

Supporting Information. 7 pages, 2 tables.

Linking Molecular and Population Stress Responses in *Daphnia magna* exposed to cadmium. Full text: 27 pages, 2 figures, 3 tables, 6,252 words.

Richard Connon ^{1,9}, Helen L.Hooper ¹, Richard M. Sibly¹, Fei-Ling Lim ^{2,12}, Lars-Henrik Heckmann ^{1,10}, David J.Moore ², Hajime Watanabe³, Annaleen Soetaert ⁴, Katie Cook ⁵, Steve J.Maund ⁶, Thomas H.Hutchinson ^{7,11}, Jonathan Moggs ², Wim De Coen ⁴, Taisen Iguchi ⁸ and Amanda Callaghan ^{1*}

Methodology

Construction of cDNA libraries

The daphnid cDNA library was constructed using a cDNA Synthesis kit according to the manufacturer's protocol (Stratagene, Tokyo, Japan). For first-strand cDNA synthesis, Superscript II reverse transcriptase (Invitrogen, Tokyo, Japan) was used in presence of 5-methyl dCTP. After a second strand synthesis, the EcoRI adaptor was ligated and digested with XhoI. The digested cDNA was then passed through a spin column (Chroma Spin-400, Clontech) to remove linkers and short cDNAs. The recovered fragments were cloned into the EcoRI and XhoI sites of pBluescript IISK(-) and transformed into the E. coli DH10B strain (Invitrogen, Tokyo, Japan) by electroporation, as stated in Watanabe *et al.*(2005)¹.

Construction of SSH libraries

Selective Subtractive Hybridisations were performed with forward and reverse subtraction using a PCR-Select cDNA Subtraction Kit (Clontech Laboratories Inc, USA). For the physico- and chemical stressors detailed above, cDNA was prepared from mRNA extracted from batches of 100 *D. magna*, between 24 and 48 h old, using a Straight A's mRNA Isolation Kit System (Novagen Inc., UK). Because of a high abundance of ribosomal RNA still being present with the mRNA sample, post-synthesised cDNA was later amplified using a Super Smart PCR cDNA Synthesis Kit (Clontech Laboratories Inc, USA). This process relatively reduces the abundance of ribosomal RNA by amplifying the copy number of poly-A synthesised product. Complementary DNA fragments were cloned with plasmid vectors pCR®4-TOPO® into competent E. coli cells using a TOPO TA Cloning Kit for Sequencing (Invitrogen, UK). Reproductive SSHs were performed as above, but using 15 adults carrying eggs against 75 under-24 h old juveniles as described in Soetaert *et al.*(2006)².

Resulting colonies were amplified using M13-Long primers (forward 5' cgacgttgtaaacgacggccag 3' and reverse 5' caggaaacagctatgacctgattacgcc 3'). The amplified products were purified using Millipore Montage 96-well cleanup plates (VWR International, UK).

Microarray construction

Single spots were pin-printed on Corning CMT-UltraGAPS glass slides (Fisher, UK), in a 17 x 18 block format with 48 blocks per microarray (grid=14,688), using an Omnigrid 100 (Genomics Solutions, USA). Dimethyl sulphoxide (DMSO) was added, to a final concentration of 50%, to the purified PCR products prior to printing. After printing, microarray slides were cross-linked by UV (150mJ/cm²) followed by baking for 2hrs at 80°C, and stored at room temperature under vacuum in total darkness until required.

A number of positive and negative hybridisation control spots were printed in each of the 48 blocks on the arrays. The positive hybridisation controls comprised of *D. magna* genomic DNA and four Spot Report System PCR products from *Arabidopsis thaliana*; *CAB*, *RCA*, *RBCL* and *LPT4* (Stratagene, USA). Negative hybridisation controls consisted of salmon sperm DNA, mouse *Cot 1*, human *Cot 1*, yeast transfer RNA (tRNA) and poly A RNA. Blank spots consisting of 50% DMSO were interspaced with the controls and jointly used to qualitatively assess the microarray hybridisation efficiency and provide orientation for the grid overlap during assessment. A common reference pool, against which all samples were hybridised, was prepared using total RNA from over 30,000 unexposed 24-48 h old *D. magna* collected from cultures over a period of 6 months.

RNA samples

RNA was obtained from populations of 240 *D. magna*, 24-48 h old, exposed for 24 h to sublethal concentrations of cadmium ranging from 0, 6.13, 20.24 and 36.79 µg L⁻¹ (Cd²⁺) in quadruplicates. Juvenile *D. magna* were used in order to avoid gene expression due to reproductive responses. Following exposure, daphnids were stored in RNAlater. (Ambion, UK) at -80°C.

Total RNA from all four biological replicates of each of the three treatments and controls was extracted using an Rneasy mini kit (Qiagen, UK). RNA quality was checked on an Agilent 2100 Bioanalyzer which showed no evidence of degradation.

mentary DNA was synthesised from 10 µg total RNA, containing four external Spot Report spike transcripts *CAB*, *RCA*, *RBCL* and *LPT4* genes (Stratagene, USA) corresponding to control spots printed in each of the 48 microarray blocks. Complementary DNA was then labelled with Alexa fluor dyes (647 and 555), using SuperScript[™] Plus Indirect cDNA labelling System (Invitrogen, UK). No technical replicates were carried out due to material limitations and no dye swaps were performed as pilot studies showed no bias whilst using a reference design.

