Transcriptomic analysis of the red and green light responses in *Columba livia domestica*

Ying Wang¹ · Haiming Yang¹ · Chen Zi² · Zhiyue Wang¹

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Abstract

In this study, 108 paired White King pigeons, randomly divided into three compartments were exposed to green light, red light, and white light followed by 15 h of light exposure, for a 6-month period. Three female birds from each group were selected and ovarian stromal tissue was collected. Pigeon reproductive data were also recorded every day. We performed transcriptome assembly on several tissue samples using Illumina Hiseq 2000 and analyzed differentially expressed genes involving follicle development mechanisms. Reproductive data confirmed that exposure to red and green lights improved pigeon reproduction. In total, approximately 158,080 unigenes with an average length of 753 bp were obtained using the Trinity program. Gene ontology, clusters of orthologous groups, and the Kyoto encyclopedia of genes were used to annotate and classify these unigenes. Large numbers of differentially expressed genes were discovered through pairwise comparisons between groups treated with monochromatic light versus white light. Some of these genes are associated with steroid hormone biosynthesis, cell cycle and circadian rhythm. Furthermore, qRT-PCR was used to detect the relative expression levels of randomly selected genes. A total of 17,419 potential simple sequence repeats were also identified. Our study provides insights into potential molecular mechanisms and genes that regulate pigeon reproduction in response to monochromatic light exposure. Our results and data will facilitate a further investigation into the molecular mechanisms behind the effects of red and green lights on follicle development and reproduction in the pigeon.

Keywords Green light · Pigeons · Red light · Reproduction · Transcriptome assembly

Introduction

Due to a high protein and low cholesterol contents, pigeon meat is considered a valuable component of the human diet (Pomianowski et al. 2009). In China, the White King pigeon (*Columba livia domestica*) has become popular as an important commercial fowl. However, the average clutch interval for these pigeons is approximately 47.44 days long (Khargharia et al. 2003), which includes non-breeding,

mating, nest-building and feeding (Bu et al. 2015). During the feeding phase, young squabs are fed crop milk which is regurgitated from the crop of each parent. Pigeons often forcibly eject their young from the nest between days 23 and 25 (Silver 1984; Vandeputte and Van 1967; Hansen 1966). Furthermore, the time from the end of yolk deposition until oviposition is 1.5 days, which is longer than for chickens and turkeys (Birrenkott et al. 1988). These characteristics affect pigeon reproduction, which requires considerable improvements to meet consumer requirements.

Light is a major environmental factor that affects poultry reproductive activities. Artificial illumination is widely used to promote reproductive performances in birds, which are photosensitive, due to the thalamofugal and tectofugal visual pathways (Huang et al. 1998). Distinct from mammals, these visual systems help birds to distinguish colors and to forage and orient position (Xiao and Frost 2009). The spectral sensitivity of birds is greater than that of humans. Prescott and Wathes (1999) reported that the transmission of longer wavelengths (> 650 nm) to the

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✉ Haiming Yang
yhmdlp@qq.com

¹ College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, Jiangsu Province, China

² Department of Pathology, Linyi People's Hospital, Linyi 276000, Shandong Province, China

avian hypothalamus was 100–1000 times greater than shorter wavelengths (40–450 nm) in pigeons (Hartwig and Van 1979). Casey et al. (1969) concluded that the reproductive development of pullets was stimulated by shorter wavelengths. Cave (1990) also suggested that green light may improve fertility of broiler breeders, while Foss and White (1983) found that red light increased egg production in laying hens. Our previous study demonstrated that the reproductive performance of pigeons was improved by supplementation with monochromatic light, in the morning and evening (Wang et al. 2015). Furthermore, our reproductive data prompted further investigations into the effects of red and green lights.

Not only the light regimen of pigeons but also the spectral sensitivity of these birds has been widely studied (Yamada et al. 1988; Westerhof et al. 1994; Case et al. 2015; Emmerton and Delhis 1980). However, the effects of monochromatic light on the reproductive performance of pigeons and the molecular regulatory mechanisms evoked by light spectra have remained largely unexplored due to a lack of genomic resources. Therefore, in this study, we examined these issues using RNA-seq, which has been used widely to discover genes involved in several biological processes (Teaniuraitemoana et al. 2014). The assembled and annotated transcriptional unigenes are useful for the identification of genes involved in light spectra function and provide insights into the underlying molecular mechanisms involved in pigeon reproduction.

