

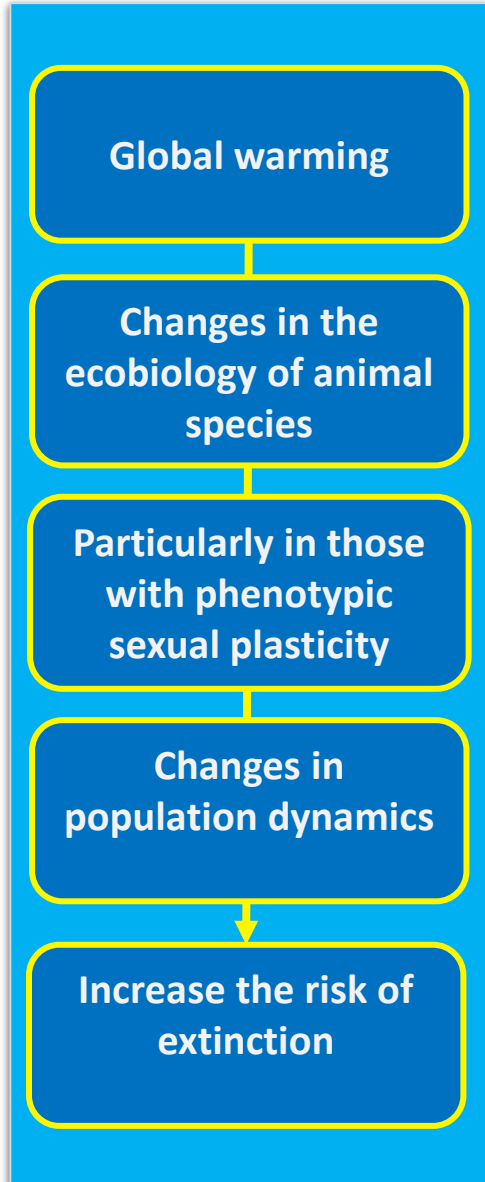
Genetic mechanism of brain-gonad axis regulating sex differentiation in sex-biased zebrafish families

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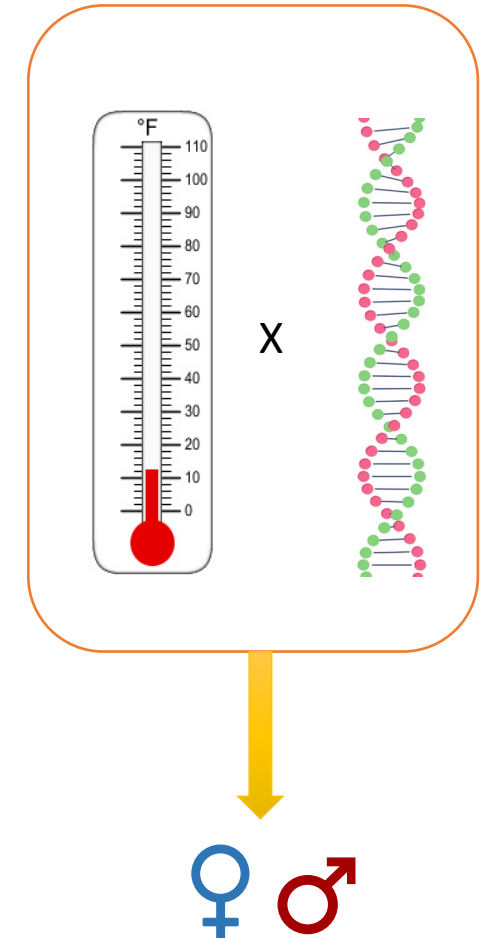
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- Phenotypic sexual plasticity in response to environmental conditions leads to skewed sex ratio, which can affect population dynamics and loss of biodiversity
- The hypothalamic-pituitary-gonadal axis is a well-known neuronal reproductive axis in vertebrates that triggers reproduction by activating the endocrine machinery
- The brain is a key factor that combines environmental stimuli with physiological responses to direct gonadal fate in animal species whose sex determination is influenced by the environment

- Zebrafish (*Danio rerio*) is an excellent research model to study the mechanism of sex determination and differentiation and the effect of environmental factors on sexual fate in vertebrates
- Sex in zebrafish is determined by interaction between genetic and environmental factors ($G \times E$) and sex ratio in this model is family-specific
- Temperature is one of the most important environmental factors that influences sex determination in zebrafish and other species with $G \times E$ sex determination mechanism



- Understanding the mechanisms underlying G×E in thermosensitive species, is of fundamental research importance for the conservation of natural populations and livestock species under rapid climate changes
- The zebrafish brain exhibits sex-specific differences at hormonal and transcriptional levels and is highly plastic even after sexual maturity

