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Comparison of Fecundity Traits between *Mauremys reevesii* and *Trachemys scripta* based on Gene Family Analysis

He Gang[1,2,3#], Wu Bin[3#], Yu Yali[2], Wang Weiping[4], Zhang Zhihong[5], He Li[2]

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ABSTRACT

Background: Chinese pond turtle (CPT, *Mauremys reevesii*) is a typical species with temperature-dependent sex determination (TSD). Pond slider turtle (PST, *Trachemys scripta*) is considered one of the worst invasive species in the world. In order to analyze the adaptive evolution of fecundity traits in CPT and TSD, the rapidly evolving gene families were obtained and functional enrichment analysis was performed. At the same time, CPT and PST species-specific genes were also enriched for their fecundity traits.

Methods: Orthofinder software was employed to search homologous genes based on protein sequence. CAFÉ software was used to obtain the expansion and contraction gene families of CPT and PST. Then, GO terms and pathway enrichment analyses were performed using TBtools software and Swissprot database.

Result: In CPT, 159 rapid expansion gene families GO enrichment results showed that the reproductive activities related pathways were mainly three significant enrichment process, spermatogenesis, steroid hormone mediated signaling pathway and male gamete generation. 2 rapidly contraction gene families showed that there was no significant enrichment pathway related to reproduction. The pathway of significant enrichment were RNA biosynthetic process, regulation of RNA metabolic process and regulation of cellular metabolic process. The gene families unique to the species were significantly enriched in pathways DNA replication-dependent nucleosome organization, DNA replication-dependent nucleosome assembly and negative regulation of megakaryocyte differentiation. In PST, 15 rapid expansion gene families GO enrichment results showed that the most significant GO terms mainly consisted of biological processes which were involved in endocrine process, regulation of endocrine process and cellular response to steroid hormone stimulus, but spermatogenesis and male gamete generation gene families also expanded. 58 rapidly contraction gene families showed that the pathways of significant enrichment were endocrine process, cellular response to steroid hormone stimulus and regulation of endocrine process. At the same time, estrogen secretion, binding of sperm to zona pellucida and sperm-egg recognition gene family also rapidly contraction. The gene families unique to the species were significantly enriched in pathways adenylate cyclase-activating G protein-coupled receptor signaling pathway, double-strand break repair and regulation of potassium ion transport.

Key words: Fecundity traits, Gene families, *Mauremys reevesii*, Rapidly evolving, *Trachemys scripta.*

INTRODUCTION

Among vertebrates, turtles have many unique characteristics providing biologists with opportunities to study novel evolutionary innovations and processes (Warren *et al.*, 2020). Chinese pond turtle (CPT, *Mauremys reevesii*), belongs to the family Geoemydidae and is widely distributed in east Asia. It is a classic TSD species and there is a male bias at low temperatures and a female bias at high temperatures (Ru *et al.*, 2017). CPT is freshwater turtle, which has crucial values in medicine and edible. In recent years, the number of wild CPT presented significantly reduced, due to the overfishing and destruction of habitat. In order to alleviate the contradiction between the huge market demand and the scarcity of wild CPT, the artificial breeding industry of CPT developed rapidly. However, the healthy development of artificial breeding was limited due to several factors, primarily containing few studies on the artificial breeding, nonstandard breeding technology and confused genealogical files of parents. Both population models and genome analysis can be used effectively for aquatic animal comparative studies (Wu *et al.* 2018, 2020, 2021a, 2021b). This study aimed to explore the potential molecular mechanism involved the adaptive evolution of fecundity traits in CPT, which can provide theoretical basis for artificial breeding and formulation of protection procedures for wild turtle. Pond slider turtle (PST, *Trachemys scripta*) is a well-known species that is native to the

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[1] Jiangxi Key Laboratory for Mass Spectrometry and Instrumentation, East China Institute of Technology, Nanchang 330013, China.

[2] Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Wuhan 430223, China.

[3] Fisheries Research Institute of Jiangxi Province, Nanchang 330000, China.

[4] Jiangxi Provincial Aquatic Biology Protection and Rescue Center, Nanchang 330000, China.

[5] Institute of Biological Resources, Jiangxi Academy of Sciences, Nanchang 330096, China.

[#] These authors contributed equally to this work.

Corresponding Author: He Li, Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Wuhan 430223, China. Email: 1170746090@qq.com

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southeastern United States where three subspecies have been identified: *T. s. scripta*, *T. s. elegans* and *T. s. troostii* (Jackson *et al.*, 2008). The invasion patterns of PST have shown that reproductive populations (in central and northern Italy) are associated to warmer climates compared with its native range (Ficetola *et al.*, 2009), while Rödder *et al.* (2009) suggested that climatic requirements during egg incubation could be a major driver for the species' native geographic distribution. PST is considered one of the worst invasive species in the world (Lowe *et al.*, 2000).