Materials and methods

Animals and light regimen

A total of 108 paired White King pigeons, approximately 24 months old, were obtained from a commercial breeder and randomly divided into nine compartments, forming groups of 12 pairs. Three separate compartments were used for this study, one was exposed to green monochromatic light (GL; 540 nm), one was exposed to red monochromatic light (RL; 660 nm) and one was exposed to white monochromatic light (WL, control; 400–760 nm) (Shenzhen Hongda Technology CO., LTD, Shenzhen, China), followed by 15 h of LED light exposure. Allocation of colored lights was accomplished in blocks of three compartments. The experiment lasted 6 months. All birds were provided with food and water in accordance with the Institutional Animal Care and Use Committee guidelines (Yangzhou University, Yangzhou, China). This study was conducted according to the Institutional Animal Care and Use Committee of the Department of Animal Science and Technology, Yangzhou University.

Sample preparation

Ovaries were isolated from nine female birds of similar body weights (mean weight = 557.02 g) and the same physiological period was selected from three groups (three birds from each group). Ovarian stromal tissues were collected at the same time of day (09:00–10:30) from each of the light groups. Pigeons were anesthetized with sodium pentobarbital, at a dose of 2.5 mg per 100 g body weight. Tissues were rapidly frozen in liquid nitrogen and stored at -80°C until analysis.

RNA extraction, cDNA library construction and sequencing

Nine RNA-seq libraries were constructed from female pigeons raised under red (R1, R2, R3), green (G1, G2, G3), or white (W1, W2, W3) light. Total RNA was isolated from each sample using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA samples were prepared following manufacturer's instructions from the Illumina's kit for transcriptome analysis. The 2100 Bioanalyzer (Agilent Technologies, Wilmington, DE, USA) was used to measure RNA integrity, purity and quantity. RNA samples yielded integrity values (RIN) of 9.5–9.8. Approximately 6 μg of total RNA from each ovary sample was purified using oligo(dT) magnetic beads, generating short fragments (approximately 200 bp). Random hexamer primers were used for first-strand cDNA synthesis to obtain double-stranded DNA. The second strand was synthesized using buffers, dNTPs, RNase H and DNA polymerase I. The double-strand cDNA was synthesized, purified and eluted using EB buffer for end-repair and poly(A) addition. The cDNA library was built after fragments were ligated with sequencing adaptors. The libraries were sequenced by a paired-end technique using the Illumina HiSeq 2000 sequencing platform, generating raw reads.

Data processing, assembly and functional annotation

To ensure data quality, we removed low-quality reads that were below the threshold quality of Q20 and lengths that were less than 35 bp. We also removed low-quality bases from 3' ends that had threshold quality values less than Q20 and reads that were below threshold lengths of 35 bp after deleting 5' end sequences containing N bases. The Trinity program (version r2013/11/10) was used for transcriptome assembly (Grabherr et al. 2011). The Trinities were clustered into unigenes using TGICL tools (Pertea et al. 2003).

The assembled unigenes were searched against the NR (NCBI non-redundant protein sequences database) (<http://>

www.ncbi.nlm.nih.gov/), Swiss-Prot (<http://www.expasy.ch/sprot/>) and KEGG (<http://www.genome.jp/kegg/>) using the BLASTX algorithm with a cut-off E-value of 1.0×10^{-5} . Gene ontology (GO) (<http://www.geneontology.org/>) unigene annotations were obtained at the second level according to cellular component, component function and biological process. Furthermore, COG (<http://www.ncbi.nlm.nih.gov/COG/>) was used to predict and classify unigene function.