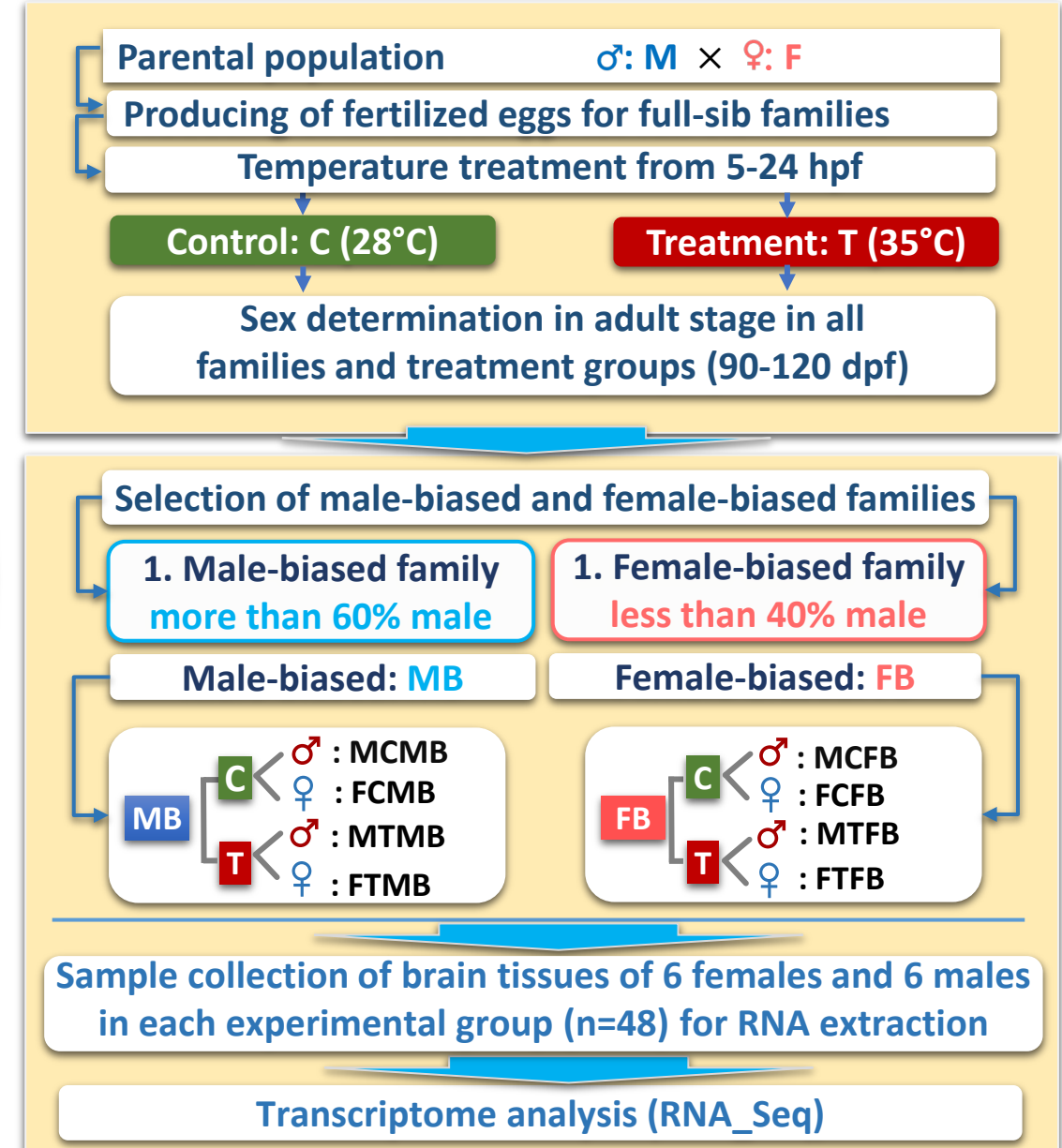
➤ **The main goal of this study was:**

- to investigate the genetic mechanism underlying phenotypic sexual plasticity in the model animal zebrafish using transcriptome profiling
- to gain new insights into the regulatory mechanism of the brain-gonadal axis on sex ratio disequilibrium in response to elevated temperature

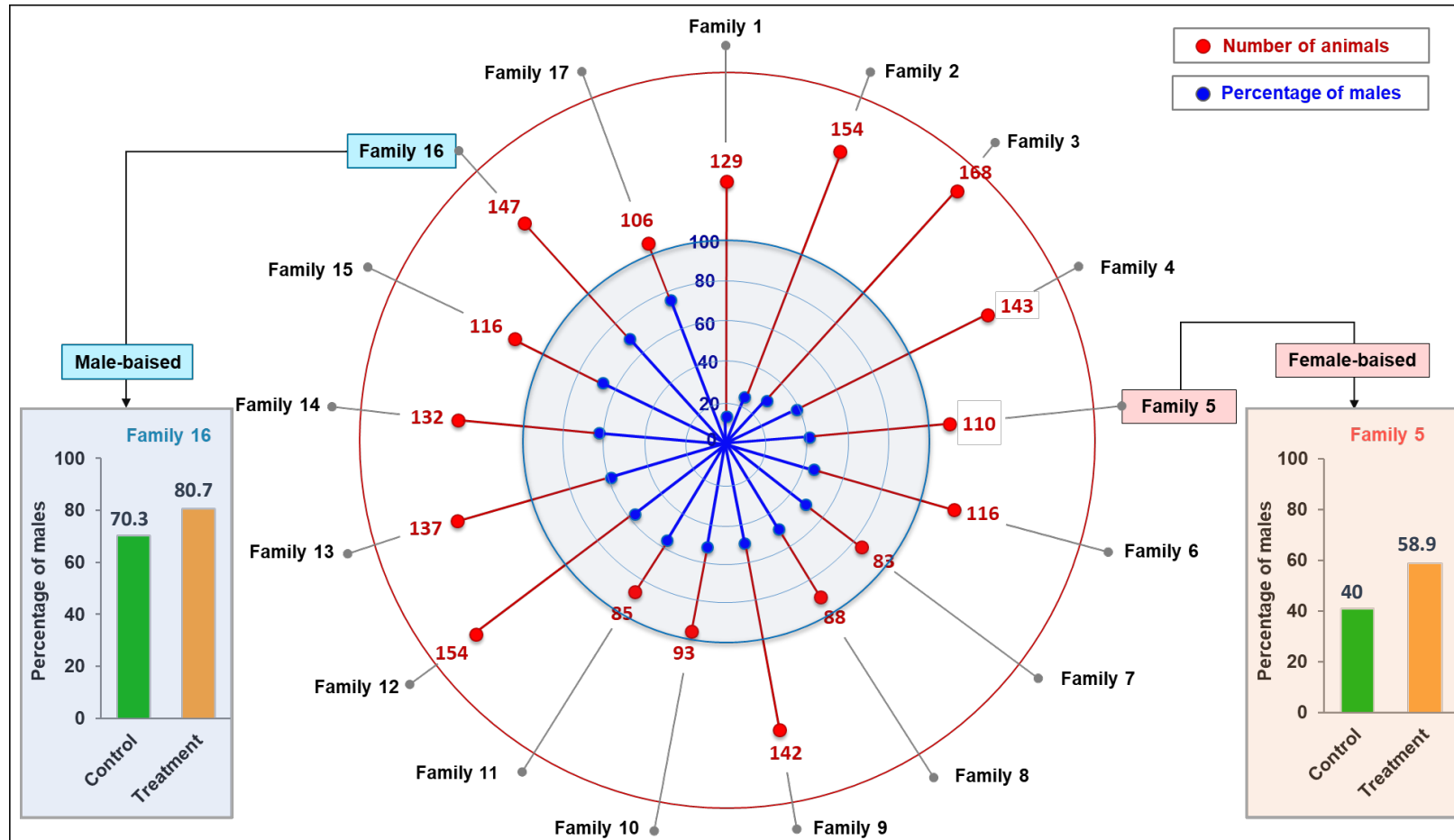


- 17 full-sib zebrafish families were produced and exposed into two different temperature incubations: 1) control group at a constant temperature of 28°C, 2) high incubation temperature group (treatment) at 35°C from 5 to 24 hpf