Turtles are considered as long-lived organism. Many individuals have been recorded as living more than 100 years (Gibbons, 1987; Shaffer *et al.*, 2013). CPT is one of the most common and widespread semi-aquatic turtles in East Asia. The unusually long lifespan of some individuals makes this turtle species a potentially useful model organism for studying the molecular basis of longevity (Yin *et al.*, 2016). Turtles have the apparent ability to escape functional impairment, especially reproductive ability and no increased in age-related mortality (Miller, 2001). Turtles are an ideal model organism for research on the molecular basis of fecundity and longevity. Following a multi-decadal tagging program at loggerhead nesting areas in Greece, 28 individual turtles have been identified with reproductive longevities greater than 21 yrs, ranging from 22 to 33 yrs (Dimitris *et al.*, 2020). A 100-year-old tortoise on Espanola, the southernmost island of the Galapagos Islands, has saved the island's endangered species of Galapagos giant tortoise by breeding so well, AFP reported. Diego, who weighs 80kg and is nearly 90 cm long, has produced more than 800 hatchlings, equivalent to 40% of the island's total Galapagos tortoises. In addition, according to a report in China's Nanfang Daily, Three Money Turtles (*Cuora trifasciata*), all over 60 years old, from the world's largest captive breeding base, can each lay more than 10 eggs a year.

MATERIALS AND METHODS

Genomic data collection and analysis work was conducted between May and October at the 2021 in Jiangxi Key Laboratory for Mass Spectrometry and Instrumentation, East China Institute of Technology.

Data availability

The assembled genome sets are available from NCBI, *Mauremys reevesii* (PRJNA699301), *Trachemys scripta* (PRJNA634151), *Gopherus evgoodei* (PRJNA559383), *Chrysemys picta* (PRJNA210179), *Chelonia mydas* (PRJNA675851), *Terrapene carolina* (PRJNA435426), *Chelonoidis abingdonii* (PRJNA611832), *Dermochelys coriacea* (PRJNA655518), *Pelodiscus sinensis* (PRJNA221645).

Orthologous family identification

To define a set of conserved genes for cross-taxa comparison, we employed Orthofinder (v2.3.3) to search homologous genes of 16 species based on protein sequence (Emms *et al.*, 2015; 2019). Toolbox for Biologists (TBtools, v1.096) software was used to obtain whole-genome representative CDS sequences from the genome and then representative protein sequences were extracted for subsequent analysis according to the corresponding relationship between the CDS sequence number and the protein sequence number (Chen *et al.*, 2020). To identify expanding and contracting gene ortholog groups across the phylogeny, the species tree was obtained from the Orthofinder (v2.3.3) (Emms *et al.*, 2017; 2018) and the time of differentiation was obtained from the website Timetree (Kumar *et al.,* 2017). We estimated the gene numbers on internal branches using a random birth and death process model implemented in the software CAFÉ (v3.0), a tool for the statistical analysis of the evolution of the size of gene families (De *et al.*, 2006).

GO enrichment and statistical analyses

After the expansion and contraction gene families of the target species were obtained by CAFÉv3.0 software, the expansion and contraction gene sets were further obtained according to the results of Orthofinderv2.3.3 software. Then, GO terms and pathway enrichment analyses were performed using TBtools and Swissprot with the default parameters. And the significantly enriched clusters associated with reproductive activities were reported (Benjamini–Hochberg corrected $q < .05$). Visualization of results were carried out using the R package 'ggplot2' (Hadley *et al.,* 2021).

RESULTS AND DISCUSSION

In 9 species, orthogroups number of genes was 183227 and percentage of genes in orthogroups was 98.3%; number of orthogroups was 18783; number of species-specific orthogroups was 329; number of genes in species-specific orthogroups was 1870; mean orthogroup size was 9.6; median orthogroup size was 9; number of orthogroups with all species present 13527; number of single-copy orthogroups was 10363. In CPT, expanded families were 805 and 159 gene families showed rapidly expansion; Contraction gene families were 653, but only 2 gene families showed rapidly contraction, as shown in Fig 1. For CPT, 2812 genes were obtained; 697 genes were lost; 17325 genes were no change; in general, CPT showed genes expansion. In PST, expanded families were 235 and 15 gene families showed rapidly expansion; Contraction gene families were 1747 and 58 gene families showed rapidly contraction, as shown in Fig 1. For PST, 555 genes were obtained; 1964 genes were lost; 16801 genes were no change; in general, PST showed genes contraction.