Microarray hybridisation

Pre-hybridisations were carried out to wash of any non-fixed cDNA resulting from printing and block background. Slides were pre-hybridised, in a solution containing 50% v/v formamide, 5x Sodium chloride-Sodium Citrate (SSC), 0.1% Sodium Dodecyl Sulphate (SDS) and 1% w/v Bovine Serum Albumin (BSA) (Sigma-Aldrich, UK) and incubated at 42°C for 1 hour. They were then washed in ultra pure water in glass staining troughs with a final dip in isopropanol before drying by centrifugation at 1,750 rpm (using a “Christ RVC 2-25” centrifuge) for 3 minutes at room temperature.

The labelled sample and reference pool cDNA were then combined and concentrated, using a vacuum centrifuge, down to ~2 µl. A 45 µl hybridisation probe solution was prepared with 22.5 µl non-aqueous formamide (Sigma-Aldrich, UK), 5 x SSC, the labelled cDNA mix and 9.25 µl hybridisation block mix containing 0.1% SDS, 0.5 mg ml⁻¹ Poly A RNA (Sigma-Aldrich, UK), 0.5 mg ml⁻¹ Yeast tRNA, 0.5 mg ml⁻¹ Salmon sperm DNA, 50 µg ml⁻¹ human and mouse Cot 1 DNA (Invitrogen, UK). The block mix was used to block both background and non-specific hybridisation from commonly repeated sequences. The probes were hybridised to each microarray under a Hybri-slip, cover-slip (Sigma-Aldrich, UK) at 42°C for 16 h in a humidified, air-tight plastic box; lined with Whatman paper, soaked in hybridisation buffer.

Post-hybridisation, slides were plunged into 2x SSC to remove the cover-slips and washed for 10 min in 0.1x SSC/0.1% SDS and a further 10 min in 0.1x SSC; the solutions were renewed after 5 min. The slides were briefly dipped in 0.05x SSC, with a final dip in isopropanol before drying by centrifugation at 1,750 rpm for 5 min at room temperature.

Microarray analysis

Scanning was performed using a GenePix 4200A microarray scanner (Axon Instruments, UK), and the image acquired was assessed using GenePix[®] Pro 5.0 to locate and quantify the spots. Genespring software was used to subtract local background, remove spots that were of low intensity and normalise slides. For details, see supplementary material.

Data were normalised using (global, within and between slide) per spot and per chip intensity-dependent (LOWESS), followed by a per gene normalisation to the control samples, within each hybridisation batch, to normalise for batch variations (For full information on standard two-colour normalizations using GeneSpring see user manual <http://www.chem.agilent.com/scripts/pds.asp?lpage=27881> .

Data were filtered on spot quality and intensity as follows: Only data flagged as present or marginal in 3 out of the 4 biological replicate slides were analysed (7,532 spots) to ensure their presence in at least 75% of the treatments. Data were further filtered by removing spots where the expression levels in cadmium treatments relative to the control varied less than 1.4 times either up or down in regulation. On a log scale this equates to

removing spots where differences were between 0.714 and 1.4 fold in all conditions (4,507 spots). Spots that did not change significantly in at least one treatment were removed by using two-sample t-test filters (unpaired, assuming equal variance). This resulted in 942 spots which were analysed individually by one-way ANOVAs, assuming equal variances and no multiple testing corrections. Genes showing significant responses ($p < 0.05$) were clustered into a gene tree using a Pearsons' correlation similarity measure. The null hypothesis was that no genes would be up or down regulated by cadmium exposure.

Gene fragment annotation

Sequencing of the 3,172 SSH ESTs (from source i and iii, see *Microarray construction* above) was carried out with M13-Long primers. Blast searches were performed on specific fragments that responded significantly to the exposure treatments. Sequences were annotated according to BlastX homology search against Uniprot Swissprot (<http://www.ebi.ac.uk/swissprot>) and Uniprot Trembl (invertebrate section, <http://www.ebi.ac.uk/trembl>) March 2006. Sequences were only annotated if they were found to have a Blast hit with the expect value smaller than 1×10^{-5} and a score above 50. GeneBank/UniProt Accession number and species' match were recorded with each annotation (table 2). Responses from EST's originating from SSH techniques were not analysed for cadmium specificity as they originated from populations exposed to much higher concentrations than those used in this experiment, however, the clone origin is specified in table 2 along with respective annotation.

MIAME Compliance

Available *D. magna* sequences can be found at <http://daphnia.nibb.ac.jp> and <http://www.biosci.reading.ac.uk/Research/eb/daphnia.htm>. Microarray images and data can be are available at <http://www.biosci.reading.ac.uk/Research/eb/daphnia.htm> and are also accessible through the public repository Array Express at the European Bioinformatics Institute (accession number E-MAXD 20). Data was also catalogued under the Natural Environment Research Council NERC EnvBase database (accession number 000054), at http://nebc.nox.ac.uk/cgi-bin/public_catalogue.cgi.