- One male-biased and one female-biased family in the adult stage were selected for transcriptome analysis (RNA_Seq)
- The brain tissue samples of 48 animals were collected for RNA_Seq analysis (24 samples in each sex-biased family)



Sex ratio of different zebrafish families and the selected sex-biased families for RNA_Seq



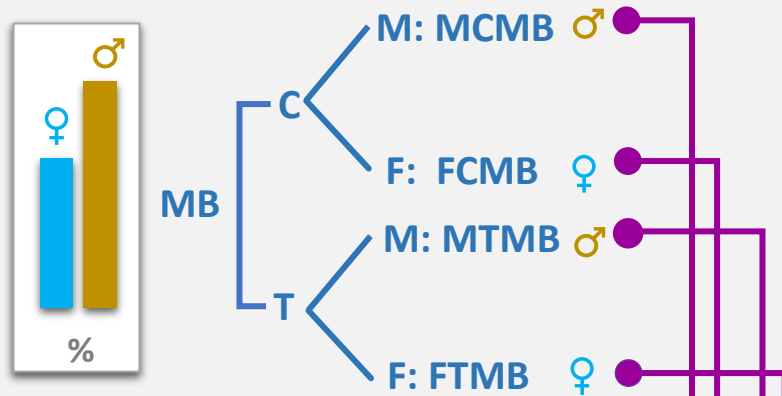
Two sex-biased families with a significant difference in sex ratio were selected for RNA_Seq: a male-biased (family 16) and a female-biased (family 5)

Strategy developed for comparative RNA_Seq analysis of different experimental groups

Male-biased vs. Female-biased family
in each sex type (Strategy 1)

experimental groups
(n=8)

Males vs. Females in each family type
(Strategy 2)

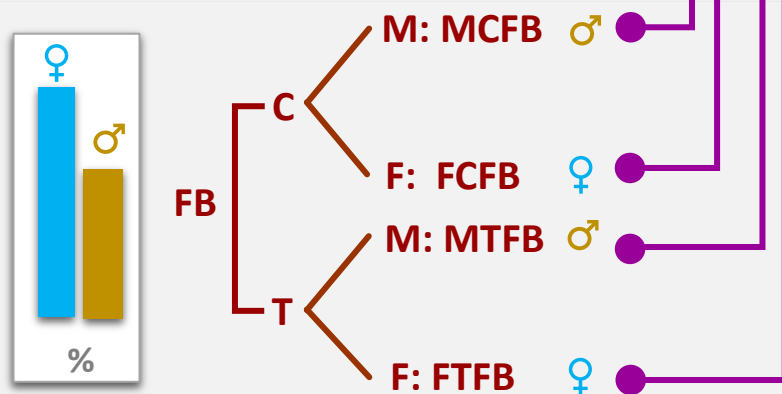
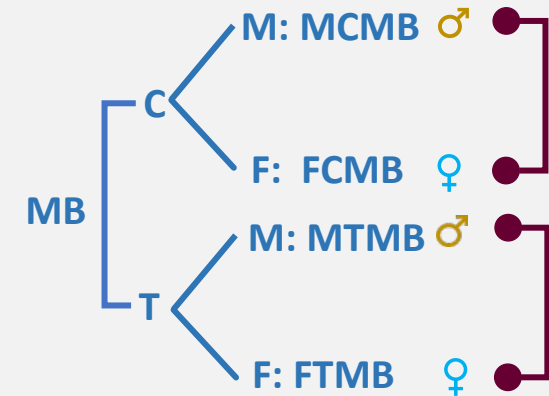


