN=KRT83 PE=1 SV=2 | 377 | 2 (9) | 15 | R.LYEEEIR.I |
|  |  |  |  |  | R.DLNMDCIVAEIK.A |
| KRT83\_MOUSE | Keratin, type II cuticular Hb3 OS=Mus musculus GN=Krt83 PE=2 SV=2 | 367 | 2 (8) | 16 | R.CCISAAPYR.G |
|  |  |  |  |  | K.LEAAVTQSEQQGEAALTDAR.C |
| ALBU\_HUMAN | Serum albumin precursor - Homo sapiens (Human) | 262 | 7 (8) | 15 | K.LVNEVTEFAK.T |
| ALBU\_PONPY | Serum albumin precursor - Pongo pygmaeus (Bornean orangutan) |  |  |  | K.TCVADESAENCDK.S |
|  |  |  |  |  | K.AAFTECCQAADK.A |
|  |  |  |  |  | K.VFDEFKPLVEEPQNLIK.Q |
|  |  |  |  |  | K.FQNALLVR.Y |
|  |  |  |  |  | K.KVPQVSTPTLVEVSR.N |
|  |  |  |  |  | K.CCTESLVNR.R |
| OMP38\_ACIBA | Outer membrane protein omp38 OS=Acinetobacter baumannii GN=omp38 PE=1 SV=1 | 186 (267\*) | 4 (4) - 6\*(6\*) | 10 (15\*) | R.LNDALSLR.T |
| OMP38\_ACIBT | Outer membrane protein omp38 OS=Acinetobacter baumannii (strain ATCC 17978 / NCDC KC 755) GN=omp38 PE=3 SV=2 |  |  |  | R.VFFDTNK.S |
|  |  |  |  |  | K.DQYKPEIAK.V |
|  |  |  |  |  | K.SALVNEYNVDASR.L |
|  |  |  |  |  | \* K.LSEYPNATAR.I |
|  |  |  |  |  | \*\* K.GDVDGASAGAEYK.Q |
| IPI00587107 | LOC772080 similar to type I hair keratin KA31 | 212 | 3 (5) | 9 | K.VTMQNLNDR.L |
|  |  |  |  |  | R.LASYLDK.V |
|  |  |  |  |  | K.ENAELECR.I |
| IPI00583111 | CTGF Connective tissue growth factor/hypertrophic chondrocyte-specific protein 24 | 195 | 4 (6) | 17 | K.QLGELCTER.D |
|  |  |  |  |  | R.VTNDNAFCR.L |
|  |  |  |  |  | K.FELSGCTSVK.T |
|  |  |  |  |  | K.FCGVCTDGR.C |
| IPI00582298 | LOC395772 40 kDa protein | 187 | 3 (4) | 8 | A.KYEDEINKR.T |
| IPI00584075 | LOC395772 Keratin, type II cytoskeletal cochleal |  |  |  | K.YEDEINKR.T |
| IPI00822243 | LOC395772 58 kDa proteína |  |  |  | R.NLDLDSIIAEVK.A |
| K2C5\_XENLA | Keratin, type II cytoskeletal OS=Xenopus laevis PE=2 SV=2 | 177 | 1 (4) | 5 | K.LAELEAALQK.A |
| PIP\_HUMAN | Prolactin-inducible protein OS=Homo sapiens GN=PIP PE=1 SV=1 | 163 | 3 (4) | 34 | K.TYLISSIPLQGAFNYK.Y |
|  |  |  |  |  | K.YTACLCDDNPK.T |
|  |  |  |  |  | R.ELGICPDDAAVIPIK.N |
| IPI00581466 | KRT75 Type II alpha-keratin IIC | 162 | 3 (4) | 8 | R.NLDLDSIIAEVK.A |
|  |  |  |  |  | R.STKQEISELNRHVQR.L |
|  |  |  |  |  | K.LLEGEECR.L |
| IPI00582298 | LOC395772 40 kDa protein | 147 | 1 (3) | 8 | K.YEDEINKR.T |
| IPI00584075 | LOC395772 Keratin, type II cytoskeletal cochleal |  |  |  | K.YEDEINKR.T |
| IPI00822243 | LOC395772 58 kDa protein |  |  |  | R.NLDLDSIIAEVK.A |
| EFTU\_XANOM | Elongation factor Tu OS=Xanthomonas oryzae pv. oryzae (strain MAFF 311018) GN=tuf1 PE=3 SV=1 | 136 | 2 (2) | 7 | K.TTVTGVEMFR.K |
| EFTU\_XANOR | Elongation factor Tu OS=Xanthomonas oryzae pv. oryzae GN=tuf1 PE=3 SV=1 |  |  |  | K.LLDQGQAGDNAGLLLR.G |
| EFTU\_XANAC | Elongation factor Tu OS=Xanthomonas axonopodis pv. citri GN=tufA PE=3 SV=1 |  |  |  |  |
| EFTU1\_XANC8 | Elongation factor Tu 1 OS=Xanthomonas campestris pv. campestris (strain 8004) GN=tuf1 PE=3 SV=1 |  |  |  |  |
| EFTU1\_XANCP | Elongation factor Tu-A OS=Xanthomonas campestris pv. campestris GN=tufA PE=3 SV=1 |  |  |  |  |
| EFTU1\_XANCB | Elongation factor Tu 1 OS=Xanthomonas campestris pv. campestris (strain B100) GN=tuf1 PE=3 SV=1 |  |  |  |  |
