Salinity-induced phenotypic plasticity in threespine stickleback sperm activation

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Abstract

Phenotypic expression may be and often is influenced by an organism's developmental environment, referred to as phenotypic plasticity. The sperm cells of teleosts have been found to be inactive in the seminal plasma and are activated by osmotic shock for most fish species, through release in either hypertonic (for marine fish) or hypotonic (for freshwater fish) water. If this is the case, the regulatory system of sperm mobility should be reversed in salt and freshwater fish. We tested this hypothesis by first activating sperm of saltwater and freshwater populations of threespine stickleback in salt- and fresh water. The sperm from saltwater stickleback could be activated in either salinity, which matches the freshwater colonization history of the species, whereas the sperm from the freshwater population acted as predicted by the osmotic shock theory and was activated in freshwater only. As the

freshwater population used here was calculated to be thousands of years old, we went on to test whether the trait(s) were plastic and sperm from freshwater males still could be activated in saltwater after individuals were exposed to saltwater. After raising freshwater stickleback in saltwater we found the mature males to have active sperm in both salt and freshwater. Further, we also found the sperm of wild-caught freshwater stickleback to be active in saltwater after exposing those mature males to saltwater for only two days. This illustrates that the ability for stickleback sperm to be activated in a range of water qualities is an environmentally induced plastic trait.

Introduction

Selection can favor flexible individuals or genotypes that are able to modify their development, physiology or behavior in response to environmental cues, a phenomenon broadly referred to as phenotypic plasticity [1]. Although phenotypic plasticity is usually thought of as adaptive, it can also be neutral or maladaptive with regards to individual fitness, depending on the environment in which the individual finds itself [2]. Adaptive plasticity can affect evolutionary dynamics because it has the potential to promote colonization and rapid divergent evolution in newly founded populations [3]. If the founding population persists and the environment is stable, these plastic traits might become genetically assimilated and expressed constitutively, rather than being plastic [4]. This loss of plasticity, also called canalization, can then represent a reproductive barrier between populations, if the isolated populations are differently canalized .

It seems phenotypic plasticity could have played an important role in the evolutionary dynamics of the threespine stickleback *Gasterosteus aculeatus* (hereafter stickleback).

Originally a marine species, the stickleback successfully colonized many newly established freshwater systems at the end of the last glacial period [5]. Most of these freshwater stickleback populations display evidence of parallel adaptation in shape and other morphological traits compared to the ancestral marine form, notably in the morphology of the bony lateral plates covering each side of the stickleback body, which are reduced in most freshwater populations [5]. Yet despite physical adaptation, all stickleback populations investigated thus far, not only those that are anadromous, have an extremely wide salinity tolerance, and can be exposed directly to a new salinity without inducing any known stress responses [6, 7].

The possibility for gametic isolation has largely been overlooked in studies of adaptation to freshwater, and little is known about actual fertilization success in nature between stickleback inhabiting different water salinities. Stickleback reproduce externally, and have gametes that might experience a dramatically different environment after being released into the surrounding water/nest [5]. There are studies on stickleback indicating that sperm are unable to activate in highly contrasting salinities, which suggested the possibility of a gametic barrier between fresh and saltwater populations [8, 9]. The objective of this study was to test whether sperm mobility could be a potential mating barrier in the threespine stickleback.

Materials and Methods:

Details concerning sampling, sample sites and keeping fish in the laboratory can be found in Supplementary 1. This study consists of four parts as seen in Figure 1. Males were immediately killed by a swift blow to the head, and the testes were removed surgically. Each

testis was cut open and diluted in either naturally occurring fresh (~0 ppt) or artificially made salt-water (30 ppt), mixed and immediately transferred to a chambered microscope where sperm movement was recorded. For further details, please see Supplementary 1.

Statistical analysis

Data were analyzed using R (R Development Core Team, 2011). The total number (n) of males analyzed for each salinity treatment is given in Figure 1. For each experimental setup we tested for differences in sperm velocity between the two treatments, freshwater and saltwater using a two-tailed t-test on mean curvilinear velocity (VCL) across treatments and within individual.

Results

The mean velocity (± standard deviation) for marine and freshwater stickleback are found in table 1 and illustrated in Figure 2. Sperm from both marine and freshwater threespine stickleback could be activated by either fresh water or salt water. However, freshwater males must acclimate to saltwater conditions before their sperm can be activated by salt water, while sperm from saltwater males can be activated immediately by either fresh- or salt water.

