Proteomic Changes Associated with Successive Reproductive Periods in Male Polychaetous Neanthes arenaceodentata

Kondethimmanahalli H. Chandramouli, Donald Reish, Huoming Zhang, Pei-Yuan Qian & Timothy Ravasi

The polychaetous annelid Neanthes acuminata complex has a widespread distribution, with the California population referred to as N. arenaceodentata. The reproductive pattern in this complex is unique, in that the female reproduces once and then dies, whereas the male can reproduce up to nine times. The male incubates the embryos until the larvae leave the male's tube 21–28 days later and commences feeding. Reproductive success and protein expression patterns were measured over the nine reproductive periods. The percent success of the male in producing juveniles increased during the first three reproductive periods and then decreased, but the number of juveniles produced was similar through all nine periods. iTRAQ based quantitative proteomics were used to analyze the dynamics of protein expression patterns. The expression patterns of several proteins were found to be altered. The abundant expression of muscular and contractile proteins may have affected body weight and reproductive success. Sperm have never been observed; fertilization occurs within the parent's tube. Proteins associated with sperm maturation and fertilization were identified, including ATPase, clathrin, peroxiredoxins and enolase, which may provide clues to the molecular mechanisms enabling males to reproduce multiple times.
individuals of the same sex will fight, whereas individuals of the opposite sex will lie side by side in a mucoid tube where ova are laid and fertilized\(^1\). A large mature worm of unknown sex is placed with a female with maturing ova visible through her body wall. The diploid numbers of chromosomes in this species complex are \(2N = 18\) (California), \(2N = 22\) (New England), and \(2N = 28\) (Hawaii)\(^3\), with \(2N = 28\) being most common to nereids\(^3\). The male N. arenaceodentata undergoes behavioral, morphological and physiological changes during reproduction and is capable of reproducing nine times\(^9\). Their fighting pattern is altered during the incubation period, in that males fight any intruder. Sperm have never been observed (Reish, pers. obs). The male's reproductive ability declines during the seventh reproductive period, after which the male is subsequently unable to fertilize ova.

Proteomic studies with N. arenaceodentata have analyzed the changes in the transition from egg to larvae\(^8\) and in males and females before and after fertilization\(^9\). Patterns of proteins and phosphoproteins were found to change as eggs become larvae\(^8\). Females, who die after fertilization, showed a 70% decrease in phosphoproteins. This study hypothesized that plasticity in protein expression patterns influences male reproductive success. The key questions were: (i) do males proceed through cycles of changes in protein expression patterns corresponding to successive reproductive periods? and (ii) are the changes in proteomic characteristics that influence male reproductive success achieved through repeated cycles of gametogenesis and sexual maturation? Male reproductive success rate was measured during each successive reproductive period. Males were found to gain body weight through six mating cycles. The number of males reproducing decreased after the fifth cycle and dropped significantly after the sixth.

### Results

#### Variation in male reproductive success during reproductive periods.

Table 1 summarizes the number of males during each reproductive period, their percent success in producing juveniles, the mean and standard deviation of the relative number of juveniles produced, and the length in days of each reproductive period. Reproductive success of male (Fig. 1), as measured by larvae present at the end

| Reproductive period | Number of pairs | Percent reproductive success | Relative number of juveniles | Experimental days |
|---------------------|----------------|-----------------------------|-----------------------------|------------------|
| 0X                  | 5              | --                          | --                          | --               |
| 1X                  | 10             | 80%                         | 2 ± 1.0                     | 37.6 ± 14        |
| 2X                  | 16             | 84.4                        | 3.3 ± 0.8                   | 65.4 ± 11        |
| 3X                  | 19             | 80.7                        | 2.6 ± 1.4                   | 89.2 ± 15        |
| 4X                  | 12             | 70.8                        | 2.4 ± 1.2                   | 148 ± 25         |
| 5X                  | 18             | 62.2                        | 2.2 ± 1.7                   | 176.5 ± 35       |
| 6X                  | 17             | 56.9                        | 2.6 ± 1.1                   | 201 ± 40         |
| 7X                  | 13             | 67                          | 2.5 ± 1.1                   | 235 ± 32         |
| 8X                  | 3              | 64.7                        | 2.7 ± 1.4                   | 260 ± 18         |
| 9X                  | 1              | 100                         | 1                           | 273              |