Differential gene expression analysis

The reads per kilobase of exon model per million mapped reads method was used to calculate and normalize the assembled unigenes, using software bowtie2 (version 2.1.0) (Mortazavi et al. 2008; Langmead and Salzberg 2012). The DESeq (2010) R package was used to calculate *P* values, fold changes and false discovery rates (FDRs) (<http://biocductor.org/packages/release/bioc/html/DESeq.html>) (Wang et al. 2010). FDRs were used to establish threshold *P* values in multiple tests and analyses. FDRs less than or equal to 0.05 and $\log_2$ ratios greater than or equal to 1 were used as thresholds to determine the significance of differences in unigene expression levels (Benjamini and Yekutieli 2001).

Identification of simple sequence repeats (SSRs)

The MISA tool (<http://pgrc.ipk-gatersleben.de/misa/>) was used to identify potential SSRs among all unigenes (Sharma et al. 2007). The identified SSRs could be classified based on repeat motifs and number of repeats, respectively, as hexamer-5, pentamer-5, tetramer-5, trimer-5, dimer-6, and mono-10.

Validation of RNA-seq results by qRT-PCR

Total RNA was isolated by TRIzol and reverse transcribed using the Fast Quant RT Kit (TIANGEN Biotech Co., LTD, Beijing, China). SuperReal PreMix (SYBR Green; TIANGEN Biotech Co., Ltd., Beijing, China) was used for qRT-PCR. PCR reactions were made up in 20 μ L volumes, consisting of 10 μ L 2 $\times$ SuperReal Premix and 0.4 μ L 50 $\times$ ROX Reference Dye. PCR was performed under the following conditions: cDNA was denatured at 95 $^{\circ}$ C for 15 min, followed by 40 cycles of 95 $^{\circ}$ C for 10 s and 60 $^{\circ}$ C for 32 s. The primers used are listed in Table S3. All measurements were conducted in triplicates. Pigeon glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA was used as an internal control and the relative gene expressions were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

Statistical analysis of reproductive performance

Egg production, fertility and birth rates of different groups were recorded during the experimental period (120 days). Data are expressed as the mean $\pm$ standard deviation. One-way analysis of variances using SPSS 13.0 software (SPSS Inc., Chicago, IL) was used to perform statistical analysis of all data, except RNA-seq data. The statistical significance of differences observed among groups was evaluated by a least significant difference post hoc multiple comparison test. $P < 0.05$ was considered statistically significant.

Results

Monochromatic lights enhance the reproductive performance of pigeons

Egg production of pigeons illuminated with monochromatic light of different wavelengths is shown in Table 1. The egg production of each pair of pigeons, over 120 days, in the group exposed to red light was greater than the control group ($P = 0.023$). Furthermore, no statistical difference was found between red and green light treatments ($P = 0.223$). The birth rate of birds exposed to red light was lower when compared with the control group ($P = 0.004$). However, no statistical difference was found between green light treatment and the control group ($P = 0.470$). Although the fertility rate of birds exposed to green light was slightly higher than the control group, it was not statistically significant ($P = 0.649$). These data suggest that monochromatic light enhances the reproductive performance of pigeons. Optimal light spectra increased egg production, making it a potentially useful tool for egg production on pigeon farms.

Table 1 Reproductive performance of pigeons under monochromatic lights

Characteristics	RL	GL	WL
Egg production ^d	9.08 $\pm$ 1.34 ^a	8.06 $\pm$ 0.96 ^{ab}	6.89 $\pm$ 0.91 ^b
Birth rate ^e	62.11 $\pm$ 4.90 ^a	73.35 $\pm$ 2.36 ^b	76.05 $\pm$ 4.88 ^b
Fertility rate ^f	84.81 $\pm$ 3.27	85.85 $\pm$ 4.41	83.81 $\pm$ 7.89

^{a-c}Mean $\pm$ standard error. Different superscripts indicate significantly different values ($P < 0.05$)

^dEgg production = Number of egg production per paired (120 days)

^eBirth rate = Number of birth eggs/number of fertility eggs (120 days)

^fFertility rate = Number of fertilized eggs/number of hatching eggs (120 days)