References cited in supplementary information.

1. Watanabe, H.; Tatarazako, N.; Oda, S.; Nishide, H.; Uchiyama, I.; Morita, M.; Iguchi, T. Analysis of expressed sequence tags of the water flea *Daphnia magna*. *Genome* **2005**, 48, 606-9.
2. Soetaert, A.; Moens, L. N.; Van der Ven, K.; Van Leemput, K.; Naudts, B.; Blust, R.; De Coen, W. M. Molecular impact of propiconazole on *Daphnia magna* using a reproduction-related cDNA array. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **2006**, 142, 66-76.

Table 1. GeneSpring direct export data expression fold ranges, per concentration (Cd 2+ $\mu\text{g.L}^{-1}$), for all 112 spots responding to cadmium exposure normalized to controls.

(Part 1 spots 1-56)

	Microarray Clone ID	Concentration 0 normalised	Concentration 6.13 Normalised	Concentration 20.24 Normalised	Concentration 36.24 Normalised
1	AD3C07	1	0.953 (0.705 to 1.506)	1.726 (0.824 to 4.424)	2.705 (1.494 to 3.896)
2	AD4C10	1 (1 to 1)	0.676 (0.463 to 0.871)	0.65 (0.566 to 0.729)	0.917 (0.684 to 1.103)
3	IGU001_S_C_000001_C03.r	1 (1 to 1)	0.754 (0.555 to 0.955)	1.251 (0.917 to 1.902)	1.49 (1.311 to 1.637)
4	IGU001_S_C_000002_E08.r	1	0.423 (0.387 to 0.461)	1.51 (0.852 to 2.188)	1.631 (0.859 to 2.69)
5	IGU001_S_C_000004_D06.r	1	1.163 (0.938 to 1.831)	0.9 (0.634 to 1.397)	0.611 (0.517 to 0.737)
6	IGU001_S_C_000006_H07.r	1 (1 to 1)	1.51 (0.871 to 3.617)	2.001 (1.39 to 3.155)	0.615 (0.395 to 1.02)
7	IGU001_S_C_000011_B02	1	1.487 (1.156 to 1.983)	1.096 (0.777 to 1.29)	1.574 (1.255 to 2.375)
8	IGU001_S_C_000012_B11	1	0.563 (0.389 to 0.701)	0.95 (0.403 to 2.061)	3.364 (1.416 to 6.618)
9	IGU001_S_C_000012_C07	1 (1 to 1)	1.976 (1.388 to 3.394)	1.157 (0.892 to 1.652)	0.811 (0.542 to 1.103)
10	IGU001_S_C_000014_H12	1	1.529 (1 to 3.5)	0.965 (0.75 to 1.099)	1.985 (1.691 to 2.64)
11	IGU001_S_C_000016_D02	1	0.504 (0.252 to 1.004)	1.904 (1.126 to 3.789)	0.905 (0.537 to 1.872)
12	IGU001_S_C_000018_E05	1	0.914 (0.439 to 3.18)	1.785 (0.944 to 3.08)	0.194 (0.132 to 0.248)
13	IGU001_S_C_000019_A01	1	1.149 (1.006 to 1.329)	0.703 (0.338 to 1.305)	0.415 (0.261 to 1.108)
14	IGU001_S_C_000020_A10	1 (1 to 1)	4.454 (1.517 to 13.07)	0.148 (0.0801 to 0.273)	0.024 (0.0222 to 0.0261)
15	IGU001_S_C_000020_E07	1 (1 to 1)	1.335 (0.703 to 2.142)	1.009 (0.381 to 2.688)	5.651 (1.169 to 15.45)
16	IGU001_S_C_000024_H03	1	3.112 (2.103 to 4.607)	6.573 (6.123 to 7.056)	3.157 (1.438 to 7.218)
17	IGU001_S_C_000027_C03	1	1.322 (0.969 to 1.617)	0.784 (0.644 to 0.966)	2.31 (1.819 to 2.785)
18	IGU001_S_C_000032_D02	1 (1 to 1)	1.296 (1.14 to 1.474)	0.427 (0.32 to 0.635)	1.295 (1.002 to 1.608)
19	IGU001_S_C_000033_C02	1	0.753 (0.47 to 1.283)	0.884 (0.775 to 1.056)	2.158 (1.193 to 4.951)
20	IGU001_S_C_000037_F05	1	1.534 (1.438 to 1.637)	0.541 (0.317 to 1.125)	1.581 (1.25 to 2.044)
21	IGU001_S_C_000038_C01	1 (1 to 1)	1.658 (1.21 to 2.466)	2.857 (2.396 to 3.74)	5.358 (2.814 to 10.2)
22	IGU001_S_C_000040_F07	1	0.396	0.41 (0.386 to 0.434)	1.255
23	IGU001_S_C_000042_E11	1 (1 to 1)	0.778 (0.442 to 1.369)	2.56 (1.873 to 3.5)	0.259 (0.172 to 0.39)