Discussion

The ability of sperm to have a somewhat wide osmotic activation tolerance has also been observed in other fish species, such as the medaka (*Oryzias latipes*) [10], and Gulf killifish (*Fundulus grandis*) [11], where the sperm of Gulf killifish and tilapia also had the ability to be activated by both hypotonic and hypertonic osmolarities. Osmolarity is not the only

97 mechanism by which sperm are activated, as both the concentration of specific ions and/or
98 pH levels have also been found to be of importance in some species [12-14]. Again, it is
99 interesting that the saltwater stickleback sperm can be activated in both salt and fresh
100 water, as salt and fresh water have the opposite concentration of critical ions such as
101 calcium and potassium [14].
102
103 Details of sperm activation in stickleback are not known, and the sperm characteristics found
104 in this study seem more similar to those of internally fertilizing fishes, e.g. ceasing to move
105 quite quickly in water, regardless of salinity. In internal fertilizing fish such as ocean pout
106 (*Macrozoares americanus L*) [15] and spotted wolfish (*Anarhichas minor*) [16], the milt
107 contains highly active sperm that are immobilized immediately upon dilution in salt water,
108 but stay active for days in the ovarian fluid of the females. In stickleback, the male builds a
109 nest where the female deposits her eggs, which are covered in gelatinous ovarian fluid. The
110 ovarian fluid contains a variety of ions [8, 17], and as the stickleback male spawns directly on
111 the eggs, which are within the nest, the ejected sperm are exposed to the ovarian fluid or a
112 dilution of that fluid rather than the surrounding water. This is presumably increasing the
113 longevity of the sperm cells by several hours [8], an important effect as fertilization of a
114 stickleback egg clutch has been found to take several minutes [18].
115
116 Sperm from freshwater males failed to activate when exposed to salt water in our
117 experimental conditions. However, when offspring of the same freshwater population were
118 raised in salt water, and more surprisingly, when wild caught freshwater males were
119 acclimated for just two days to salt water, the freshwater males' sperm could be activated
120 by either fresh or salt water. This indicates that phenotypic plasticity in sperm activation has

been maintained during the almost 8,000 years that this population has been living in fresh water, and there has not been environmental assimilation causing sperm to be activated in only fresh water. The degree of phenotypic plasticity in sperm of salt-water males is unclear and was not tested, given their robust activation in both salinity treatments. Phenotypic plasticity in sperm activation has also been observed in tilapia [19] and the range of osmolarity that activated sperm motility shifted higher and broadened as the acclimation salinity of the fish increased for Gulf killifish [11]. Spermatogenesis usually starts in adult stickleback males immediately after the reproductive season, meaning the sperm can be mature (thought to be a fixed state) for several months prior to spawning season [20]. It is therefore quite surprising that the sperm of mature, wild caught freshwater stickleback have the ability to adapt to sudden changes in osmolarity and go from almost zero activation in salt water when being fresh water adapted, to having fully active sperm in salt water after being acclimated to salt water for only a few days. One likely explanation for the plastic activation of the sperm cells could be a change of the ion concentration in the seminal plasma that is surrounding the sperm while in storage [21] - if the seminal plasma increases in osmolarity when the fish is exposed to saltwater, the spermatozoa could change such that they become active already in the testis, as is the case for internally fertilizing fish [15, 16]. However, while sperm of internally breeding fishes loses the ability to move instantly in pure salt water (not tested in fresh water) [16], the sperm of saltwater stickleback and salt water-acclimated freshwater stickleback are motile also in both salt and fresh water. Spermatozoa mobility signaling is a complex and highly orchestrated process that has not been extensively studied in fish [22]. More work is needed to determine which physiological acclimation mechanisms the stickleback use to change the interpretation of the osmotic stress signals from hypo- to hyperosmolarity, and vice versa, to activate the spermatozoa mobility.

Ethics

This research was approved by the National Animal Research Authority in Norway, permit number 4442.

Data accessibility

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Authors´ contributions

A.T, A.B.M and T.L designed the study. All authors contributed to data collection. E.R.A.C analyzed the sperm velocity videos, A.T analyzed the data and wrote the first draft of the paper. All authors contributed towards subsequent drafts critically and approved the final version of the manuscript. All authors agree to be held accountable for the content in this manuscript.

Competing interests

The authors declare no competing interests.

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Table 1: Mean velocity (μm/s) and standard deviation for each group, t-test, degrees of freedom (df) and p-value of t-test for the comparisons. There was no significant difference in the sperm velocity between the two and seven-day exposure ($F_{2\ \&\ 21}$= 0.47, p= 0.63, so these were combined in the t-test, referred to as “acclimated combined” in the table. The results are illustrated in Figure 2.

Figure Legends:

Figure 1. Experimental design. The fish were wild caught from their natural environment, either saltwater or freshwater, and taken into holding tanks in the lab, then: a) saltwater fish in saltwater b) freshwater fish in freshwater c) freshwater fish bred and grown in saltwater d) freshwater fish held in saltwater for two and seven days. e) illustrate the data collection, at the experimental start both testes were removed surgically. One was activated in saltwater and the other was activated in freshwater, n = number of testis activated for each

group. Several activity parameters were recorded by video. In all panels, dark grey indicates saltwater and light grey indicates fresh water.

Figure 2. Results. Sperm velocity in salt- and freshwater for the four experimental setups expressed as boxplots of the mean VCL of each individual, calculated from 4 or 6 filmed locations. Shown are 25%-75% quantiles (boxes), median (black horizontal line), mean (*), 95% limits (bars), and outliers (open circles). As there were no significant differences between the two- and seven-day saltwater-exposed treatments, the data were pooled and illustrated as one plot (d).

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