Transcriptome profiling by RNA-seq

To further determine the effects of monochromatic light on pigeon reproductive performance, we performed RNA-seq analysis using ovaries isolated from female pigeons exposed to monochromatic red, green, or white light. Transcriptome sequencing generated 11.60 million, 13.91 million and 11.90 million reads from red, green and white light-treated

pigeon ovaries, respectively. Detailed results from sequencing analysis and assembly are shown in Table 2. A total of 14.31 Gb, 17.21 Gb, and 14.69 Gb of clean bases were obtained from red, green and white treatments, respectively. Trinity software was used to assemble the reads into a transcriptome because a reference genome was not yet available for *Columba livia domestica* (Grabherr et al. 2011; Rosenkranz et al. 2008). The transcriptome consisted of 158,080

Table 2 Summary of the sequence assembly of cDNA derived from ovaries of pigeons exposed to monochromatic red (R), green (G), or white (W) light

Sample name	Raw reads	Clean reads	Clean bases (bp)	Valid rate (%)	Q30 (%)	GC content (%)
R1	34,308,030	33,956,666	4,233,261,678	98.71	94.20	53.00
R2	46,189,550	45,791,510	5,703,000,324	98.77	93.39	54.00
R3	35,457,214	35,120,026	4,373,461,594	98.67	93.10	56.00
G1	34,824,252	34,496,654	4,307,674,927	98.95	93.75	53.00
G2	52,137,574	51,753,588	6,449,024,891	98.95	94.11	54.00
G3	52,177,132	51,806,014	6,457,456,976	99.00	94.29	53.00
W1	42,935,054	42,523,434	5,298,145,890	98.71	93.79	55.00
W2	33,365,916	33,090,892	4,132,171,509	99.07	93.2	54.00
W3	42,670,976	42,283,004	5,258,718,979	98.59	92.07	55.00

Fig. 1 Distribution of unigene length in *Columba livia domestica*

unigenes (mean length: 753 bp) with an N50 of 939 bp. The length of the unigenes ranged from 201 to 24,681 bp (Fig. 1). Sequences were deposited into the NCBI Transcriptome Shotgun Assembly Sequence Database (R1: SRR2148830, R2: SRR2148831, R3: SRR2148832; G1: SRR2148833, G2: SRR2148834, G3: SRR2148835; W1: SRR2094777, W2: SRR2094789, W3: SRR2094799).

Annotation of predicted proteins

BLASTX searches against the NR and Swiss-Prot protein databases were conducted using a cut-off E-value of 10^{-5} . A total of 30,515 (19.30%) unigenes showed high homology with sequences in the NR database, while 22,421 (14.18%) unigenes were matched to known genes in Swiss-Prot (Table 3). The NR database search identified a large number of unigenes that matched sequences from *Columba livia* (27.59%), *Haliaeetus leucocephalus* (5.08%), *Pseudopodoces humilis* (2.01%), *Gallus gallus* (1.91%), *Anas*

platyrhynchos (1.47%), *Aptenodytes forsteri* (1.20%), *Nipponia nippon* (1.14%), and *Melopsittacus undulatus* (0.85%) (Fig. 2).

GO, COG, and KEGG classifications

The GO, COG, and KEGG classifications were used to characterize the function of *Columba livia domestica* transcripts. A total of 9362 unigenes were allocated to GO categories, which were categorized into 64 functional groups (Fig. 3) (Kanehisa et al. 2008). Biological processes, cellular components and molecular functions were the three main ontologies identified, with ‘cellular processes’, ‘cell parts’, and ‘binding’ found to be the most common categories across groups. ‘Metabolic processes’, ‘cell’, and ‘catalytic activity’ groups were also high in all groups. In addition, some genes were found to be associated with ‘cell killing’, ‘mitochondrion-association adherens complexes’, and ‘morphogen activity’.

Table 3 Functional annotation of *Columba livia domestica* unigenes

Annotation database	NR	Swiss-Prot	COG	KEGG	GO	ALL
Unigenes	30,515	22,421	14,956	15,185	9362	158,080

NR non-redundant database, Swiss-Prot Swiss protein database, COG cluster of orthologous groups, KEGG Kyoto encyclopedia of genes and genomes, GO gene ontology

Fig. 2 Species distribution for homology search of Illumina sequences against the NR database. Of the matched genes, 27.59% showed similarities with *Columba livia*, followed by *Haliaeetus leucocephalus* (5.08%), *Pseudopodoces humilis* (2.01%), *Gallus gallus* (1.91%), *Anas platyrhynchos* (1.47%), *Aptenodytes forsteri* (1.20%), *Nipponia nippon* (1.14%), *Melopsittacus undulatus* (0.85%), and others (32.25%)