Table 1. Summary of reproductive success in *Neanthes arenaceodentata*. *Mean and standard deviation.*

Figure 1. Male *Neanthes arenaceodentata*. Inbred population which was established in 1964 collected in Los Angeles Harbor, California, USA.
of the reduced incubation period, was over 80% through the third period (1X–3X). This reproductive success rate started to decline from the fourth (70.8%) through the sixth (56.9%) reproductive period (4X–6X), accompanied by a decrease in the number of juveniles. The reproductive success rate and number of juveniles increased up to 67% through reproductive periods 7X and 8X. One male, approximately one year of age, reproduced successfully through nine periods (9X) but produced fewer than 50 juveniles.

**The proteome of male Neanthes arenaceodentata.** A total of 266 proteins were identified from 2250 spectra, of which 691 were unique. Proteins accounted for an average of 1.88 unique peptides, 2.59 unique spectra, 12.8 total spectra and 10.7% sequence coverage (Supplementary Table S1). Figure 2 lists the percentages of proteins belonging to each Gene Ontology (GO) term, such as biological process (BP), molecular function (MF), and cellular component (CC). The two most prominent BPs were cellular and metabolic processes, representing 70.5% and 53% of proteins, respectively. Ten percent of proteins were related to reproduction. Over 70% of proteins were associated with binding activity, followed by structural (15%) and transport (10.5%) activity of MF. Most proteins were localized to the cytoplasm, with 33% and 15.5% associated with the membrane and nucleus, respectively. Proteins were quantified relative to virgin males (0X), with 113 reporter ion intensities adjusted to 1. Twenty proteins showed changes in expression pattern through the first to eight reproductive periods (1X–8X). Fifteen of these proteins were up-regulated and 5 were down-regulated. Supplementary Table S2 shows the average fold changes and standard deviations of differentially expressed proteins obtained from three replicates. A heat map (Fig. 3) showed clusters of down-regulated (green) and up-regulated (red) proteins during successive reproductive stages (1X–8X), with the dark color representing reference values for 0X. Consistent changes were observed for the replicates.

**Changes in expression patterns of muscular and metabolic proteins.** Table 2 lists the differentially expressed proteins, along with a description of the number of peptides, spectra and sequence coverage matched to each protein. The expression of six actin family proteins, muscle-type A1, actin, actin-1 (cytoplasmic), and actin-2, was increased from the first to sixth reproductive period (1X–6X), but decreased during periods 7X–8X (Fig. 4a). In contrast, actin-muscle and acrosomal process isoform expression decreased from 1X to 6X, but increased during 7X and 8X (Fig. 4a). The three muscle proteins, tropomyosin, myosin-6, and myosin heavy chain, increased, while collagen alpha-2 decreased, during all reproductive periods (1X–8X) (Fig. 4b). Expression of clathrin heavy chain, ATP synthase and calcium ATPase was up-regulated during all reproductive periods (1X–8X) (Fig. 5a). Two antioxidant proteins, pyridoxal biosynthesis protein (PDX1) and peroxiredoxin-2 (PDX2), exhibited opposite expression patterns. PDX1 increased during all reproductive periods, whereas PDX2 expression was decreased from 1X to 6X, but increased thereafter (Fig. 5a). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and enolase were dramatically up-regulated from 1X to 6X, but were reduced during 7X and 8X (Fig. 5b). The expression of phosphoglycerate mutase decreased during the first, sixth, seventh and eighth reproductive periods. Fifteen proteins involved in spermatogenesis, gonad development, oogenesis, and ovulation were identified. Table 3 lists proteins involved in reproductive success, along with a description of the number of peptides, spectra and sequence coverage matched to each protein.
of peptides, spectra and sequence coverage. Several of these proteins, as marked by*, were differentially expressed during reproductive periods (1X–8X).