Functional enrichment analysis of 2403 genes in CPT (genes of rapidly expansion 159 gene families) was executed. Only clusters associated with reproductive activities enrichment with $p < 0.05$ were listed (Fig 2). The most significant GO terms mainly consisted of biological processes which were involved in spermatogenesis, steroid hormone mediated signaling pathway and male gamete generation. Gonadal maintenance and gametogenesis are highly regulated and coordinated biological processes. Functional enrichment analysis of 347 genes in PST (genes of rapidly expansion 15 gene families) was executed. Only clusters associated with reproductive activities enrichment with p<0.05 were listed (Fig 3). The most significant GO terms mainly consisted of biological processes which were involved in endocrine process, regulation of endocrine process and cellular response to steroid hormone stimulus, but spermatogenesis and male gamete generation gene families also expanded. Rapid expansion of genes associated with spermatogenesis, male gamete production and steroid hormone could be genetic adaptation of TSD and multiple paternity in CPT and PST (Xiong, 2019). Both adult testes and ovaries play central roles in the reproductive system by secreting steroid hormones and producing gametes (Hossain *et al.*, 2012). Estrogen and steroid signaling are involved in the sex determination process of TSD animals (Dodd *et al.* 2008; Ramsey *et al.* 2009). Estrogen is also involved in the sex reversal process of TSD animals. During egg incubation, treating embryos at male incubation temperature with exogenous estrogen can make the original male develop into female larvae (Crews *et al.* 1996). Reproductive behavior is one of the most important behaviors of animals. Only through the complex reproductive behavior, can ensure the success of reproduction, maintain the continuation and development of the population. Jennion and Petrie (2000) summarized the genetic advantages brought by multiple paternity to offspring: multiple paternity not only increases the genetic diversity of offspring, but also increases the fitness of selection and reproductive success rate. Sperm competition refers to the competition between sperm from two or more males for the right to inseminate eggs. Sperm competition is competition at the cellular level of species, a necessary process of sexual selection and the ultimate form of competition between males (Pomiankowski *et al.* 2021). The rapid expansion of sperm-related genes is conducive to sperm competition, as the most important part of sexual

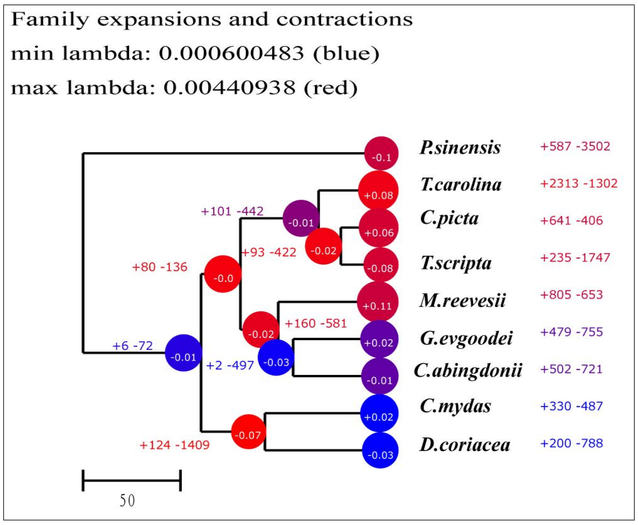

Fig 1: Orthologous family analysis.