Male Control Male-Biased

Female Control Male-biased

Male Treat Male-biased

Female Treat Male-biased

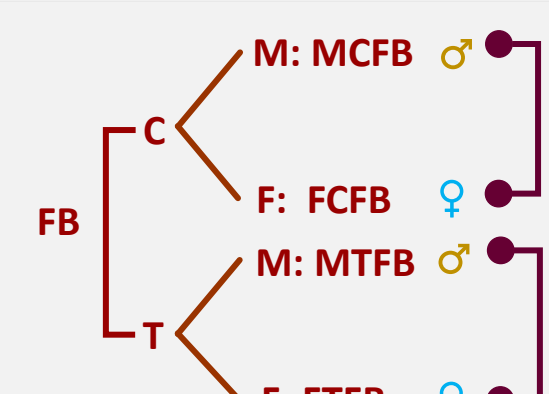


Male control Female-biased

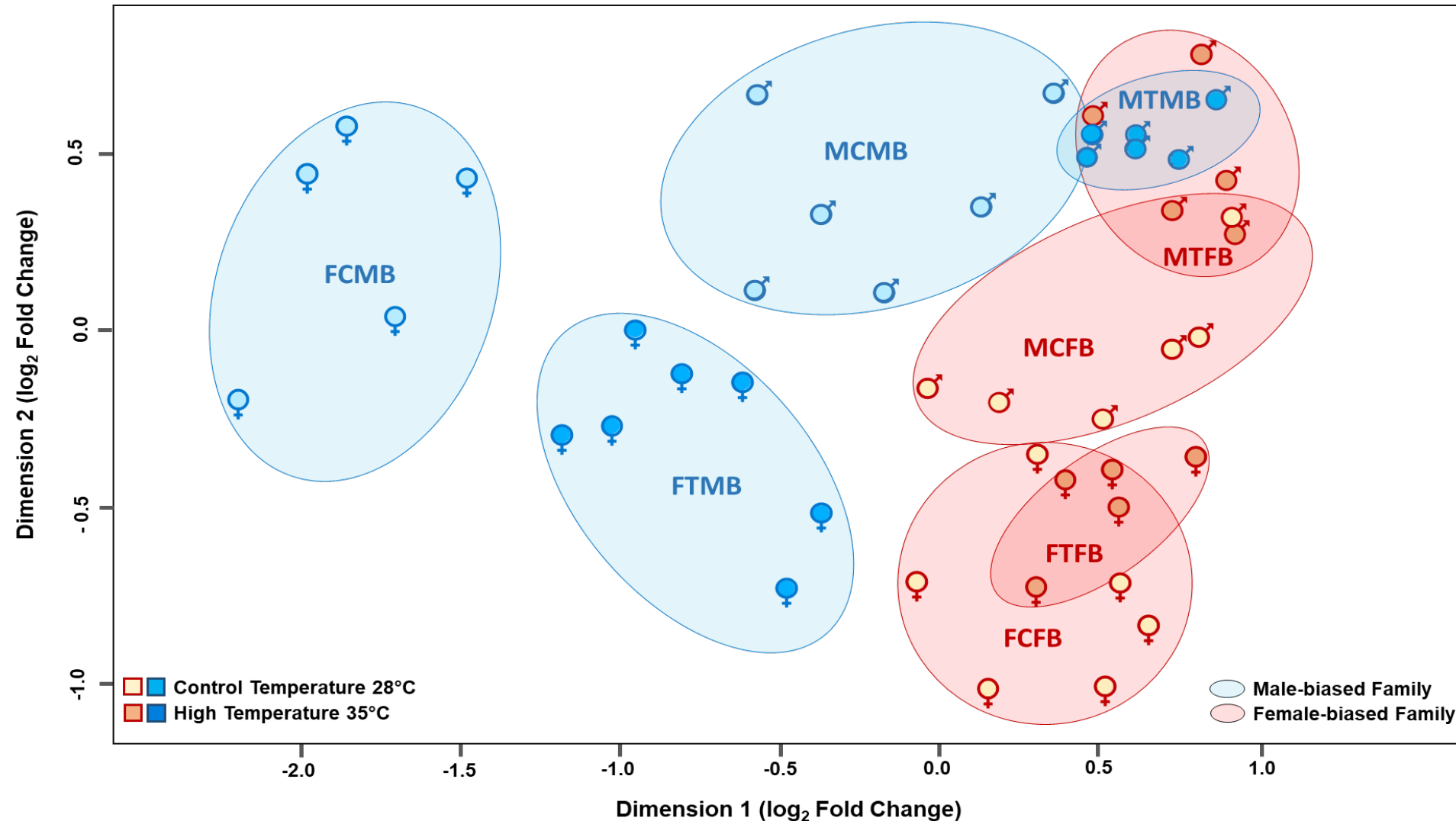
Female Control Female-biased

Male Treat Female-biased

Female Treat Female-biased

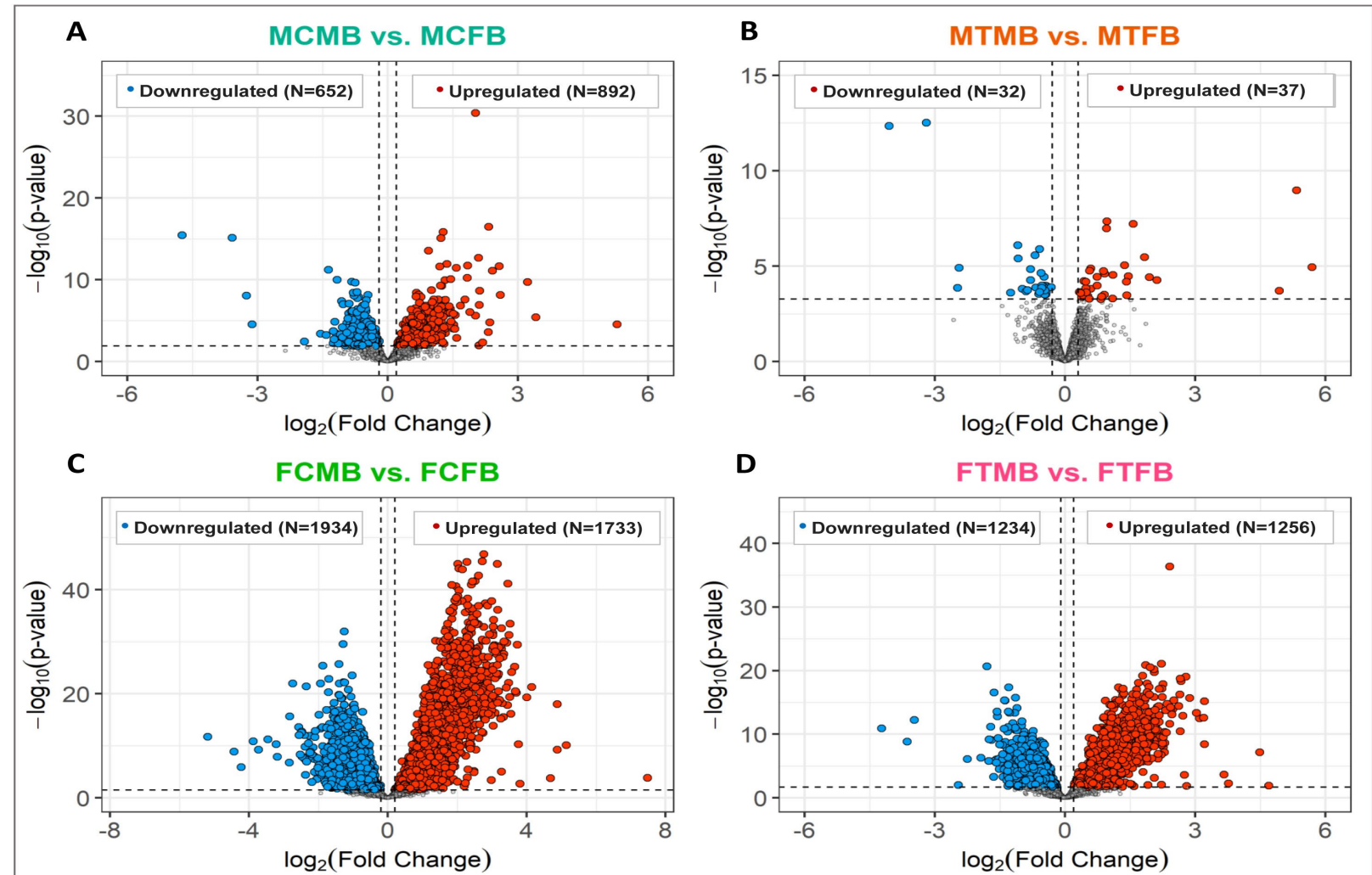


Multidimensional scaling of overall transcriptome profiles in individual samples



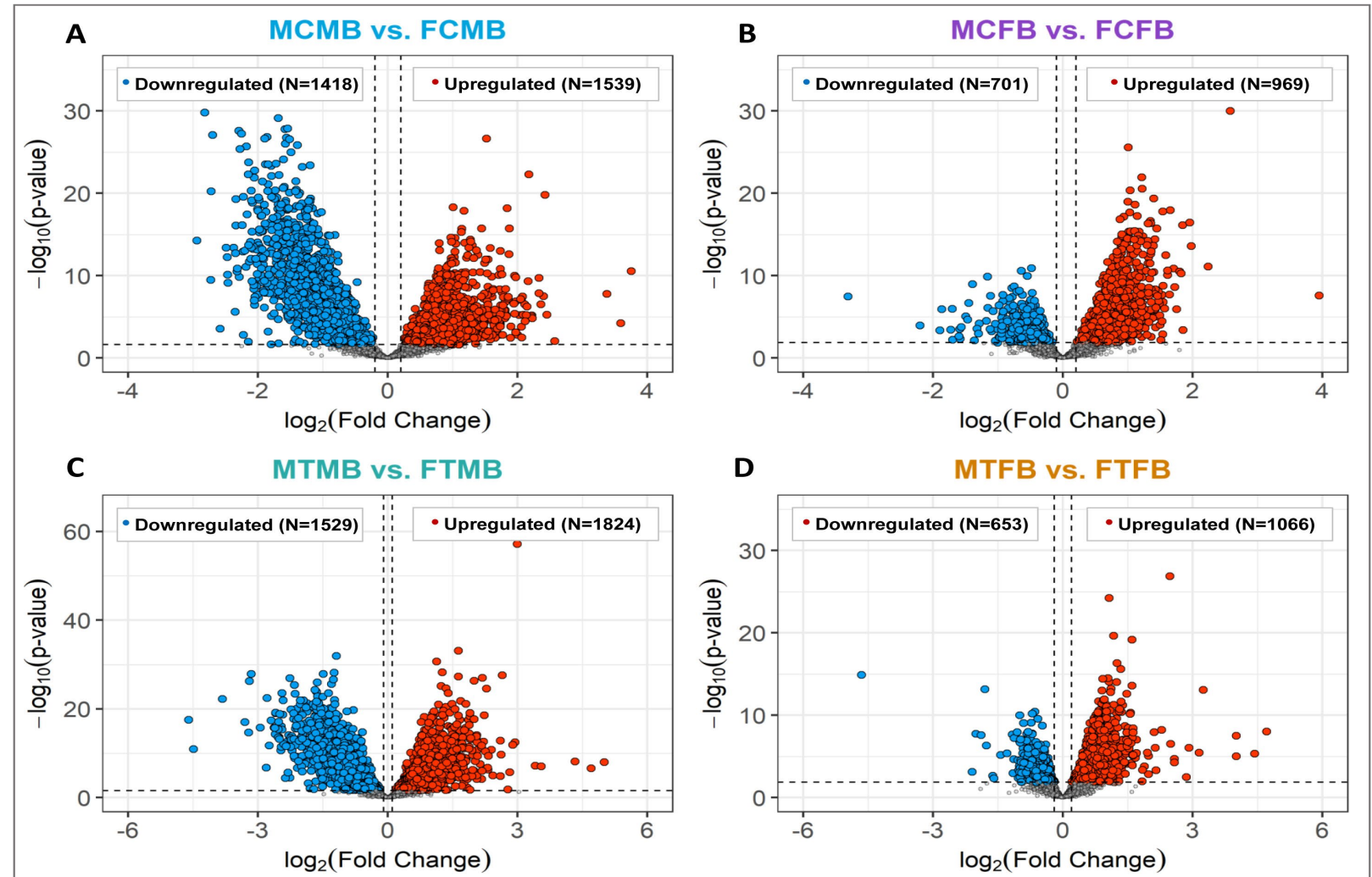