**Discussion**

In this study, an iTRAQ-based quantitative proteomics approach was used to identify proteomic changes during successive reproductive periods in male *N. arenaceodentata*. Differentially expressed proteins involved in development, muscular activity, gametogenesis and sperm maturation were identified. *N. arenaceodentata* reproductive success was found to be associated with changes in protein expression patterns, with these proteins possibly influencing gametogenesis and sexual maturation.

**Body weight and reproductive success.** Several proteins that influenced reproductive success of males were identified. The abundant expression of muscular and contractile proteins through the sixth reproductive period highlighted the importance of body weight and successful pairing for reproductive success. Further, proteins associated with gametogenesis and sexual maturation may play a crucial role in determining the reproductive capacity of males. The decrease in the number of larvae produced following the seventh reproductive period may be related to reductions in body weight, number of sperm and/or health. The higher reproductive rate, as shown by increased numbers of juveniles, may have resulted from the increase in body weight during the first six mating periods. Age was also found to negatively influence reproductive success rate, as shown by the decrease starting during the fifth mating period. Furthermore, only one male reached the ninth reproductive period, producing fewer than 50 juveniles.

**Complexity of worm proteome.** Proteomics tools have been used to investigate protein expression dynamics during early development, and to assess reproductive patterns in male and female *N. arenaceodentata*. Previous studies are extended by proteomic changes in males occur through nine reproductive cycles. The extraction and purification of proteins from polychaetes poses a challenge in proteomic studies. For example, polychaetes are covered with self-secreted mucilage or slime. Further, *Neanthes* adults are 2–4 cm in length and largely composed of cytoskeletal elements. Protein extraction from whole worms results in a high degree of complexity, which may prevent the identification of less abundant proteins by mass spectrometry. Proteomes degrade soon after lysis, requiring the addition of protease inhibitors during sample preparation. Extraction of proteins was improved by homogenization, followed by precipitation with methanol and chloroform, enhancing protein recovery when compared with...
our previous study. Of the 266 proteins identified, 20 were differentially expressed and 15 were involved in reproductive success.

**Roles of muscular and contractile proteins.** Increased expression of proteins associated with muscular and contractile properties during the first six reproductive periods resulted in an increase in worm body weight, suggesting a direct correlation between worm size. This finding would support the argument that the larger the worms; the higher the mating success, both in laboratory and field populations. Larger worms are generally more successful in fighting and pairing with other worms18. Several elastic or cytoskeletal proteins associated with body musculature, mechanical support and cellular signaling are present in polychaetes19–21. During the incubation period, the body of the male undulates to renew water10, and males fight off other individuals of both sexes to protect their embryos1. The abundant

**Table 2. Proteins differentially expressed during successive reproductive periods in male polychaetous annelid Neanthes arenaceodentata.**