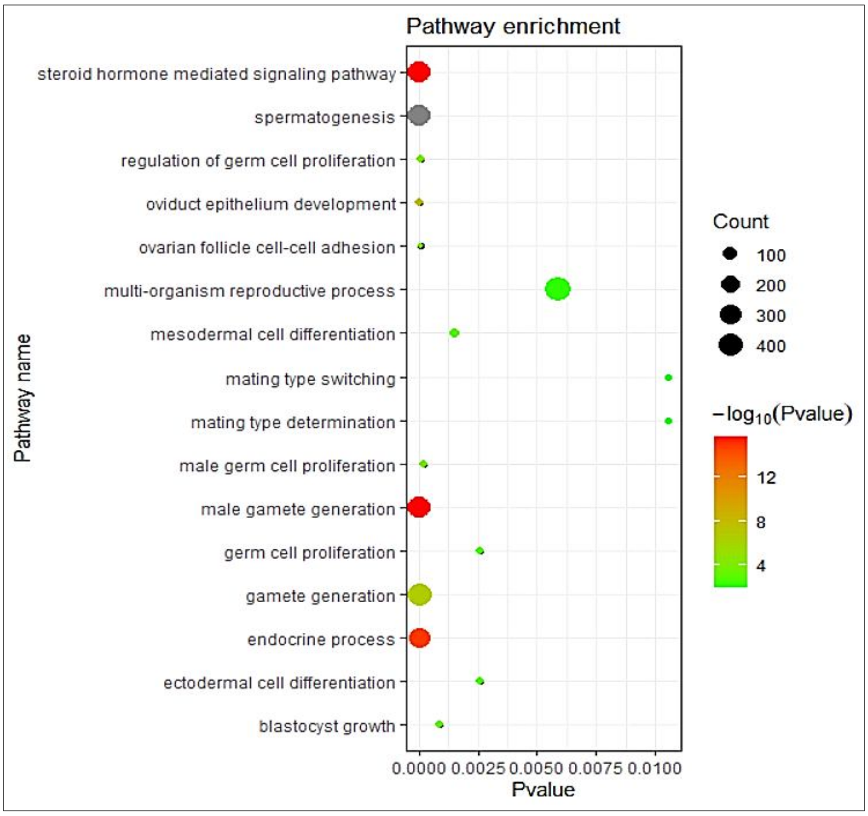

Fig 2: GO and pathway enrichment analysis of rapidly expansion genes in *Mauremys reevesii* (only clusters associated with reproductive activities enrichment with p < .05 were listed).

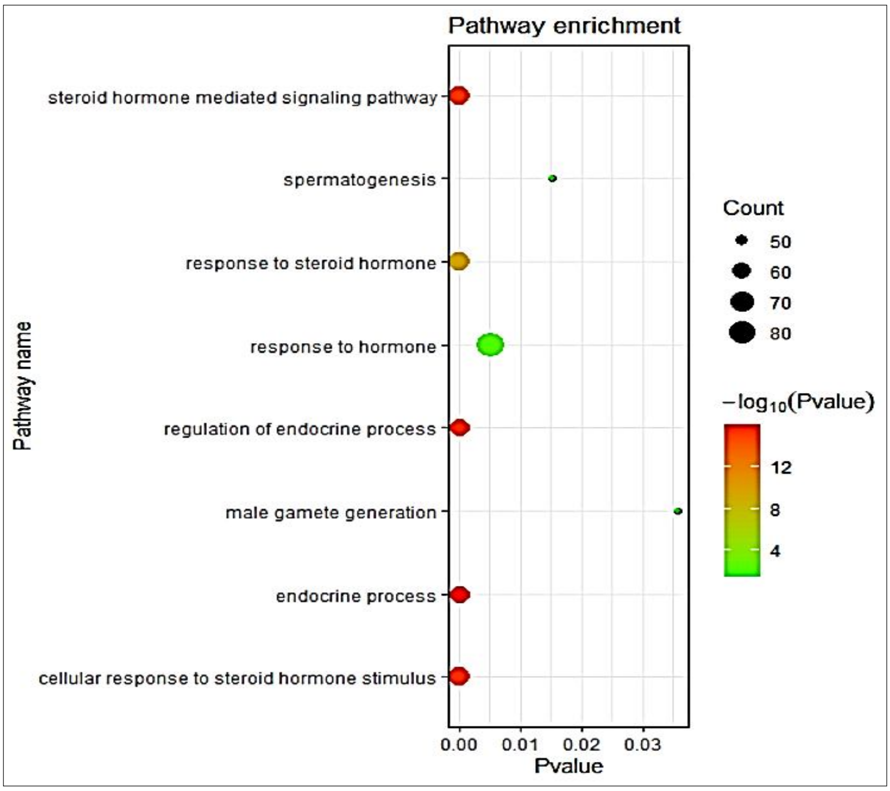

Fig 3: GO and pathway enrichment analysis of rapidly expansion genes in *Trachemys scripta* (only clusters associated with reproductive activities enrichment with $p < .05$ were listed).