24	IGU001_S_C_000042_F07	1	1.163 (1.054 to 1.416)	1.357 (1.14 to 1.512)	1.484 (1.051 to 1.945)
25	IGU001_S_C_000043_E01	1 (1 to 1)	19.35 (11.1 to 33.72)	10.41 (7.011 to 15.45)	4.263 (2.604 to 6.978)
26	IGU001_S_C_000044_A01	1 (1 to 1)	0.881 (0.797 to 0.974)	1.711 (1.539 to 1.903)	1.492 (1.107 to 2.13)
27	IGU001_S_C_000044_E04	1	1.054 (0.423 to 1.832)	1.021 (0.623 to 1.644)	2.887 (1.179 to 4.853)
28	IGU001_S_C_000045_E09	1	3.867 (3.405 to 4.775)	1.897 (0.974 to 3.057)	1.705 (1.191 to 2.442)
29	IGU001_S_C_000046_E03	1 (1 to 1)	1.504 (1.439 to 1.608)	1.581 (1.352 to 1.775)	1.705 (1.083 to 3.002)
30	IGU001_S_C_000046_H06	1	0.687 (0.655 to 0.74)	1.357 (1.349 to 1.366)	0.452 (0.32 to 0.777)
31	IGU001_S_C_000047_G03	1	1.191 (1.107 to 1.33)	1.07 (0.988 to 1.336)	1.69 (1.216 to 3.221)
32	IGU001_S_C_000048_B10	1	0.797 (0.59 to 0.984)	1.444 (1.308 to 1.717)	1.883 (1.471 to 2.411)
33	IGU001_S_C_000048_C06	1	0.659 (0.338 to 1.157)	0.992 (0.855 to 1.172)	1.598 (1.213 to 1.947)
34	IGU001_S_C_000048_G05	1 (1 to 1)	1.117 (0.85 to 1.33)	1.249 (1.009 to 1.54)	1.517 (1.23 to 1.971)
35	IGU001_S_C_000049_C10	1 (1 to 1)	0.132 (0.0337 to 0.73)	1.248 (0.867 to 1.798)	0.11 (0.028 to 0.451)
36	IGU001_S_C_000049_D08	1 (1 to 1)	1.323 (1.082 to 1.741)	1.496 (1.056 to 1.798)	1.521 (1.225 to 2.006)
37	IGU001_S_C_000049_F02	1 (1 to 1)	1.168 (1.07 to 1.22)	1.53 (1.376 to 1.663)	1.01 (0.652 to 1.666)
38	IGU001_S_C_000052_E08	1 (1 to 1)	1.22 (0.948 to 1.479)	1.629 (1.427 to 2.101)	1.479 (1.245 to 1.765)
39	IGU001_S_C_000053_D10	1	0.608 (0.511 to 0.753)	1.923 (1.193 to 2.478)	0.388 (0.272 to 0.552)
40	IGU001_S_C_000053_G07	1	1.059 (0.914 to 1.313)	1.464 (1.181 to 2.038)	1.321 (0.986 to 1.767)
41	IGU001_S_C_000054_D11	1	1.15 (0.936 to 1.422)	1.562 (1.225 to 1.786)	1.305 (0.991 to 1.736)
42	IGU001_S_C_000054_F10	1	1.142 (0.855 to 1.325)	1.637 (1.519 to 1.975)	1.362 (1.015 to 1.659)
43	IGU001_S_C_000055_B09	1 (1 to 1)	1.027 (0.933 to 1.235)	1.404 (1.293 to 1.52)	1.197 (0.852 to 1.612)
44	IGU001_S_C_000055_G01	1	1.384 (0.949 to 1.674)	1.492 (1.256 to 1.795)	1.549 (1.159 to 1.96)
45	IGU001_S_C_000056_B04	1 (1 to 1)	0.98 (0.852 to 1.305)	1.579 (1.396 to 1.93)	1.161 (0.716 to 1.553)
46	JD7D07	1	0.306 (0.217 to 0.71)	0.677 (0.403 to 1.205)	1.572 (0.779 to 2.508)
47	R01CDF1C01	1 (1 to 1)	1.879	2.606 (1.879 to 3.615)	5.876 (4.593 to 7.517)
48	R03CDF3B06	1	4.298 (3.925 to 4.706)	5.318 (4.306 to 6.567)	1.29 (1.151 to 1.482)
49	R05CDR1B12	1	1.096 (0.881 to 1.332)	1.584 (1.321 to 1.828)	1.309 (0.868 to 1.744)
50	R10CAR1F09	1 (1 to 1)	1.058 (0.669 to 1.786)	1.076 (0.786 to 1.938)	2.621 (1.29 to 6.74)
51	R10CAR1G06	1 (1 to 1)	1.627 (1.113 to 2.085)	1.589 (1.21 to 2.297)	1.807 (1.519 to 2.691)
52	R16LFR1D10	1 (1 to 1)	2.855 (1.379 to 4.866)	1.498 (1.237 to 2.019)	2.563 (1.728 to 4.131)
53	R16LFR1F12	1	0.549 (0.159 to 1.95)	1.774 (0.886 to 4.117)	2.68 (2.369 to 2.877)
54	R18BF2A03	1	3.487 (2.195 to 9.48)	1.629 (0.542 to 3.841)	1.232 (0.698 to 1.953)
55	RAC06	1 (1 to 1)	0.526 (0.349 to 0.818)	0.284 (0.0949 to 0.539)	1.914 (1.675 to 2.187)
56	RBB03	1	1.293 (0.718 to 1.866)	2.374 (1.62 to 4.786)	3.056 (1.984 to 6.245)