| Protein name                                      | Expression during reproduction | Accession numbers | Molecular weight (KDa) | Identification probability (%) | Unique peptides | Unique spectrum | Total spectrum | Sequence coverage (%) |
|--------------------------------------------------|--------------------------------|-------------------|------------------------|-------------------------------|----------------|----------------|----------------|-----------------------|
| Actins                                           |                                |                   |                        |                               |                |                |                |                       |
| Actin, muscle-type A1                            | up-regulated                    | ACT1_ BOMMO       | 41.80                  | 100                           | 4              | 5              | 9              | 52.4                  |
| Actin (Fragment)                                 | up-regulated                    | ACT_ PROCL        | 36.00                  | 99                            | 1              | 2              | 3              | 20.2                  |
| Actin, cytoplasmic                               | up-regulated                    | ACTC_ STYPL       | 42.00                  | 98                            | 1              | 2              | 4              | 43.2                  |
| Actin-2                                          | up-regulated                    | ACT2_ SACKO       | 42.00                  | 99.7                          | 2              | 2              | 7              | 51.9                  |
| Actin, muscle                                    | down-regulated                  | ACTM_ STRPU       | 41.00                  | 99.9                          | 1              | 1              | 3              | 33.4                  |
| Actin, acrosomal process isoform                 | down-regulated                  | ACTA_ LIMPO       | 42.00                  | 99.2                          | 1              | 1              | 2              | 44.9                  |
| Muscle proteins                                  |                                |                   |                        |                               |                |                |                |                       |
| Tropomyosin                                      | up-regulated                    | TPM_ HALDV        | 32.80                  | 100                           | 2              | 2              | 11             | 7.3                   |
| Myosin-6                                         | up-regulated                    | MYH_ MOUSE        | 223.00                 | 100                           | 4              | 6              | 24             | 2.6                   |
| Myosin heavy chain, striated muscle              | up-regulated                    | MYS_ AEQIR        | 222.00                 | 100                           | 8              | 13             | 128            | 3.5                   |
| Collagen alpha-2(IV) chain                       | down-regulated                  | COA2_ BOVIN       | 25.00                  | 99.9                          | 1              | 2              | 23             | 4.8                   |
| ATPase                                           | ATP synthase subunit alpha, mitochondrial | up-regulated | ATPA_ CAEEL | 57.70                  | 100                           | 2              | 2              | 14             | 15.2                  |
| ATP synthase subunit beta                        | up-regulated                    | ATPB_ MAGSA       | 50.60                  | 99                            | 1              | 3              | 4              | 9.7                   |
| Sarcoplasmic/endoplasmic reticulum calcium ATPase 1 | up-regulated            | AT2A1_ CHICK      | 109.00                 | 100                           | 4              | 7              | 13             | 7.9                   |
| Clathrin heavy chain                             | up-regulated                    | CLH_ DROME        | 191.00                 | 100                           | 2              | 2              | 15             | 1.5                   |
| Antioxidants                                     | Pyridoxal biosynthesis protein (PDX1) | up-regulated | PDX1_ GINBI | 32.90                  | 100.00%                      | 4              | 6              | 15             | 10                    |
| Peroxiredoxin-2 (PDX2)                          | down-regulated                  | PRDX2_ BOVIN      | 21.90                  | 98.60%                        | 1              | 1              | 7              | 5.5                   |
| Glyceraldehyde-3-phosphate dehydrogenase         | up-regulated                    | GP3_ BRUMA        | 36.00                  | 99.9                          | 1              | 2              | 6              | 4.1                   |
| Glyceraldehyde-3-phosphate dehydrogenase 1       | up-regulated                    | G3P1_ YEAST       | 35.70                  | 98.1                          | 1              | 4              | 11             | 2.4                   |
| Enolase                                          | up-regulated                    | ENO_ MASBA        | 48.00                  | 99.2                          | 1              | 1              | 2              | 3.1                   |
| 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase | down-regulated | GPMA_ THETN      | 28.70                  | 99.8                          | 1              | 2              | 14             | 4.4                   |
expression of non-muscle actins facilitates high speed contractions in mature males. Three connecting proteins have been identified in muscle tissue of Neanthes sp. The contractile function of muscle tissue relies on the interactions between actins and myosins. Myosins synergistically modulate reproduction by interacting with other myosin isoforms and with actins. These proteins showed varied expression patterns before and after spawning in N. arenaceodentata. The up-regulation of several myosins and actins during reproduction may facilitate the increased body weight essential for muscular functions during mating suggesting that increased reproductive effort in males may be mediated by muscular and contractile proteins. Down-regulation of other actin proteins, including actin muscle and actin acrosomal process isoforms, after the seventh reproductive period may lead to reduced body weight decreasing reproductive effort. Sperm competition and production are reduced as males age.