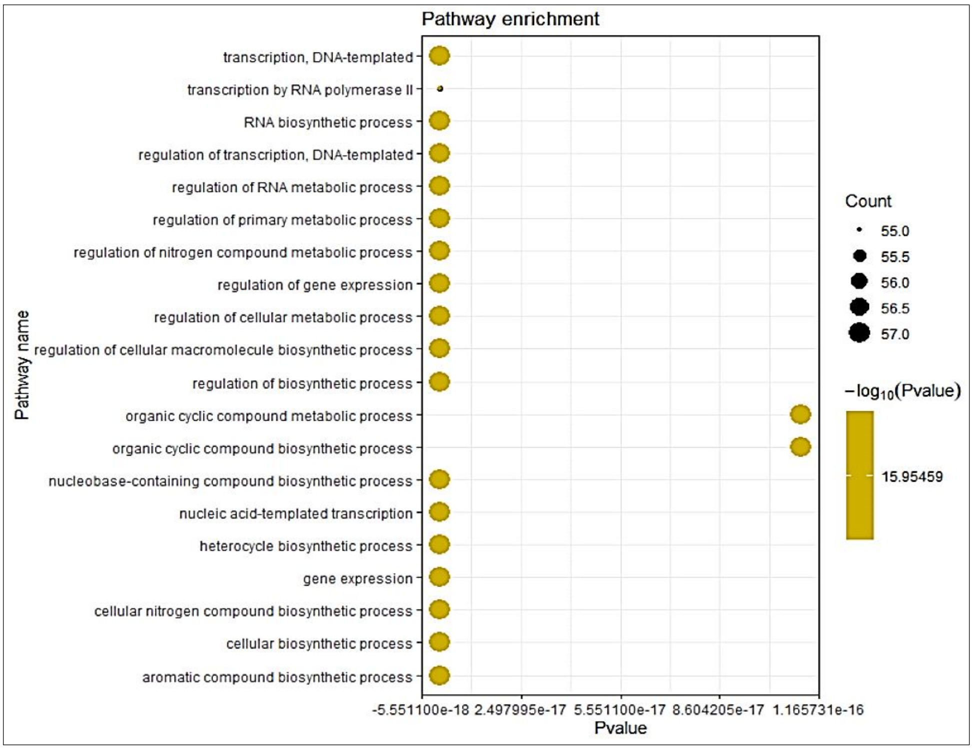

Fig 4: GO and pathway enrichment analysis of rapidly contraction genes in *Mauremys reevesii* (top 20 pathways with the most significant enrichment were listed).

selection. Sperm competition is of great significance to sexual behavior mechanism, sexual organ morphology and genetic structure and diversity of CPT population. Therefore, it is important to study sperm competition in CPT and further research on the mechanism and influence of sperm competition should be carried out gradually.

Functional enrichment analysis of 128 genes (2 gene families of CPT rapidly contraction and the gene set was

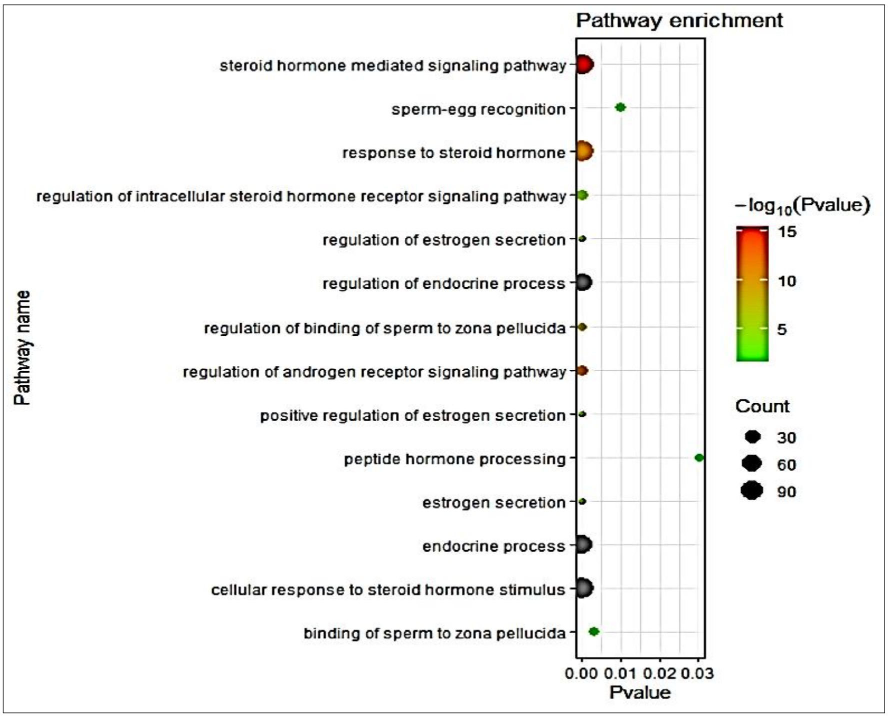

Fig 5: GO and pathway enrichment analysis of rapidly contraction genes in *Trachemys scripta* (only clusters associated with reproductive activities enrichment with $p < .05$ were listed).