Fig. 3 GO classification map. The abscissa represents the second-level GO term of the three GO categories: biological process, cellular component, and molecular function

Annotated unigenes were further grouped using the COG database, which demonstrated the effectiveness of the annotation process and completion of the transcriptome library. Our analysis classified 14,956 unigenes into 25 COG categories (Fig. 4). The cluster for ‘signal transduction mechanisms’ represented the largest group (4092, 27.36%), followed by ‘general function predictions’ (3643, 24.36%) and ‘post-translational modifications, protein turnover and chaperones’ (1727, 11.55%). The ‘nuclear structure’ (69, 0.0046%) and ‘cell motility’ categories (58, 0.0039%) constituted the smallest groups.

All unigenes were mapped to reference canonical pathways in the KEGG database (Fig. 5) (Zhao et al. 2011). We found 15,185 unigenes matched to 338 KEGG pathways. The most highly enriched pathways were those related to metabolism ($n=2008$), regulation of actin cytoskeleton ($n=1108$) and PI3K/Akt signaling ($n=838$). Enriched pathways also included those involved in circadian rhythm, circadian entrainment, Wnt signaling, estrogen signaling, reproduction and ovarian steroidogenesis (Table S1) (Martin et al. 2010; Conesa et al. 2005). Taken together, these GO,

COG, and KEGG annotations are invaluable for identifying potential genes to better understand the effects of different light wavelengths on pigeons.

Identification of SSRs

All unigenes in this study were used to identify SSRs. A total of 17,419 potential SSRs were identified in 158,080 unigenes, which included two types of mononucleotide, four types of dinucleotide, ten types of trinucleotide, tetranucleotides, pentanucleotides and hexanucleotide SSRs. Mononucleotides SSRs were the most abundant microsatellite repeat unit (12,139, 69.69%), followed by dinucleotide SSRs (2626, 15.08%), trinucleotide SSRs (2317, 13.30%), tetranucleotide SSRs (308, 1.77%), pentanucleotide SSRs (21, 0.12%) and hexanucleotide SSRs (8, 0.05%) (Table 4). Our data demonstrate that A/T accounted for approximately 83.49% of the mononucleotide SSRs. Meanwhile, AC/GT accounted for 48.78% of the dinucleotide SSRs and AGG/CCT accounted for 37.33% of the trinucleotide SSRs.

Fig. 4 Results from Cluster of Orthologous Groups (COG) analysis of 14,956 annotated unigenes

Monochromatic light exposure affects gene expression

A previous study established that exposure to different monochromatic light wavelengths affects the reproductive performance of pigeons, possibly due to the degree by which the wavelength can penetrate the hypothalamus (Hartwig and Van 1979). The hypothalamic region of the brain, via secretion of gonadotrophin receptor hormone, is associated with reproductive performance in birds (Lewis and Morris 2000). All treatments were compared to the WL control: fold changes > 1.0 in $\log_2$ ratios and FDRs < 0.05 were regarded as significant differences (Cai et al. 2015). Therefore, we compared the effects of exposure to red, green, or white light on gene expression in pigeons (Fig. 6). The results demonstrated that unigenes affected by green and white light exposures numbered 3279 (of which 1520 were upregulated and 1759 were downregulated), while red and white light treatments modulated 1993 unigenes (1076 were upregulated and 917 were downregulated) (Table S2). Furthermore, a total of 455 unigenes (227 were upregulated and 228 were downregulated) were regulated by red and green light treatments. Our analysis also revealed that 849 upregulated genes were induced only by green light, while 689 genes were induced only by red light. These results indicate that monochromatic light exposure had significant effects on gene transcription in pigeons.