Proteins involved in sexual maturation and fertilization. Nereids cease feeding at the end of maturation and depend on fat catabolism and muscle protein to fuel the growth of gametes. In Neanthes, coelomic cells synthesize lipids required for the development of spermatogonia. The abundant expression of glycolytic enzymes in males may indicate an increase in energy metabolism and related sexual maturation. GAPDH, enolase and phosphoglycerate mutase are enzymes involved in the process of sperm maturation. GAPDH activity in sperm affects energy metabolism, maturation and fertilization, whereas the sperm-specific enolase isoform may play a major role during different stages of maturation.
maturation. Differences in the glycosylation patterns of enolase-phosphatase E1 and 14-3-3 probably play important roles in polychaete male reproductive strategies. ATP synthases display different expression profiles during sexual reproduction. Abundant expression of ATP synthase in males may influence reproduction through oxidative phosphorylation. Increased mitochondrial ATPase activity in *N. virens* males may increase the reductive state of enzymes in the respiratory chain, facilitating the production of reactive oxygen species (ROS) and generating oxidative stress. Altered expression of pyridoxal proteins in polychaetes may compensate for oxidative stress.

The bodies of polychaetes are covered by a cuticle made of collagen fibers. A differential distribution of collagen fibers has been reported in male *Teleostei* fish during spawning. The numbers of collagen fibers are reduced due to degradation by proteinases. The clathrin protein complex plays an important role in oocyte maturation through spindle stabilization. Other reproductive proteins, including 40S ribosomal protein S2, spectrin alpha chain, dynein heavy chain, nitric oxide synthase, and 26S protease regulatory subunit, are involved in oogenesis and ovulation. T-complex protein 1 subunit and eukaryotic translation initiation factor facilitate the binding of sperm to the zona pellucida. No significant changes were observed in the expression patterns of these proteins, suggesting that their abundant synthesis was not required to achieve reproductive success.

In conclusion, the differentially expressed reproductive proteins identified in this study were found, for the first time, to be involved in sperm maturation and fertilization during successive reproductive periods. For example, ATP synthase subunit alpha, clathrin heavy chain and calcium-transporting ATPase were up-regulated, whereas actin, acrosomal process isoform and peroxiredoxin were down-regulated. Nevertheless, the expression of energy producing glycolytic proteins, such as GAPDH, enolase, and
phosphoglycerate mutase, which also participate in sperm maturation, was unchanged. These findings suggest that the reproductive proteins identified in males are highly conserved and possibly have evolutionary implications in relation to sperm formation. However, such assumption warrants further investigations.

Neanthes males exhibit different protein expression patterns which influence their reproductive capacity. Males capable of reproducing many times must proceed through cycles of protein expression during gametogenesis and sexual maturation. The majority of nereidid individuals of both sexes undergoes metamorphosis at maturity, spawn in the water column and die. It would be of interest to learn if both sexes undergo protein changes similar to those in male Neanthes arenaceodentata.

Materials and Methods

Experimental worms. All worms were derived from the same inbred population, which was established from six animals collected in Los Angeles Harbor in 1964. Those six specimens have undergone over 200 generations of reproduction without addition of new worms. Immature worms were taken from the laboratory colony of Neanthes arenaceodentata. Juveniles were taken from the laboratory aquaria, placed in separate petri dishes and fed resoaked dried algae (Enteromorpha sp.) as needed. The sex of each worm was determined about one month later by the behavioral assay described above. Each male-female pair was placed in a one gallon (3.78 l) glass jar containing 750 ml of seawater, which was aerated, with the worms fed the green alga Enteromorpha sp. To insure that at least 10 males were obtained during each reproductive period, 140 male-female pairs were mated; however, 10 males were not obtained during the eighth and ninth reproductive periods (Table 1). The pairs were examined 3–5 times per week and their reproductive state was noted. The female was removed after the eggs were laid. The male incubation period lasts about 3–4 weeks, at which time the larvae leave the male’s tube and commence feeding. At the end of the first reproductive period, males are approximately 60 to 85 days old. However, since the male often eats the larvae, the young were removed at days 16 to 18. The number of larvae present was approximated numerically, with values of 1–4 indicating 1–50, 51–100, 101–200 and >200 larvae, respectively. A female with maturing ova was taken from the laboratory colony and placed with a male after each reproductive period. A minimum of five males from each reproductive period and five virgin males (Fig. 1) were frozen, except for periods 8 and 9 because fewer numbers reached that stage. Frozen