Differentially expressed genes (DEGs) comparing male-biased versus female-biased family (Strategy 1)

- a total of **1,544** DEGs in the control group and **69** DEGs in the treatment group in the male brains
- a total of **3,667** DEGs in the control group and **2,490** DEGs in the treatment group in the female brains

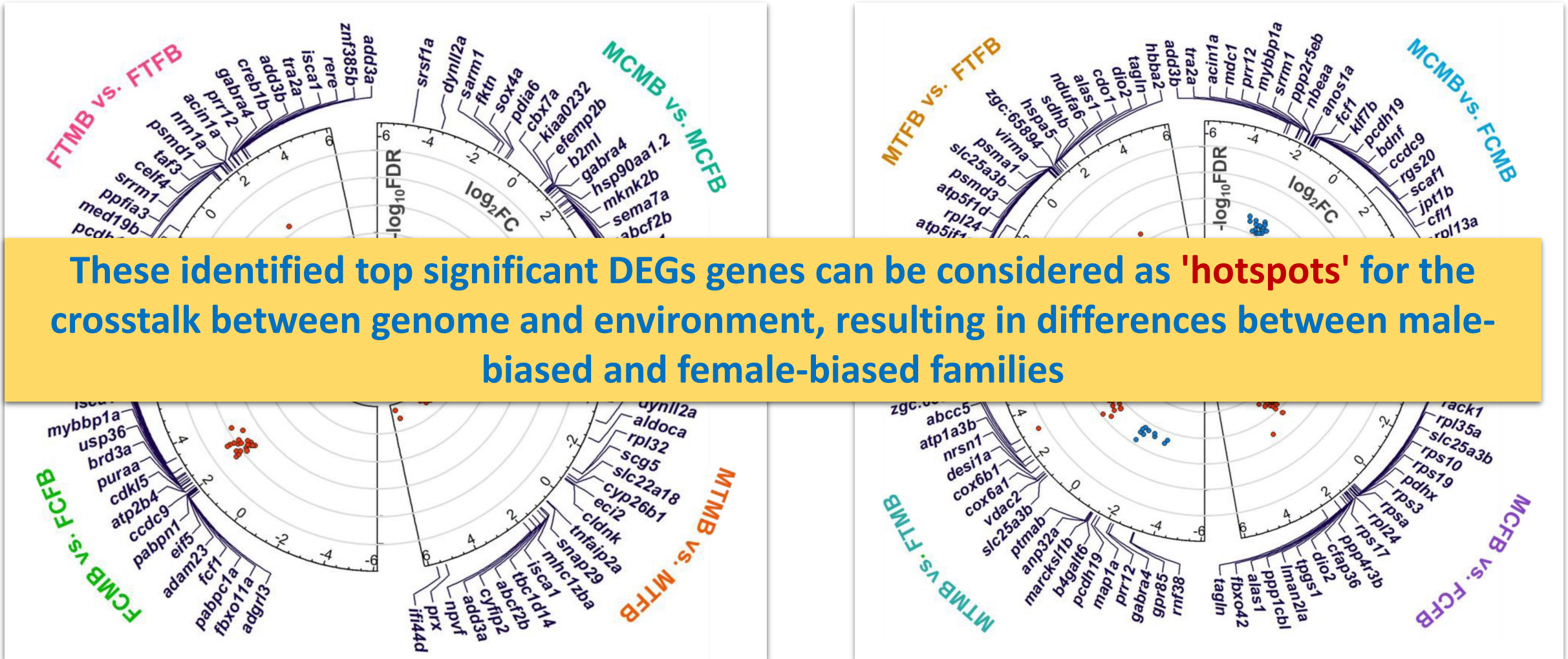


Differentially expressed genes (DEGs) comparing brain of males versus females within sex-biased families (Strategy 2)