Finally, we performed qRT-PCR analysis to confirm RNA-seq and computational analysis. Genes related to

cell cycle (*E2F transcription factor 1 (E2F1)*, *histone deacetylase 2 (HDAC2)*), circadian rhythm (*nuclear receptor ROR β* , *PER*), ovarian steroidogenesis (*bone morphogenetic protein 15 (BMP15)*), steroid hormone biosynthesis (*11 β -hydroxysteroid dehydrogenase type 1 (HSD11B1)*, *cytochrome P450*), and TGF β signaling (*BMP receptor type 2 (BMPR2)*, *Smad10*) were validated by qRT-PCR. Our qRT-PCR results supported the data produced by the transcriptome analysis (Table S4). For instance, exposure to red light led to the upregulation of *HSD11B1* and *BMP15* expression, while green light produced the opposite effect. Moreover, qRT-PCR verified that several genes were differentially expressed by all three wavelengths.

Discussion

Our results demonstrated that monochromatic lights affect the reproductive performance of pigeons. Compared with white light, red light improved egg production, which was in agreement with observations made by Foss and White (1983). However, contrary to Lewis et al. (2007), our study suggested that green light also stimulated egg production. Gongruttanatum (2011) concluded that red light exposure increased the concentration of blood estradiol and the growth rate of ovarian follicles in hens, while Liu et al. (2015) indicated that green light increased estrogen levels in chickens, thereby providing potential mechanistic insights into monochromatic light-induced biological responses.

Fig. 5 Pathway assignment based on the Kyoto encyclopedia of genes and genomes (KEGG) database. **a** Metabolism, **b** genetic information processing, **c** environmental information processing, **d** cellular processes, **e** organismal systems

Our transcriptome analysis also suggested that most DEGs were involved in hormone synthesis. Contrary to Jones et al. (1982), our study showed that green light also increased egg production. Harrison et al. (1969) inferred pullets reached sexual maturity 4–5 months earlier under green light, when compared with red or white light treatment; however, egg production was inferior. Although birth rate opposed that of egg production, monochromatic light supplementation had the same effect on the patterns of both indices (Wang et al. 2015). This effect on birth rate may be related to monochromatic light penetration. Halevy et al. (2006) found that monochromatic green light penetrated the eggshell and had a direct effect on muscle development in the embryo. Furthermore, RNA-seq analysis provided insights into the reproductive performance of pigeons exposed to monochromatic light. Consistent with our previous study, genes involved in circadian rhythm were affected by monochromatic light supplementation. In addition, previous RNA-seq results indicated that monochromatic light modulates circadian genes (*RORβ*, *PER*) (Yang et al. 2006; Nicholas and Paul 2002).

TGFβ signaling has been shown to play an important role in folliculogenesis (Drummond 2005), a process that involves specification of primordial germ cells by bone morphogenetic proteins (BMPs). BMPs regulate cellular functions and play a role in mammalian reproductive function (Wozney et al. 1988). In this study, *BMP15* was differentially expressed under different light conditions. *BMP15* promotes granulosa cell processes and is involved in early follicle growth (Shimasaki et al. 2002; Yan et al. 2001). Moore and Shimasaki (2005) found that heterozygous *BMP15* mutant sheep exhibited increased fertility, suggesting that BMP15 pro-peptides act in a dominant-negative fashion. Our results demonstrate that *BMP15* is upregulated by red light exposure, but downregulated when exposed to green light, suggesting this gene may be associated with fertility rates. Furthermore, as part of the TGFβ signaling pathway, *BMPR2* is essential for controlling post-implantation of embryos (Nagashima et al. 2013). Our study supports this finding. These and other regulatory genes will be excellent candidates for future research.

Table 4 Summary of simple sequence repeats (SSRs) identified from the ovary transcriptome of *Columba livia domestica*

SSR type	Repeats	Total number	Proportion of total SSRs (%)	
Mononucleotide	Total	12,139	69.69	
	A/T	10,135	58.18	
	C/G	2004	11.50	
Dinucleotide	Total	2626	15.08	
	AC/GT	1281	7.35	
	AG/CT	503	2.89	
	AT/AT	814	4.67	
	CG/CG	18	0.10	
Trinucleotide	Total	2317	13.30	
	AAC/GTT	173	0.99	
	AAG/CTT	115	0.66	
	AAT/ATT	259	1.49	
	ACC/GGT	138	0.79	
	ACG/CGT	10	0.06	
	ACT/AGT	10	0.06	
	AGC/CTG	448	2.57	
	AGG/CCT	865	4.97	
	ATC/ATG	103	0.59	
	CCG/CGG	196	1.13	
	Tetranucleotide	Total	308	1.77
	Pentanucleotide	Total	21	0.12
Hexanucleotide	Total	8	0.05	