| Proteins | Reproductive function | Accession number | Molecular weight (kDa) | Identification probability (%) | Unique peptides | Unique spectrum | Total spectrum |
|----------|-----------------------|------------------|------------------------|--------------------------------|----------------|----------------|---------------|
| Actin, acrosomal process isoform | spermatogenesis | ACTA_LIMPO | 42 | 99.2 | 1 | 1 | 2 |
| ATP synthase subunit alpha | spermatid development | ATPA_DROME | 59 | 100 | 11 | 17 | 62 |
| 40S ribosomal protein S2 | oogenesis | RS2_DROME | 29 | 100 | 5 | 3 | 5 |
| Clathrin heavy chain | sperm individualization | CLH_DROME | 191 | 100 | 2 | 2 | 15 |
| Spectrin alpha chain | oocyte construction | SPTCA_DROME | 278 | 98.6 | 1 | 1 | 2 |
| T-complex protein 1 subunit | binding of sperm to zona pellucida | TCPH_MOUSE | 60 | 100 | 2 | 2 | 10 |
| Peroxiredoxin-4 | spermatogenesis | PRDX4_HUMAN | 31 | 99.9 | 2 | 2 | 9 |
| Calcium-transporting ATPase 4 | spermatogenesis | AT2B4_RAT | 133 | 99.4 | 1 | 2 | 5 |
| Dynamin heavy chain | ovarian fusome organization | DYHC_DROME | 530 | 99.9 | 2 | 2 | 2 |
| 14-3-3 protein zeta | oocyte microtubule polarization | 1433Z_DROME | 28 | 99.2 | 1 | 1 | 1 |
| Nitric oxide synthase | ovulation from ovarian follicle | NOS3_HUMAN | 133 | 97.7 | 1 | 1 | 3 |
| Eukaryotic translation initiation factor 5A | spermatogenesis | IF5A2_HUMAN | 17 | 99.8 | 1 | 1 | 2 |
| ATP synthase subunit beta | gonad development | ATPB_CAEL | 58 | 99.7 | 2 | 3 |
| T-complex protein 1 subunit beta | binding of sperm to zona pellucida | TCPB_BOVIN | 57 | 99.8 | 1 | 1 | 2 |
| 26S protease regulatory subunit 4 | sorocarp development | PRS4_DICDI | 49 | 99.8 | 1 | 1 | 1 |