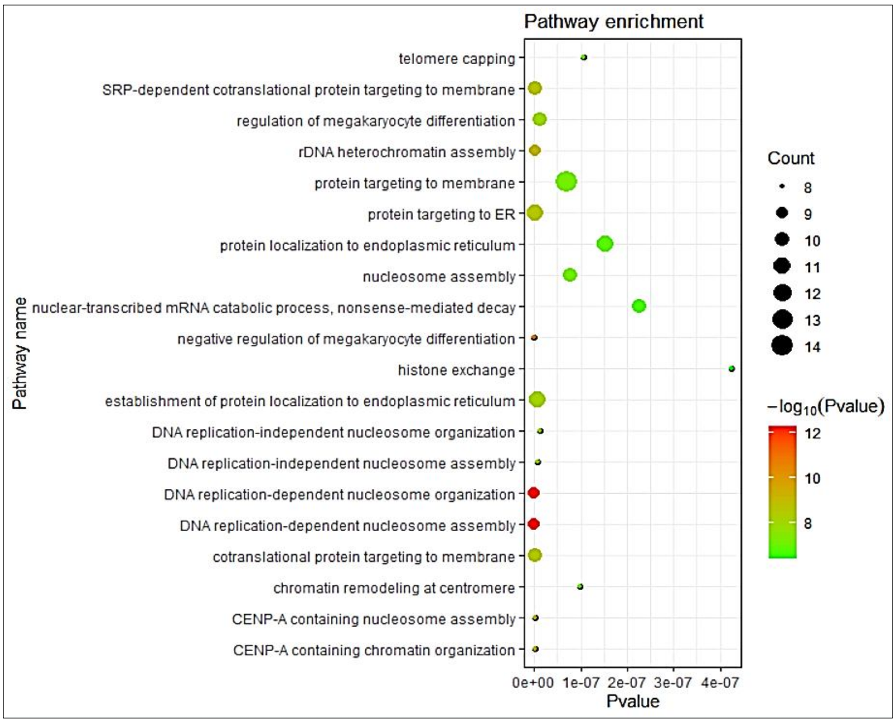

Fig 6: GO and pathway enrichment analysis of unique genes in *Mauremys reevesii* (top 20 pathways with the most significant enrichment were listed).

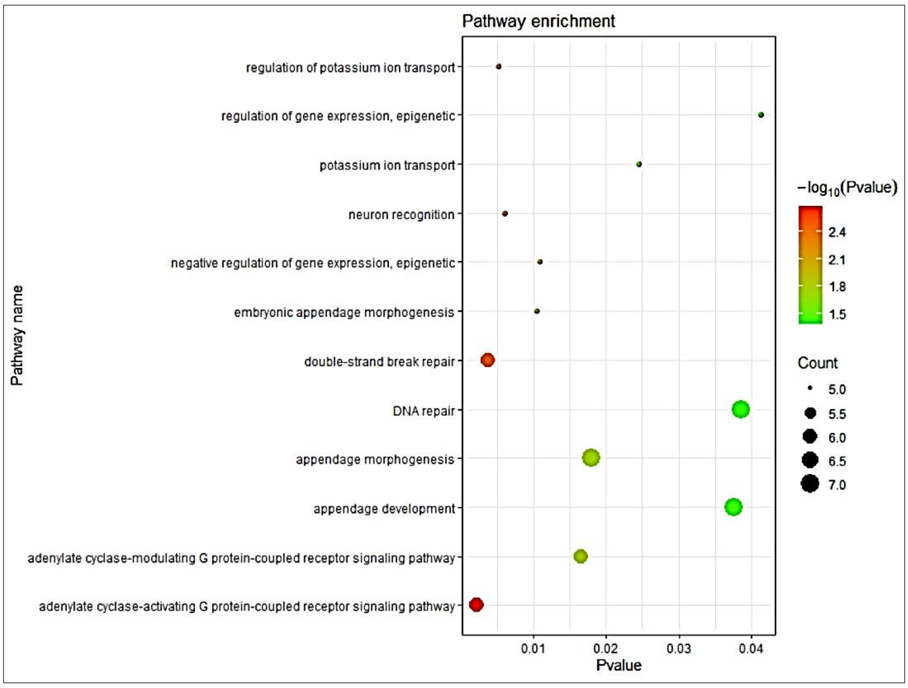

Fig 7: GO and pathway enrichment analysis of unique genes in *Trachemys scripta.*

the corresponding *Gopherus evgoodei* genes) was executed. There was no significant enrichment pathway related to reproduction. The pathway of significant enrichment were RNA biosynthetic process, regulation of RNA metabolic process and regulation of cellular metabolic process (Fig 4). Functional enrichment analysis of 667 genes (58 gene families of PST rapidly contraction and the gene set was the corresponding *Chrysemys picta* genes) was executed. Only clusters associated with reproductive activities enrichment with $p<0.05$ were listed (Fig 5). The pathways of significant enrichment were endocrine process, cellular response to steroid hormone stimulus and regulation of endocrine process. At the same time, estrogen secretion, binding of sperm to zona pellucida and sperm-egg recognition gene family also rapidly contraction. Many of examples demonstrate the apparent ability to escape functional impairment, especially reproductive ability and no increased in age-related mortality. CPT, was considered as long-lived organism. The rapid contraction of genes associated with RNA biosynthetic process, regulation of RNA metabolic process and regulation of cellular metabolic process could be genetic adaptation to CPT growth slow, long reproductive life characteristics. Compared with CPT, sexually mature females of PST were larger and the egg weight and width were also bigger. This may be due to the rapidly contraction of the estrogen-secretion-related gene family, leading to the need for individuals to reach a larger size to become sexually mature, with larger eggs and more adaptable offspring. Particular attention should be paid to the rapidly contraction of the sperm-egg recognition related gene family in PST, which may indicate that it is more prone to interspecific hybridization, increasing the risk and harm of biological invasion.