(Part 2 spots 57-112)

	Microarray Clone ID	Concentration 0 normalised	Concentration 6.13 Normalised	Concentration 20.24 Normalised	Concentration 36.24 Normalised
57	RBD06	1 (1 to 1)	0.92 (0.839 to 1.015)	1.185 (0.786 to 1.396)	1.488 (1.18 to 2.015)
58	RCD11	1 (1 to 1)	1.013 (0.604 to 1.464)	1.103 (0.763 to 1.413)	1.658 (1.426 to 2.004)
59	RRC03	1 (1 to 1)	1.261 (0.847 to 1.876)	1.205 (0.611 to 2.421)	3.173 (1.757 to 4.356)
60	WTH01A01NCLL0001_B11	1 (1 to 1)	0.405 (0.275 to 0.597)	2.105 (1.857 to 2.387)	2.236 (1.08 to 4.63)
61	WTH01A01NCLL0001_B21	1	0.394 (0.334 to 0.5)	0.345 (0.253 to 0.511)	0.451 (0.309 to 0.688)
62	WTH01A01NCLL0001_L22	1 (1 to 1)	0.988 (0.55 to 1.176)	0.798 (0.682 to 0.944)	0.616 (0.503 to 0.733)
63	WTH01A01NCLL0001_M22	1 (1 to 1)	1.016 (0.432 to 1.747)	0.397 (0.268 to 0.776)	1.711 (1.284 to 2.042)
64	WTH01A01NCLL0001_N07	1 (1 to 1)	1.822 (1.323 to 2.604)	0.803 (0.596 to 1.035)	0.696 (0.501 to 0.883)
65	WTH01A01NCLL0001_N15	1	2.472 (0.905 to 6.505)	8.779 (4.822 to 15.98)	1.582 (1.044 to 3.023)
66	WTH01A01NCLL0001_O20	1 (1 to 1)	1.584 (1.172 to 2.201)	0.953 (0.812 to 1.147)	1.373 (1.213 to 1.725)
67	WTH01A01NCLL0002_E09	1	3.355 (1.316 to 9.877)	2.638 (0.877 to 12.09)	0.437 (0.287 to 0.722)
68	WTH01A01NCLL0002_H08	1 (1 to 1)	1.872 (1.382 to 2.946)	2.133 (1.458 to 3.144)	3.475 (1.492 to 13.36)
69	WTH01A01NCLL0002_P09	1	3.604 (1.955 to 5.727)	1.086 (0.785 to 1.503)	3.413
70	WTH01A01NCLL0003_C22	1	0.567 (0.361 to 0.848)	1.013 (0.786 to 1.407)	1.441 (0.772 to 2.052)
71	WTH01A01NCLL0003_F24	1	0.909 (0.25 to 2.158)	0.539 (0.392 to 0.64)	0.0644 (0.01 to 0.232)
72	WTH01A01NCLL0003_H20	1 (1 to 1)	0.583 (0.568 to 0.611)	0.513 (0.368 to 0.623)	0.655 (0.639 to 0.679)
73	WTH01A01NCLL0004_A06	1	0.105 (0.0587 to 0.187)	1.401 (1.193 to 1.646)	1.33
74	WTH01A01NCLL0004_I13	1	0.757 (0.425 to 1.008)	0.774 (0.578 to 1.465)	2.198 (1.198 to 3.731)
75	WTH01A01NCLL0004_I18	1	2.064 (1.827 to 2.635)	1.225 (0.751 to 2.478)	0.936 (0.437 to 1.331)
76	WTH01A01NCLL0004_K21	1 (1 to 1)	1.094 (0.725 to 1.579)	0.615 (0.432 to 0.763)	1.277 (1.024 to 1.59)
77	WTH01A01NCLL0004_O16	1	0.707 (0.583 to 0.909)	0.766 (0.65 to 0.996)	0.677 (0.577 to 0.853)
78	WTH01A01NCLL0005_C13	1	3.362 (1.748 to 4.819)	0.63 (0.398 to 0.997)	0.688 (0.355 to 1.427)
79	WTH01A01NCLL0005_D16	1 (1 to 1)	0.722 (0.301 to 1.305)	0.694 (0.655 to 1.234)	0.25 (0.114 to 0.739)
80	WTH01A01NCLL0005_O10	1 (1 to 1)	0.422	0.626 (0.39 to 1.147)	0.0243
81	WTH01A01NCLL0006_M08	1 (1 to 1)	3.101 (1.001 to 6.978)	0.486 (0.155 to 1.296)	13.41
82	WTH01A01NCLL0006_M23	1	0.812 (0.675 to 0.97)	0.867 (0.771 to 0.941)	0.654 (0.42 to 0.792)
83	WTH01A01NCLL0007_A17	1 (1 to 1)	0.527 (0.342 to 0.771)	1.465 (1.013 to 2.945)	1.549 (1.236 to 2.018)
84	WTH01A01NCLL0007_D20	1 (1 to 1)	0.457 (0.284 to 0.764)	1.034 (0.586 to 2.629)	0.224 (0.122 to 0.72)