Table 3. Proteins involved in fertilization and spermatogenesis in male polychaetous annelid Neanthes arenaceodentata. *differentially expressed proteins.
Proteome extraction, digestion and iTRAQ labeling. Two to three males from each reproductive period were suspended in lysis buffer containing 8 M urea and protease inhibitor. The lysates were homogenized (Wheaton Homogenizer, USA) and sonicated (Q Sonica, LLC, USA), and the contaminants were removed by precipitating lysates with methanol:chloroform (1:4, v/v). The pellets were vacuum dried and the protein concentrations measured using a 2-D Quant kit (GE Healthcare, UK). Protein (60 μg) pellets from each reproductive period were reduced, alkylated, and digested with trypsin (Promega, USA) at an enzyme:protein ratio of 1:40 for 16 h at 37 °C. The peptides were desalted using Sep-Pak C18 Vac cartridges (Water Corporation, USA) and labeled using iTRAQ Reagents-8plex Kit (Applied Biosystems, USA). The peptides were suspended in 27 μL of dissolution buffer, 50 μL isopropanol were added to each reagent and the solution transferred to the corresponding peptide samples. The reagents 113–114, 116–119 and 121 were labeled to 0X, 1X, 4X, 5X, 6X, 7X, and 8X, respectively depending on the reproductive period. Since only one male reached the ninth reproductive stage, this stage was not analyzed because the amount of tissue was insufficient. The reporter-peptide mixture was incubated for 60 min and all peptide samples labeled individually were pooled and dried. Samples were fractionated by cation exchange chromatography (SCX), with 15 peptide fractions desalted using Sep-Pak C18 Vac cartridges. The dried peptides were resuspended in 20 μL of LC sample buffer (97% H2O, 3% ACN, 0.1% formic acid) and desalted using C18 Zip-Tips (Millipore Ltd, USA). Six replicates of each sample were analyzed by mass spectrometry (LTQ-Orbitrap Velos; Thermo Scientific, Germany).

Protein identification and quantitation. The MS spectra were searched against the SwissProt database (538,010 sequences) using the search engine Mascot v2.4.0 (Matrix Sciences Ltd, UK). The variable modifications were set to iTRAQ8plex (Y) and oxidation (M), and the fixed modifications to Carbamidomethyl (C). Proteins were identified using Scaffold v4.3.2 (Proteome Software Inc. USA) software. Peptide and protein thresholds were set at 94% and 90%, respectively, a one peptide minimum, a 1.5% protein Prophet false discovery rate (FDR) and Scaffold delta-mass correction. Results were quantitated using the Scaffold Q+ algorithm. The reference reporter 113 was normalized to produce a 1:1 fold change as described by Zhang et al. Replicates variations were evaluated by unsupervised multivariate principal component analysis (PCA), using the Multiple Array Viewer (MeV).