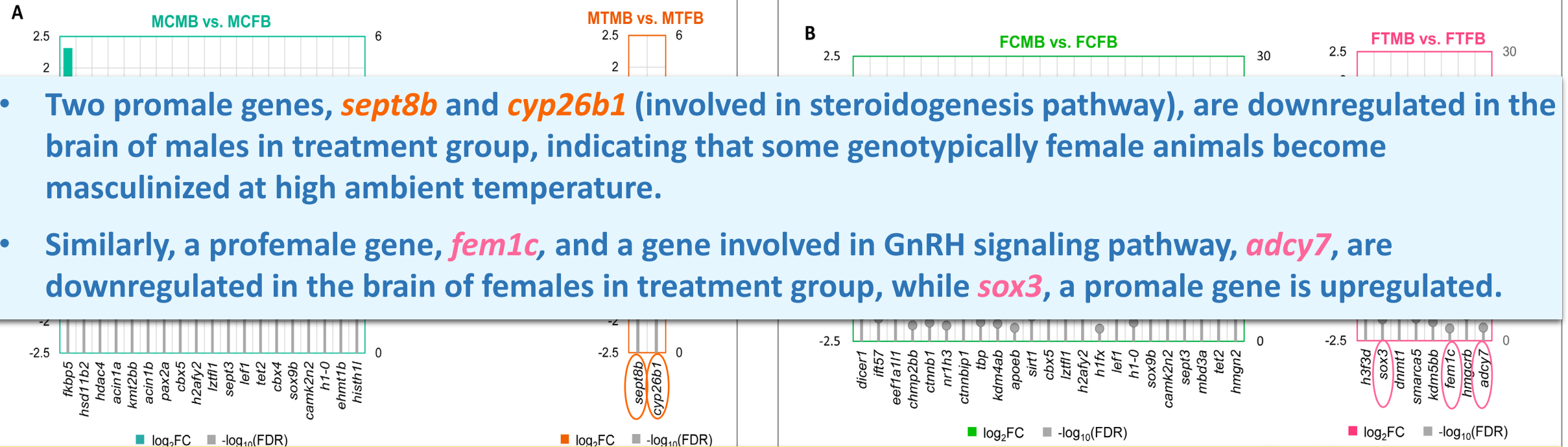
- a total of **2,957** and **1,670** DEGs in the male-biased and female-biased families, respectively, in the control group
- a total of **3,353** and **1,719** DEGs in the male-biased and female-biased families, respectively, in the treatment group



Top significant differentially expressed genes (DEGs) in sex-biased zebrafish families



Unique identified reproduction-related genes between sex-biased zebrafish families



Differential expression of sex-related genes in response to high environmental temperature can affect the phenotypic sexual dimorphism, resulting in **sex-reversed females (masculinized)** and **females with expressing a male-like transcriptome**

Unique identified reproduction-related genes within sex-biased zebrafish families

- Many promale genes such as *sept3* (spermatogenesis gene), *adcy7* (GnRH signaling pathway gene), *dkk3a* (testicular differentiation gene) were upregulated in male-biased family.
- By contrast, several promale genes were downregulated in female-biased family such as *fkbp5* (a steroid hormone biosynthesis gene), *lztfl1* (involved in spermatogenesis and testicular differentiation, and *hsd11b2* (a gene involved in steroidogenesis pathway), while *sox9b* (a gene for ovary development) was upregulated in this family.



The identified family-specific reproduction-related genes can influence the trends of sex determination pathways towards maleness or femaleness

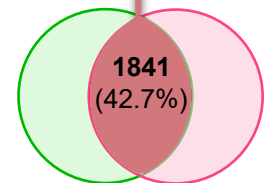
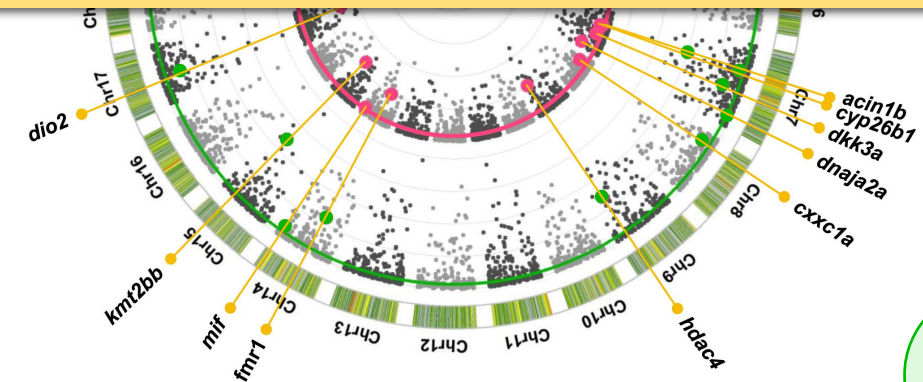
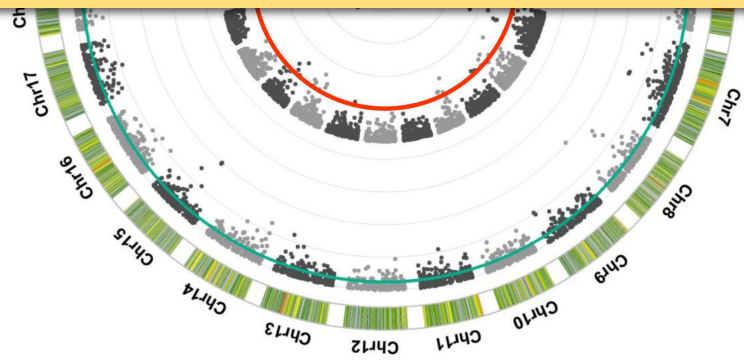
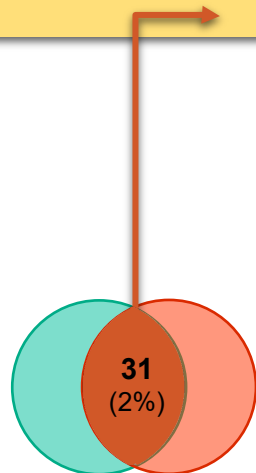
Common identified reproduction-related genes between sex-biased zebrafish families