85	WTH01A01NCLL0007_H14	1	0.313 (0.181 to 0.538)	0.287 (0.235 to 0.422)	0.402 (0.185 to 0.664)
86	WTH01A01NCLL0007_I18	1 (1 to 1)	1.205 (0.871 to 1.682)	1.701 (0.968 to 2.308)	0.84 (0.636 to 1.244)
87	WTH01A01NCLL0007_K13	1	0.929 (0.599 to 1.565)	0.602 (0.492 to 0.724)	1.171 (1.009 to 1.456)
88	WTH01A01NCLL0009_A23	1	0.832 (0.556 to 1.104)	0.997 (0.792 to 1.373)	1.491 (1.417 to 1.617)
89	WTH01A01NCLL0009_E18	1 (1 to 1)	1.269 (1.096 to 1.375)	0.661 (0.412 to 0.913)	1.145 (0.857 to 2.249)
90	WTH01A01NCLL0009_G08	1	1.028 (0.559 to 1.428)	1.472 (0.814 to 2.613)	3.907 (1.142 to 9.913)
91	WTH01A01NCLL0009_I03	1	0.639 (0.524 to 0.702)	0.599 (0.487 to 0.868)	1.091 (0.917 to 1.678)
92	WTH01A01NCLL0009_N17	1	0.729 (0.563 to 0.943)	0.406 (0.207 to 0.584)	0.772 (0.459 to 1.294)
93	WTH01A01NCLL0009_P13	1 (1 to 1)	1.038 (0.941 to 1.187)	0.696 (0.665 to 0.746)	0.985 (0.92 to 1.103)
94	WTH01A01NCLL0010_B22	1	3.164 (1.284 to 4.56)	1.799 (0.921 to 3.439)	1.493 (1.058 to 1.832)
95	WTH01A01NCLL0010_C14	1	1.41 (1.19 to 2.326)	1.792 (0.885 to 2.504)	0.724 (0.447 to 1.131)
96	WTH01A01NCLL0010_D24	1 (1 to 1)	1.261 (0.765 to 3.525)	0.576 (0.473 to 0.798)	1.529 (0.882 to 2.829)
97	WTH01A01NCLL0010_G07	1 (1 to 1)	0.723 (0.543 to 0.878)	0.653 (0.614 to 0.683)	0.593 (0.465 to 0.801)
98	WTH01A01NCLL0010_G24	1	2.618 (1.998 to 3.811)	1.202 (0.518 to 1.93)	0.834 (0.638 to 1.091)
99	WTH01A01NCLL0010_I14	1 (1 to 1)	0.673 (0.498 to 1.014)	0.424 (0.104 to 0.808)	0.0414 (0.01 to 0.48)
100	WTH01A01NCLL0010_K02	1 (1 to 1)	0.528 (0.425 to 0.6)	0.505 (0.346 to 0.67)	0.426 (0.227 to 0.596)
101	WTH01A01NCLL0012_B07	1 (1 to 1)	3.602 (2.551 to 4.867)	1.011 (0.765 to 1.336)	2.482 (1.267 to 4.865)
102	WTH01A01NCLL0012_C06	1 (1 to 1)	2.789 (1.92 to 5.762)	4.777 (1.996 to 9.687)	1.244 (0.497 to 3.03)
103	WTH01A01NCLL0012_G14	1 (1 to 1)	0.377 (0.249 to 0.858)	1.022 (0.305 to 2.573)	0.212 (0.125 to 0.287)
104	WTH01A01NCLL0012_H24	1 (1 to 1)	1.242 (0.707 to 1.717)	0.821 (0.57 to 1.106)	0.681 (0.542 to 0.853)
105	WTH01A01NCLL0012_K18	1 (1 to 1)	0.809 (0.692 to 1.012)	0.828 (0.725 to 0.995)	0.666 (0.536 to 0.778)
106	WTH01A01NCLL0012_M22	1	0.757 (0.619 to 0.9)	0.849 (0.643 to 1.215)	0.561 (0.415 to 0.872)
107	WTH01A01NCLL0013_D14	1	0.37 (0.264 to 0.517)	2.139 (1.886 to 2.426)	0.967 (0.739 to 1.266)
108	WTH01A01NCLL0014_C10	1 (1 to 1)	0.712 (0.604 to 0.932)	0.629 (0.363 to 0.781)	1.264 (0.97 to 1.889)
109	WTH01A01NCLL0014_F22	1 (1 to 1)	1.032 (0.762 to 1.605)	0.768 (0.596 to 0.864)	0.686 (0.559 to 0.807)
110	WTH01A01NCLL0014_G19	1	0.975 (0.388 to 1.765)	5.066 (3.311 to 7.813)	1.608 (1.041 to 3.769)
111	WTH01A01NCLL0014_K13	1	0.674 (0.503 to 1.302)	0.913 (0.496 to 1.332)	1.604 (1.265 to 2.085)
112	WTH01A01NCLL0014_M12	1	0.203 (0.0894 to 0.389)	0.0731	0.667 (0.394 to 1.13)