References

1. Reish, D. J. The life history of the polychaetous annelid Neanthes sanda (delle Chiaie), including a summary of development in the family Nereidae. Proc. Sci. 11, 216–228 (1957).
2. Reish, D. J. & Gerlinger, T. V. A view of the toxicological studies with polychaetous annelids. Bull. Mar. Sci. 60, 584–607 (1997).
3. Sutton, R. et al. Chemical signal mediated premetting reproductive isolation in a marine polychaete, Neanthes arenaceodentata. J. Chem. Ecol. 31, 1685–1676 (2005).
4. Fletcher, N., Storey, E. J., Johnson, M., Reish, D. J. & Hardege, J. D. Experience matters: females use smell to select experiment males for parental care. PLoS ONE. 4, e762 (2009).
5. Lee, R. E., Walker, A. & Reish, D. J. Characterization of lipotetin in eggs of the polychaete Neanthes arenaceodentata. Comp Biochem. Physiol. B. Biochem Mol. Biol. 40, 381–386 (2005).
6. Rosen, G. & Miller, K. A post exposure feeding assay using the marine polychaete Neanthes arenaceodentata suitable for laboratory and in situ exposure. Environ. Toxicol. Chem. 30, 730–737 (2011).
7. Winckel, C. J., Walencja, J. E. & Jacobs, D. K. Conocal analysis of nervous system architecture in direct-development juveniles of Neanthes arenaceodentata (Nannelidae). Front. Zool. 7, 17 (2010).
8. Chandramouli, K. H., Reish, D. & Qian, P.-Y. Gel-based and gel-free identification of proteins and phosphoproteins during egg-to-larva transition in polychaete Neanthes arenaceodentata. PLoS ONE. 7, e38814 (2012).
9. Chandramouli, K. H., Ravasi, T., Reish, D. & Qian, P.-Y. Proteomic changes between male and female worms of the polychaete Neanthes arenaceodentata before and after spawning. PLoS ONE. 8, e72990 (2013).
10. Reish, D. J., DeCallibus, K., Dewart, J. & Bube, C. Reproductive longevity in two species of polychaetous annelids. Zoosymposium. 2, 391–395 (2009).
11. Gianì, V. C., Yamaguchi, E., Boyle, M. J. & Seaver, E. C. Somatic and germline expression of pwi during development and regeneration in the marine polychaete annelid Capitella teleta. EvoDevo. 2, 10 (2011).
12. Chatelain, E. H., Breton, S., Lemieux, H. & Blé, P. U. Epitoky in Nereis (Neanthes) virens (Polychaeta: Nereididae): a story about sex and death. Comp Biochem Physiol B. Biochem Mol Biol. 149, 202–208 (2008).
13. Fontés, M., Coulon, J., Delgrossi, M. H. & Thouny, Y. Muscle dedifferentiation and contractile protein synthesis during post-traumatic regeneration by Owenia fusiformis (polychaeta annelid). Cell Differ. 13, 267–82 (1983).
14. Giangrande, A., Licciano, M., Pagliara, P. & Gamb, M. C. Gametogenesis and larval development in Sabella spallanzanii (Polychaeta: Sabellidae) from the Mediterranean Sea. Mar. Biol. 136, 847–861 (2000).
15. Branchini, B. R., Befnek, C. E., Southworth, T. L., Rawat, R. & Dehney, D. D. Chemical analysis of the luminous slime secreted by the marine worm Chaetopterus (Annelida, Polychaeta). PLoS ONE. 9, 247–251 (2014).
16. Chandramouli, K. H., Mok, F. S., Wang, H. & Qian, P.-Y. Phosphoproteome analysis during larval development and metamorphosis in the spionid polychaeta Pseudopolydora vexillosa. BMC Dev Biol. 11, 31 (2011).
17. Chandramouli, K. H., Qian, P.-Y. & Ravasi, T. Proteomics insights: proteins related to larval attachment and metamorphosis of marine invertebrates. Front. Mar. Sci. 1, 52 (2014).
18. Bridges, T. S., Farrar, J. D., Gamble, E. V. & Dillon, T. M. Intraspecific density effects in Nereis (Neanthes) arenaceodentata Moore (Polychaeta: Nereididae). J Exp Mar Biol Ecol. 195, 221–233 (1996).
19. Chandramouli, K. H., Soo, L. & Qian, P.-Y. Differential expression of proteins and phosphoproteins during larval metamorphosis of the polychaete Capitella sp. I. Proteome Sci. 9, 51 (2011).

males were shipped to King Abdullah University of Science and Technology, Saudi Arabia, for proteomic analysis.
Reproductive Periods in Male Polychaetous Neanthes arenaceodentata: Proteomic Changes Associated with Successive Reproductive Success Measurement

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How to cite this article

Competing financial interests: The authors declare no competing financial interests.

Supplementary information accompanies this paper at http://www.nature.com/srep

Additional Information

This study was supported by the King Abdullah University of Science and Technology and a grant award [SA-C0040/UK-C0016] from the King Abdullah University of Science and Technology to P.-Y. Qian and Timothy Ravasi.

Author Contributions

T.R., D.R., P.Y.Q., K.H.C.: Conceived and designed the experiments, D.R.: Collection of worms and reproductive success measurement, K.H.C.: Performed the experiments: H.Z.: MS analysis, K.H.C.: Analyzed and interpreted the data, wrote this article. D.R.: Editing and proofreading. All authors reviewed the manuscript.

Acknowledgements

This study was supported by the King Abdullah University of Science and Technology and a grant award [SA-C0040/UK-C0016] from the King Abdullah University of Science and Technology to P.-Y. Qian and Timothy Ravasi.

Additional Information

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Chandramouli, K. H. et al. Proteomic Changes Associated with Successive Reproductive Periods in Male Polychaetous Neanthes arenaceodentata. Sci. Rep. 5, 13561; doi: 10.1038/srep13561 (2015).

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