The gene families unique to CPT were significantly enriched in pathways DNA replication-dependent nucleosome organization, DNA replication-dependent nucleosome assembly and negative regulation of megakaryocyte differentiation (Fig 6). Nucleosome has become one of the important research contents in epigenetics. Xiong (2019) confirmed that tortoise has ZZ/ZW sex determination system and speculated that its sex determination mechanism may be GSD+TE type. Epigenetic inheritance has a certain influence on early embryonic gonadal development and plays a role in the maintenance of adult gonadal function. The gene families unique to PST were significantly enriched in pathways adenylate cyclase-activating G protein-coupled receptor signaling pathway, double-strand break repair and regulation of potassium ion transport (Fig 7). This may be related to the special mechanism of energy balance, antioxidant stress and acid-base balance in PST under hypoxia, which provides valuable reference for sports medicine research and the exploration of physiological and biochemical mechanisms related to anti-aging medicine.

CONCLUSION

The rapid expansion of sperm-related genes is conducive to sperm competition, as the most important part of sexual selection. Sperm competition is of great significance to sexual behavior mechanism, sexual organ morphology and genetic structure and diversity of freshwater turtles such as *Mauremys reevesii* and the *Trachemys scripta*. The rapid contraction of genes could be genetic adaptation to *Mauremys reevesii* growth slow and long reproductive life characteristics. Compared with *Mauremys reevesii*, sexually mature females of *Trachemys scripta* were larger and the egg weight and width were also bigger. This may be due to the rapidly contraction of the estrogen-secretion-related gene family and the rapidly contraction of the sperm-egg recognition related gene family in *Trachemys scripta*, which may indicate that it is more prone to interspecific hybridization, increasing the risk and harm of biological invasion. *Mauremys reevesii* may be a good model for studying TSD and multiple paternity, while *Trachemys scripta* is one of the representative species for studying the physiological and ecological mechanisms of turtle crossbreeding, locomotion and aging medicine.

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Conflicts of interest

The authors declare no conflict of interest.

Ethics declaration

Data acquisition in the model was conducted in accordance with the Regulations of the People's Republic of China for the Implementation of Wild Aquatic Animal Protection promulgated in 1993, adhering to all ethical guidelines and legal requirements in China.

Data availability declaration

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Chen, C.J., Chen, H. and Zhang, Y., *et al.* (2020). TBtools: An integrative toolkit developed for interactive analyses of big biological data. Molecular Plant. 13(8): 1194-1202.

Crews, D. (1996). Temperature-dependent sex determination: The interplay of steroid hormones and temperature. Zoological Science. 13(1): 1-13.

De, B.T., Cristianini, N. and Demuth, J.P., *et al.* (2006). CAFE: A computational tool for the study of gene family evolution. Bioinformatics. 22(10): 1269-1271.

Dimitris, M., Dean, C.J., Lourenço, G. *et al.* (2020). Riggall "Reproductive Longevity of Loggerhead Sea Turtles Nesting in Greece,". Chelonian Conservation and Biology. 19(1): 133-136.

Dodd, K.L., Wibbels, T. (2008). Estrogen inhibits caudal progression but stimulates proliferation of developing müllerian ducts in a turtle with temperature-dependent sex determination. Comparative Biochemistry and Physiology. Part A, Molecular and Integrative Physiology, 150(3): 315- 319.

Emms, D.M. and Kelly, S. (2015). OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. Genome Biology. 16: 157.

Emms, D.M. and Kelly, S. (2017). STRIDE: Species tree root inference from gene duplication events. Mol. Biol. Evol. 34(12): 3267-3278.

Emms, D.M. and Kelly, S. (2018). STAG: Species tree inference from all genes. bioRxiv https://doi.org/10.1101/267914.

Emms, D.M. and Kelly, S. (2019). OrthoFinder: Phylogenetic orthology inference for comparative genomics. Genome Biology. 20: 238.

