# Olfactory Signals Involved in Kin Recognition in Zebrafish

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## Kin recognition

In many species - invertebrates and vertebrates - social interaction differs according to the genetic relatedness of individuals. Individuals that are able to assess the genetic relatedness of conspecifics can preferentially allocate resources towards related individuals and avoid inbreeding.

Several mechanisms have been proposed by which individuals may discriminate kin. One way to identify possible relatives is to treat any conspecific that shares a particular location or degree of familiarity as kin. Kin recognition requires more stringent criteria when proximity and familiarity with conspecifics are not sufficiently reliable to detect true genetic relatedness. In a more specific method of kin recognition known as phenotype matching, an individual learns a template of its own phenotype [1] and/or that of its familiar kin [2], and later compares the phenotypes of unfamiliar animals with this template [3]. Such phenotype matching depends on a consistent correlation between phenotypic and genotypic similarity, so that detectable traits are more alike among close relatives than among more distantly related or unrelated individuals [4].

### Olfactory imprinting and kin recognition in zebrafish

We have addressed the question of how animals acquire the ability to recognize kin by studying the development of olfactory kin preference in zebrafish (*Danio rerio*). We exposed zebrafish eggs directly after fertilization to different conditions: We either kept a single larva isolated in a small beaker or exposed larvae to olfactory and visual cues of their siblings at different days of their development. When 10 to 15 days old, we tested larvae for their olfactory preference of kin in a two channel choice flume with a steady driven flow. Each channel of the flume contained holding water of either kin or non-kin. We recorded the position of the fish in one or the other water flow every 10 s during two 3-min periods separated by a 1-min transition period to switch water sources as a control for possible (non-olfactory) side bias of the fish. Our results show that larvae could only recognize and preferred kin when having been exposed to kin during a 24-hour time window on day six post fertilization (dpf) [5]. Exposing larvae to kin at any other day than at 6 dpf does not induce any recognition of kin later in life; isolated larvae did also not express any kin recognition later in life [6,7].

Surprisingly, larvae do not imprint on the cues of non-kin during this sensitive phase for imprinting, indicating that a predisposition for the olfactory cues might exist or that larvae might use additional cues [5].

Our results on kin recognition in juvenile and adult zebrafish suggest that juveniles prefer to shoal with relatives while adults might use the same kin recognition mechanism to avoid mating with close relatives. Indeed, in the same choice flume adult females preferred olfactory cues of foreign males over those of brothers [7].

### Identification of olfactory signals triggering kin recognition

Extreme genetic diversity of genes of the major histocompatibility complex (MHC) provides the underlying mechanism for kin recognition [8]. The MHC is a cluster of genes that has been intensively studied for its importance in immune reactions and immune recognition [9], and shows striking similarity among many vertebrates. Variation in MHC genes contributes also to unique individual odors (odortypes). MHC/peptide complexes expressed at cell surfaces are thought to be shed from the cell surface and their fragments appear in the urine and other body fluids. In recent studies we could identify peptide ligands of MHC receptors as signals triggering imprinting and kin recognition [10]. When adding mixtures of nine amino acid long peptides we could evoke later kin recognition in zebrafish with specific MHC class II alleles.

#### Benefits of associating with kin

We examined the potential benefits of kin preference by comparing growth rate, shoaling, and aggressive behavior in juvenile zebrafish housed in groups of either familiar kin or unfamiliar non-kin [11]. Over an observation period of 5 days animals grew 33 % more in kin groups; however, neither shoaling nor the frequency of aggressive interactions was different in groups of related versus unrelated individuals. Shoaling behavior increased with increasing observation time and increasing age, while aggressive behavior remained the same. We conclude that associating with kin probably creates a less stressful environment that allows for higher growth rates, which can lead to higher direct fitness based on increased survival and earlier reproduction. Kin recognition leading to kin-structured groups may therefore be under positive selection

#### References

- 1. Mateo, M. Johnston, E. (2000). Kin recognition and the 'armpit effect': evidence of self-referent phenotype matching. *Proceeding of the Royal Society of London B* **267**, 695-700.
- 2. Sherman, P.W., Reeve, H.K., Pfennig, D.W. (1997). Recognition systems. Pages 69-96 *in J. R. Krebs* and N. B. Davies, editors. *Behavioral ecology: an evolutionary approach*. Blackwell Scientific, Oxford UK.
- 3. Tang-Martinez, Z. (2001). The mechanisms of kin discrimination and the evolution of kin recognition in vertebrates: A critical re-evaluation. *Behavioural Processes* **53**, 21-40.
- 4. Holmes, W., Sherman, P. (1983). Kin recognition in animals. The American Scientist 71, 46-55.
- 5. Gerlach, G., Hodgins-Davis, A., Avolio, C., Schunter, C. (2008). Kin recognition in zebrafish: A 24-hour window for olfactory imprinting. *Proceedings of the Royal Society, London B* **275**, 2165-2170.
- 6. Mann, K.D., Turnell, E.R., Atema, J., Gerlach, G. (2003). Kin recognition in juvenile zebrafish (*Danio rerio*) based on olfactory cues. The Biological Bulletin **205**, 224-225.
- 7. Gerlach, G., Lysiak, N. (2006). Kin recognition and inbreeding avoidance in zebrafish, *Danio rerio*, is based on phenotype matching. *Anim. Behav.* **71**, 1371-1377.
- 8. Hepper, P.G., Cleland, J. (1999). Developmental aspects of kin recognition. *Genetica* **104**, 199-205.
- 9. Klein, J. (1986). Natural history of the major histocompatibility complex and human evolution. *Trends in Genetics* **6**, 7-11.
- 10. Hinz, C., Namekawa, I., Behrmann-Godel, J., Oppelt, C., Dittmar, J., Jäschke, A., Friedrich, R.W., Gerlach, G. (submitted). A peptide makes you like your kin: MHC peptide ligands can evoke olfactory imprinting on kin in zebrafish.
- Gerlach, G., Hodgins-Davis, A., MacDonald, B., Hannah, R.C. (2007). Benefits of kin association: related and familiar zebrafish larvae (*Danio rerio*) show improved growth. *Behavioral Ecology and Sociobiology* 61, 1765-1770.