MCMB vs. MCFB ∪ MTMB vs. MTFB

FCMB vs. FCFB ∪ FTMB vs. FTFB

Chromosome density
0
2
4
6
8
10
12
14
16
18
20
22

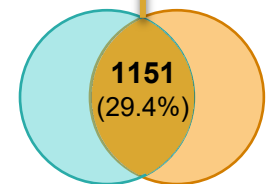
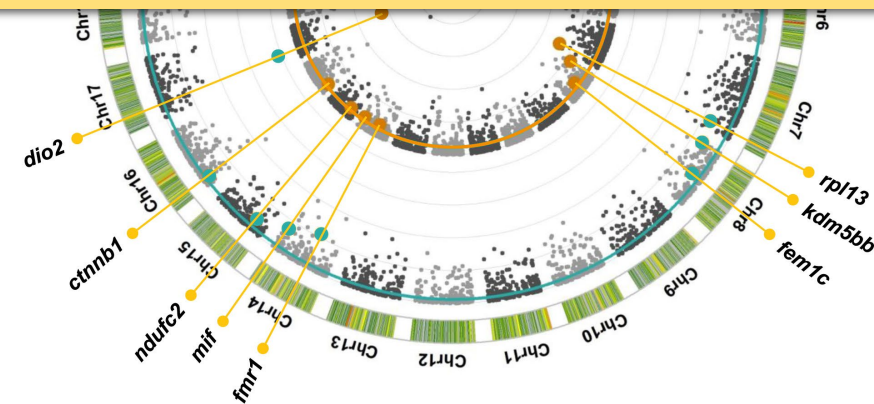
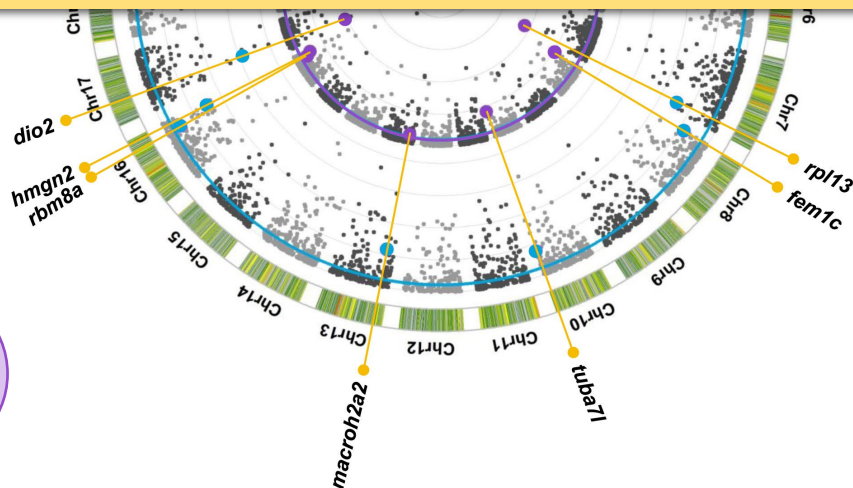
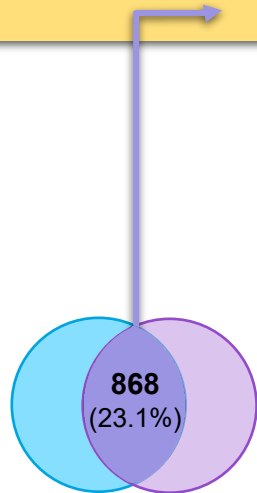
Differences in gene expression between male-biased and female-biased families independent from environmental temperature effect



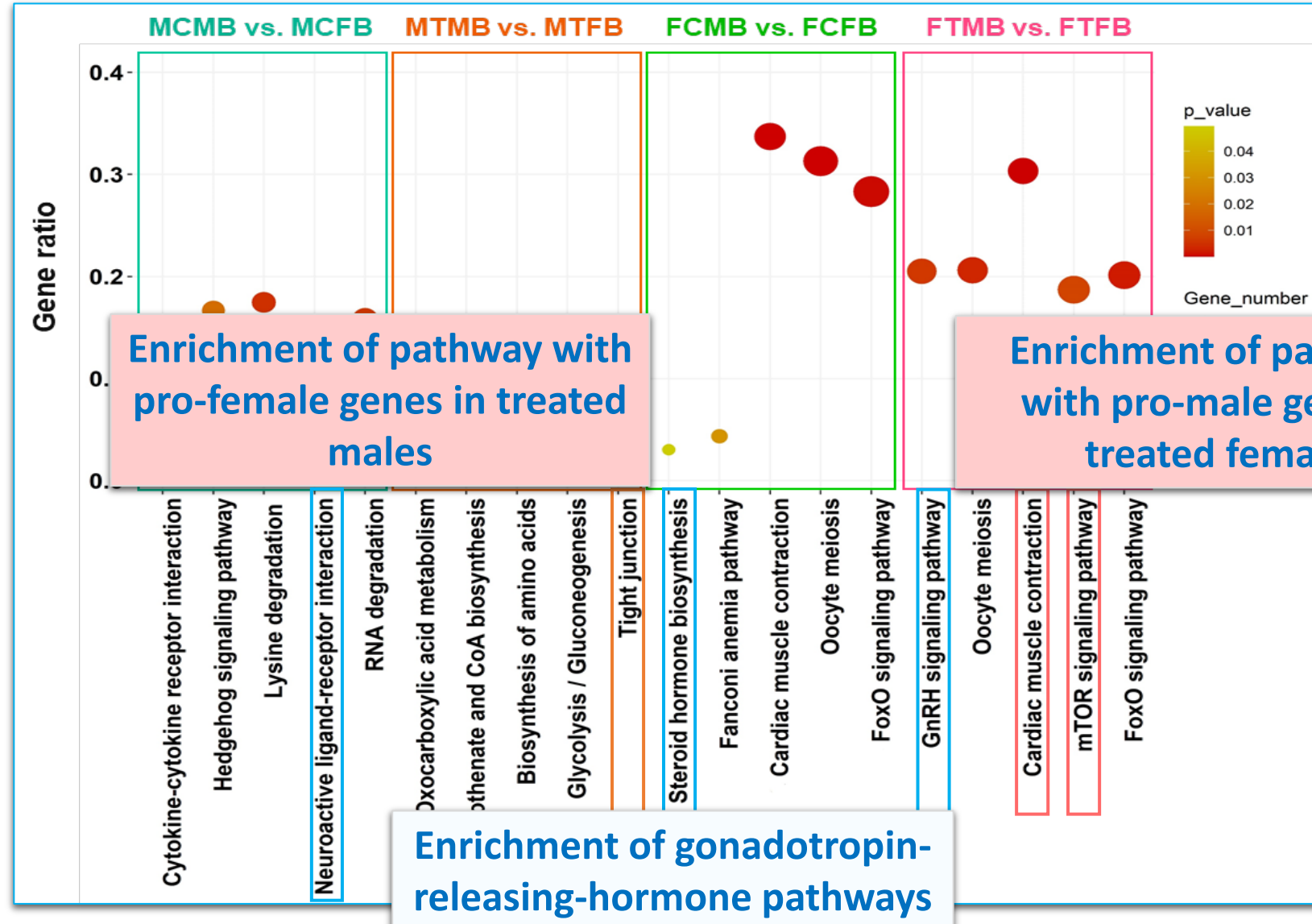
Common identified reproduction-related genes within sex-biased zebrafish families