Steroid hormone biosynthesis is important for reproduction (Sanderson 2006) and is controlled by several highly substrate-selective cytochrome P450 enzymes and a number of steroid dehydrogenases and reductases. *HSD11B1* belongs to a family of steroid dehydrogenases that regulate ruminant pregnancy duration (Simmons et al. 2010). *HSD11B1* expression was upregulated in the endometrium

of cattle between days 7 and 13 of pregnancy, during conceptus elongation (Forde et al. 2009). *HSD11B1* catalyzes the interconversion of inactive cortisone and active cortisol, which acts on the pituitary gland to reduce responsiveness to GnRH, and reduce the frequency of LH pulses, which are vital processes for ovulation (Michael et al. 2003; Oakley et al. 2009). In our study, we found that *HSD11B1* was upregulated by red light exposure but downregulated by green light, suggesting a possible involvement of this gene or protein product in egg production. Moreover, *HSD11B2* was identified in the pigeon transcriptome. P450 enzymes are involved in estrogen metabolism and are physiologically significant for maintaining estrogen homeostasis (Tsuchiya et al. 2005; Simpson et al. 1994). These genes are related to ovarian steroidogenesis and steroid hormone biosynthesis pathways, suggesting these pathways are involved in the molecular mechanism of monochromatic light-mediated hormone synthesis. Further exploration is needed to unravel the interplay between these genes.

The cell cycle pathway also plays a critical role in the regulation of monochromatic light. *E2F1* regulates progression through G1/S transition and is essential for cell cycle proliferation and development (Wu et al. 2001). Hou et al. (2002) demonstrated *E2F1*-induced apoptosis in response to mitogenic or non-mitogenic stress signals. Wilting et al. (2010) reported that *HDAC2*-regulated p53–p21-independent pathways were critical for maintaining cell cycle progression. Interestingly, Ma et al. (2012) showed that deletion of either *HDAC1* or *HDAC2* had little effect on oocyte development, whereas deletion of both genes arrested follicle development at the secondary follicle stage. Cobrinik (2005) showed that HDACs play a role in E2F function in the cell cycle, and that both proteins cooperate in regulating genes involved in cell cycle progression (Zhang et al. 2003). Both these genes were downregulated following monochromatic light exposure in our study, indicating that HDACs and E2Fs may also

Fig. 6 Differential gene expression induced by monochromatic light exposure. Gene expression was analyzed in pigeons treated with red (R), green (G), or white (W) light. Venn diagrams are shown of dif-

ferentially downregulated (a) and upregulated (b) genes among the treatment groups

function in this process. Further research into the molecular mechanisms and roles of the genes identified in our study will provide a better understanding on the effects of different light wavelengths on *Columba livia domestica* reproduction.

Conclusions

Monochromatic light affects the reproductive performance of pigeons, with red light improving egg production. Our RNA-seq analyses demonstrate that monochromatic light affects the transcription of genes involved in steroid hormone biosynthesis during reproduction. The expression patterns of *BMP15* and *HSD11B1* were consistent with the effects of red light on pigeon reproductive performances, indicating these genes are involved in red light-mediated egg production. Genes associated with the cell cycle (*E2F1*), circadian rhythm (*ROR β* , *PER*) and TGF β signaling (*BMPT2*, *Smad10*) were also differentially expressed under monochromatic light exposure. Our study provides new insights into the molecular mechanisms behind monochromatic light exposure in pigeons.

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Data availability Data are available at NCBI Transcriptome Shotgun Assembly Sequence Database (R1: SRR2148830, R2: SRR2148831, R3: SRR2148832; G1: SRR2148833, G2: SRR2148834, G3: SRR2148835; W1: SRR2094777, W2: SRR2094789, W3: SRR2094799).

Compliance with ethical standards

Conflict of interest The author(s) declare no competing financial interests.

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