Table 2. List of 57 annotated genes responding to a 24 h cadmium exposure indicating the spot origin; whether from cDNA library or SSH and relevant stressor. Supplementary to table 2 in publication, thus gene list is presented in same order.

Cluster	Clone ID	Clone Origin - Stressor	Gene most similar to	Species Match	Accession No.	E-Value
1	R10CAR1F09	SSH - Calcium	Actin	<i>Daphnia magna</i>	AJ292554.1	<1.E-05
1	WTH001_0004_I13	cDNA Library - no exposure	Heat Shock Protein 20	<i>Locusta migratoria</i>	ABC84493	1.00E-40
1	IGU001_0047_G03	cDNA Library - no exposure	NADH dehydrogenase subunit 3	<i>Daphnia pulex</i>	DQ340837	<1.E-05
1	WTH001_0009_G08	cDNA Library - no exposure	Stubarista	<i>Drosophila erecta</i>	AB032438	1.00E-91
1	IGU001_0048_B10	cDNA Library - no exposure	Ribosomal protein S14E	<i>Dascillus cervinus</i>	AJ783882	<1.E-05
2	R10CAR1G06	SSH - Calcium	Actin, muscle A2	<i>Bombyx mori</i>	X06363	4.00E-44
2	R05CDR1B12	SSH - Cadmium	Cytochrome c oxidase subunit 1	<i>Daphnia pulex</i>	DQ340836	1.00E-65
2	R01CDF1C01	SSH - Cadmium	Ribosomal protein L22	<i>Tribolium castaneum</i>	XP_970247	9.00E-34
2	IGU001_0054_F10	cDNA Library - no exposure	Ribosomal protein S3A	<i>Culicoides sonorensis</i>	AY603568	8.10E-61
2	WTH001_0002_H08	cDNA Library - no exposure	Trypsin	<i>Aplysina fistularis</i>	AF486488	4.20E-29
2	IGU001_0038_C01	cDNA Library - no exposure	Chymotrypsin B2	<i>Litopenaeus vannamei</i>	Y10665	5.90E-34
2	IGU001_0042_F07	cDNA Library - no exposure	GM2 ganglioside activator protein	<i>Danio rerio</i>	AAH92784	2.0.E-07
2	IGU001_0024_H03	cDNA Library - no exposure	Glycogen synthase	<i>Apis mellifera</i>	XM_624704	<1.E-05
2	IGU001_0049_D08	cDNA Library - no exposure	Gram-negative bacteria binding protein	<i>Anopheles gambiae</i>	AAAB01008859	<1.E-05
3	WTH001_0004_A06	cDNA Library - no exposure	Haemoglobin 2	<i>Daphnia magna</i>	AB021136	3.00E-161
3	WTH001_0013_D14	cDNA Library - no exposure	Heat Shock Protein 70	<i>Petrobia massilliana</i>	AF026520	<1.E-05
3	WTH001_0001_B11	cDNA Library - no exposure	28S ribosomal RNA	<i>Daphnia magna</i>	AF346515	0.00E+00
3	WTH001_0007_A17	cDNA Library - no exposure	Ribosomal protein L32	<i>Spodoptera frugiperda</i>	AF400195	<1.E-05
3	IGU001_0002_E08	cDNA Library - no exposure	ATP synthase a chain	<i>Daphnia melanica</i>	ABD19465	<1.E-05
4	WTH001_0009_I03	cDNA Library - no exposure	T-complex protein 1, alpha subunit	<i>Paleosuchus palpebrosus</i>	AF143496	3.00E-105
4	WTH001_0004_K21	cDNA Library - no exposure	Low-density lipoprotein receptor domain class A	<i>Drosophila melanogaster</i>	AE003516	<1.E-05
4	RAC06	SSH - Cadmium	Low-density lipoprotein receptor domain class A	<i>Drosophila pseudoobscura</i>	CH379070	<1.E-05
4	WTH001_0014_M12	cDNA Library - no exposure	Haemoglobin 1	<i>Daphnia magna</i>	U67067	5.00E-87
4	WTH001_0014_C10	cDNA Library - no exposure	Histone H1	<i>Mytilus galloprovincialis</i>	AY267739	3.00E-09
4	WTH001_0009_P13	cDNA Library - no exposure	Speckled-type POZ protein	<i>Homo sapiens</i>	AK021919	<1.E-05
4	IGU001_0037_F05	cDNA Library - no exposure	ATP synthase a chain	<i>Daphnia melanica</i>	ABD19465	<1.E-05
4	WTH001_0007_K13	cDNA Library - no exposure	Glutamate dehydrogenase	<i>Salmo salar</i>	AJ532825	<1.E-05