Ficetola, G.F., Thuiller, W., Padoa-Schioppa, E. (2009). From introduction to the establishment of alien species: Bioclimatic differences between presence and reproduction localities in the slider turtle. Diversity and Distributions. 15: 108-116.

Gibbons, J. (1987). Why do turtles live so long? BioScience. 37: 262-269.

Hadley, W., Winston, C., Lionel, H. *et al.* (2021). Package 'ggplot2'. Create Elegant Data Visualisations Using the Grammar of Graphics. Version 3.3.4

Hossain, M.M., Sohel, M.M., Schellander, K. and Tesfaye, D. (2012). Characterization and importance of microRNAs in mammalian gonadal functions. Cell Tissue Res. 349: 679-690.

Jackson, J.T., Starkey, D.E., Guthrie, R.W., Forstner, M.R.J. (2008). A mitochondrial DNA phylogeny of extant species of the genus Trachemys with resulting taxonomic implications. Chelonian Conserv Biol. 7: 131-5.

Jennion, M.D. and Petrie, M. (2000). Why do females mate multiply? A review of the genetic benefits. Biological reviews of the Cambridge Philosophical Society. 75(1): 21-64.

Kumar, S., Stecher, G. and Suleski, M., *et al.* (2017). TimeTree: A resource for timelines, timetrees and divergence times. Molecular Biology and Evolution. 34: 1812-1819.

Lowe, S., Browne, M., Boudjelas, S. *et al.* (2000). 100 of the World's Worst Invasive Alien Species. A selection from the Global Invasive Species Database. Published by the Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN). Available at http://www.issg.org/pdf/publications/worst_100/english_100_worst.pdf .

Miller, J. (2001). Escaping senescence: demographic data from the three-toed box turtle (*Terrapene carolina triunguis*). Experimental Gerontology. 36: 829-832.

Pomiankowski, A., Wedell, N. (2021). Sexual selection: Large sex combs signal male triumph in sperm competition. Current Biology. 31(10): 478-481.

Ramsey, M., Crews, D. (2009). Steroid signaling and temperature-dependent sex determination- Reviewing the evidence for early action ofestrogen during ovarian determination in turtles. Seminars in Cell and Developmental Biology. 20(3): 283- 292.

Rödder, D., Schmidtlein, S., Veith, M., Lötters, S. (2009). Alien invasive slider turtle in unpredicted habitat: A matter of niche shift or of predictors studied? PLOS ONE. 4(11): e7843.

Ru, W., Hua, L., Wei, Y., Li, W., Cao, D., Ge, Y., *et al.* (2017). The Effect of the *Cyp19a1* Gene Methylation Modification on Temperature-dependent Sex Determination of Reeves' Turtle (*Mauremys reevesii*). Asian Herpetol. Res. 8(3): 213-220.

Shaffer, H.B., Minx, P., Warren, D.E., *et al.* (2013). The western painted turtle genome, a model for the evolution of extreme physiological adaptations in a slowly evolving lineage. Genome Biology. 14: 1-23.

Warren, B.S., James, F.P, Theodore J.P. *et al.* (2020). An annotated chromosome-level reference genome of the red-eared slider turtle (*Trachemys scripta elegans*). Genome Biology and Evolution. 4(12): 456-462.

Wu, B., Wang, H.H. and Fu, H.Y. *et al.* (2018). Estimating some population parameters and stock assessment of Dark Sleeper *Odontobutis potamophila* in the Gaosha River, Wuyuan County, Jiangxi Province. Indian Journal of Animal Research. 52(5): 664-668.

Wu, B., Wang, W.P. and Wang, H.H. *et al.* (2020). A retrospective Analysis on the Population Viability of the Yangtze River Dolphin or Baiji (*Lipotes vexillifer*). Indian Journal of Animal Research. DOI: 10.18805/ijar.B-1238.

Wu, B., Xu, J.G. Wang, J.M. *et al.* (2021a). Population viability analysis of Yangtze Finless Porpoise in the Yangtze main steam suggesting that a total ban on productive fishing could be decisive. Indian Journal of Animal Research. 55(12): 1515-1520.

Wu, B., Wang, W., Zhang, H. (2021b). The analysis of reproduction-related genes provides insights into the adaptive evolution of fecundity traits in Yangtze Finless Porpoise. Indian Journal of Animal Research. DOI: 10.18805/IJAR.BF-1418.

Xiong, L. (2019). The sex determination system and epigenetic studies on early development and adult function maintenance of gonad in *Mauremys reevesii* Wuhu. Anhui Normal University.

Yin, H., Nie, L., Zhao, F., *et al.* (2016). De novo assembly and characterization of the Chinese three-keeled pond turtle (*Mauremys reevesii*) transcriptome: Presence of longevity-related genes. Peer. J. 4: e2062.