| IPI00821633 | RARRES1 Ovocalyxin-32 | 131 | 2 (2) | 8 | Y.INSHEASPSRPL.A |
|  |  |  |  |  | Y.LLAQVSSVK.Q |
| IPI00599020 | SKIV2L2 Putative uncharacterized protein | 128 | 4 (4) | 4 | I.AMDIKAAK.R |
|  |  |  |  |  | R.LGFATSSDVIEMKGRVAC.E |
|  |  |  |  |  | R.LGFATSSDVIEMKGRVACEI.S |
|  |  |  |  |  | A.IGNTELENK.F |
| IPI00593841 | SIRT2 Putative uncharacterized protein | 127 | 3 (5) | 10 | G.VEPGGSLR.R |
|  |  |  |  |  | R.VLDELSLAGI.A |
|  |  |  |  |  | V.FFGENLPSR.F |
| IPI00681344 | LOC771972 similar to Kunitz-like protease inhibitor | 126 | 2 (4) | 11 | R.SVLPEKDDFHPR.T |
|  |  |  |  |  | R.VFVHSSCGGNANNFR.T |
| IPI00583721 | CACNA1C similar to calcium channel, voltage-dependent, L type, alpha 1C subunit | 126 | 2 (4) | 2 | I.ALNDTTEINR.N |
|  |  |  |  |  | C.WKSQEELK.D |
| K2C74\_HUMAN | Keratin, type II cytoskeletal 74 OS=Homo sapiens GN=KRT74 PE=1 SV=2 | 120 | 1 (3) | 5 | R.FLEQQNQVLETK.W |
| TITIN\_MOUSE | Titin - Mus musculus (Mouse) | 111 | 3 (6) | 0.2 | K.MQFKNNVASLVINKVDHSDV.G |
|  |  |  |  |  | E.VQWLR.N |
|  |  |  |  |  | F.LSDNLTNDSCK.L |
| IPI00814562 | LOC431315 similar to Scale keratin | 110 | 2 (2) | 23 | K.TSVAVPQPIAESCNELCAR.Q |
|  |  |  |  |  | R.KLWDTCGPC.- |
| IPI00589189 | NPAT similar to NPAT | 98 | 3 (3) | 3 | D.KDVVLDHVNAQAQPPQ.R |
|  |  |  |  |  | I.LSSPAKSPNK.T |
|  |  |  |  |  | A.QTAKSLPQKERNENSSFPVDSAPSSAK.T |
| PHBP\_UNKP | Phosphate-binding protein OS=Unknown prokaryotic organism PE=1 SV=1 | 96 (237\*) | 1 (2) - 3\* (4\*) | 7 (12\*) | K.LIQVPSVATSVAIPFR.K |
|  |  |  |  |  | \*\*\*R.SGPIQVVYR.A |
|  |  |  |  |  | \*\*\*\*R.AESSGTTELFTR.F |
| IPI00681344 | LOC771972 similar to Kunitz-like protease inhibitor | 91 | 2 (2) | 8 | R.SVLPEKDDFHPR.T |
|  |  |  |  |  | R.VFVHSSCGGNANNFR.T |
| IPI00592402 | MYO9A similar to myosin-IXa | 86 | 2 (2) | 1 | K.LQLEKTKC.Y |
|  |  |  |  |  | S.VALSSLRP.V |
| IPI00581368 | LOC395256 Ovocleidin-116 | 78 | 2 (2) | 4 | R.TQPEVASAPSTVGK.A |
|  |  |  |  |  | R.LGQAARPEVAPAPSTGGR.I |
| UP01\_VITRO | Unknown protein 1 (Fragment) OS=Vitis rotundifolia PE=1 SV=1 | 76 | 1 (1) | 92 | -.TNAENEFVTIK.K |
| UP18\_PSEMZ | Unknown protein 18 (Fragment) OS=Pseudotsuga menziesii PE=1 SV=1 |  |  |  |  |
| VMO1\_HUMAN | Vitelline membrane outer layer protein 1 homolog OS=Homo sapiens GN=VMO1 PE=1 SV=1 | 89 | 2 (2) | 10 | P.GDDTALNGIR.L |
|  |  |  |  |  | L.GDNTAANNVR.F |
| OPRI\_PSEAE | Major outer membrane lipoprotein precursor (Outer membrane lipoprotein I) - Pseudomonas aeruginosa | 67 | 1 (1) | 12 | R.LTATEDAAAR.A |
| HSP02\_PSEMZ | Putative heat shock protein 2 (Fragment) OS=Pseudotsuga menziesii PE=1 SV=1 | 49 | 1 (1) | 100 | -.ELLSEINR.- |

*\*Modification:*Ser->Thr; \*\* *Modification:* Ser->Ile; \*\*\* *Modification:* Gln->Glu; *\*\*\*\*Modification:* Ala->Ser.

**Online resource 4**

Lineal relationship between protoporphyrin IX concentration and amount of eggshell proteins (kDa from electrophoresis gel bands) found in cotton samples used for wiping eggs. Correlations: 105 kDa band, *r* = - 0.51, *P* = 0.197; 69 kDa band, *r* = - 0.61, *P* = 0.108; 45 kDa band, *r* = - 0.71, *P* = 0.050; 38 kDa band, *r* = - 0.13, *P* = 0.764; 20 kDa band, *r* = - 0.79, *P* = 0.020. 