4	WTH001_0009_E18	cDNA Library - no exposure	Trypsin	<i>Aplysina fistularis</i>	AF486488	<1.E-05
5	WTH001_0006_M08	cDNA Library - no exposure	Cu/Zn-superoxide dismutase	<i>Hylobates lar</i>	AB087268	<1.E-05
5	WTH001_0001_O20	cDNA Library - no exposure	Receptor for activated protein kinase C	<i>Tribolium castaneum</i>	XP_973579	1.00E-84
5	WTH001_0012_B07	cDNA Library - no exposure	Ubiquitin	<i>Homo sapiens</i>	AF108460	<1.E-05
5	WTH001_0002_P09	cDNA Library - no exposure	Glucose-6-phosphatase	<i>Xenopus laevis</i>	BC045085	<1.E-05
5	IGU001_0027_C03	cDNA Library - no exposure	Carboxypeptidase A1	<i>Mus musculus</i>	BC052661	3.50E-15
6	WTH001_0004_I18	cDNA Library - no exposure	Chitinase	<i>Aedes aegypti</i>	AF026492	<1.E-05
6	WTH001_0010_G24	cDNA Library - no exposure	DD5 (structural constituent of cuticle)	<i>Marsupenaeus japonicus</i>	AB049147	1.00E-06
6	IGU001_0012_C07	cDNA Library - no exposure	Cytochrome c oxidase subunit 1	<i>Daphnia pulex</i>	AAB53197	<1.E-05
6	WTH001_0005_C13	cDNA Library - no exposure	Astacin (zinc metalloprotease)	<i>Drosophila melanogaster</i>	AAM29490	2.00E-33
6	IGU001_0020_A10	cDNA Library - no exposure	Ubiquitin-like/S30 ribosomal fusion protein	<i>Lysiphlebus testaceipes</i>	AY961508	9.90E-38
6	WTH001_0014_F22	cDNA Library - no exposure	Elongation factor 2	<i>Aedes aegypti</i>	AY064104	<1.E-05
6	WTH001_0001_N07	cDNA Library - no exposure	rRNA intron-encoded homing endonuclease	<i>Oryza sativa</i>	AF139989	<1.E-05
7	WTH001_0010_C14	cDNA Library - no exposure	Cuticular protein precursor	<i>Tenebrio molitor</i>	AJ000044	<1.E-05
7	R18BF2A03	SSH - Ibuprofen	Cuticle extracellular matrix structural constituent	<i>Anopheles gambiae</i>	AAAB01008980	6.00E-23
7	IGU001_0049_F02	cDNA Library - no exposure	Cytochrome P450 monooxygenase	<i>Apis mellifera</i>	DQ244075	<1.E-05
7	IGU001_0006_H07	cDNA Library - no exposure	Ferritin 1-like protein A	<i>Daphnia pulex</i>	ABK91577	6.00E-52
7	WTH001_0002_E09	cDNA Library - no exposure	Glutathione peroxidase	<i>Mus musculus</i>	AK029731	<1.E-05
7	WTH001_0007_I18	cDNA Library - no exposure	Ribosomal protein S20	<i>Oncorhynchus mykiss</i>	AJ312336	<1.E-05
7	WTH001_0012_C06	cDNA Library - no exposure	Endoglucanase 2	<i>Reticulitermes speratus</i>	AB019095	2.00E-37
7	IGU001_0043_E01	cDNA Library - no exposure	ATP synthase a chain	<i>Daphnia melanica</i>	ABD19465	1.60E-30
8	WTH001_0012_K18	cDNA Library - no exposure	16S ribosomal RNA	<i>Daphnia magna</i>	AY921452	0.00E+00
8	WTH001_0010_I14	cDNA Library - no exposure	Haemoglobin 3	<i>Daphnia magna</i>	AB021137	1.00E-80
8	WTH001_0005_O10	cDNA Library - no exposure	Haemoglobin 2	<i>Daphnia magna</i>	AB021136	2.00E-112
8	WTH001_0001_L22	cDNA Library - no exposure	Cytochrome b	<i>Daphnia pulex</i>	DQ340836	<1.E-05
8	WTH001_0006_M23	cDNA Library - no exposure	Cytochrome c oxidase subunit 1	<i>Daphnia pulex</i>	ABD19215	<1.E-05
8	IGU001_0046_H06	cDNA Library - no exposure	28S ribosomal RNA	<i>Daphnia magna</i>	AF346515	0.00E+00
8	IGU001_0049_C10	cDNA Library - no exposure	Ribosomal protein L7	<i>Biphylus lunatus</i>	AM049000	<1.E-05
8	WTH001_0007_D20	cDNA Library - no exposure	Succinate dehydrogenase flavoprotein	<i>Anopheles gambiae</i>	AAAB01008849	<1.E-05
9	WTH001_0010_K02	cDNA Library - no exposure	NADH dehydrogenase subunit 2	<i>Daphnia pulex</i>	ABA02335	<1.E-05