MCMB vs. FCMB $\cup$ MCFB vs. FCFBMTMB vs. FTMB $\cup$ MTFB vs. FTFBChromosome density
0
2
4
6
8
10
12
14
16
18
20
22

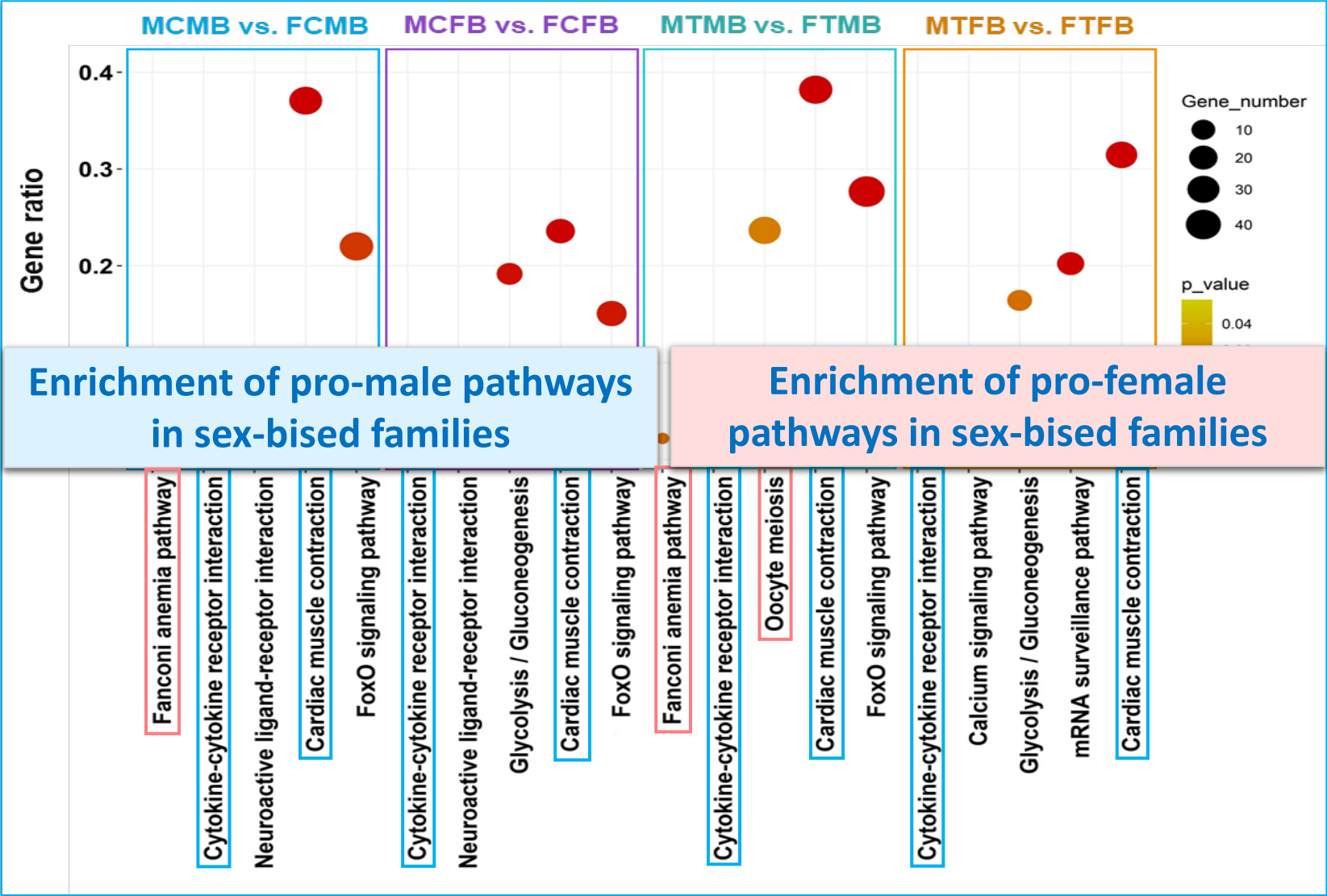
Differences in gene expression between male- and female-brains independent from family structure effect



Enriched KEGG pathways in male-biased versus female-biased family



Enriched KEGG pathways in brain of males versus females within sex-biased families



- Our study provides new insights into the importance of the brain-gonadal axis as a transducer of environmental stimuli that cause skewed sex ratio and generate sex-biased families in model animal, the zebrafish
- A highly skewed sex ratio may have a detrimental effect on the viability of populations and can lead to a greater risk of extinct in animal species with $G \times E$ sex-determining system under rapid global warming
- Our findings lead us to conclude that the effect of high ambient temperature in sex-biased zebrafish families can affect the phenotypic sexual dimorphism, resulting in sex-reversed females (masculinized) and females with ovaries expressing a male-like transcriptome

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**THANK YOU VERY MUCH
FOR YOUR ATTENTION**