COLLOQUIO

PROVA N. 1

Domanda 1 (max 20 punti):

Quale disegno sperimentale è necessario per verificare l'effetto dell'esposizione continua (un mese) al 2 diverse temperature elevate (superiori a quelle di allevamento di 1 e 2 gradi rispettivamente).

Domanda 2 (max 15 punti):

Domanda statistica: la/il candidata/o descriva quali test statistici userebbe per confrontare le medie di un carattere continuo in tre gruppi indipendenti.

Domanda 3 (max 15 punti):

La/il candidata/o illustri se e quale tipo di permesso etico è necessario per raccogliere osservazioni comportamentali di pesci nel loro ambiente naturale.

PROVA N. 2

Domanda 1 (max 20 punti):

Quale disegno sperimentale utilizzerebbe per valutare l'effetto dell'inquinamento delle acque dei fiumi (es. livello di pesticidi) sulla riproduzione dei pesci.

Domanda 2 (max 15 punti):

Domanda statistica: la/il candidata/o descriva quali test statistici userebbe per confrontare due misure di un carattere rilevate prima e dopo il trattamento sperimentale.

Domanda 3 (max 15 punti):

La/il candidata/o illustri se e quale tipo di permesso etico è necessario per raccogliere osservazioni comportamentali in acquario su due gruppi di pesci tenuti in vasche con e senza arricchimenti ambientali.

PROVA N. 3

Domanda 1 (max 20 punti):

Quale disegno sperimentale adotterebbe per misurare il livello di adattamento di popolazioni di pesci che vivono in ambienti ad alta e bassa temperatura.

Domanda 2 (max 15 punti):

Domanda statistica: la/il candidata/o descriva quali test statistici userebbe per confrontare due caratteri (es. peso e lunghezza) misurati negli individui di un gruppo.

Domanda 3 (max 15 punti):

La/il candidata/o illustri se e quale tipo di permesso etico è necessario per raccogliere dati sulla qualità dell'eiaculato in due gruppi di pesci allevati in acquario.

PROVA 1

ORIGINAL ARTICLE

doi:10.1111/j.1558-5646.2008.00575.x

EVOLUTION OF MATE CHOICE FOR GENOME-WIDE HETEROZYGOSITY

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Received July 1, 2008
Accepted October 23, 2008

The extent to which indirect genetic benefits can drive the evolution of directional mating preferences for more ornamented mates, and the mechanisms that maintain such preferences without depleting genetic variance, remain key questions in evolutionary ecology. We used an individual-based genetic model to examine whether a directional preference for mates with higher genome-wide heterozygosity (*H*), and consequently greater ornamentation, could evolve and be maintained in the absence of direct fitness benefits of mate choice. We specifically considered finite populations of varying size and spatial genetic structure, in which parent–offspring resemblance in heterozygosity could provide an indirect benefit of mate choice. A directional preference for heterozygous mates evolved under broad conditions, even given a substantial direct cost of mate choice, low mutation rate, and stochastic variation in the link between individual heterozygosity and ornamentation. Furthermore, genetic variance was retained under directional sexual selection. Preference evolution was strongest in smaller populations, but weaker in populations with greater internal genetic structure in which restricted dispersal increased local inbreeding among offspring of neighboring females that all preferentially mated with the same male. These results suggest that directional preferences for heterozygous or outbred mates could evolve and be maintained in finite populations in the absence of direct fitness benefits, suggesting a novel resolution to the lek paradox.

KEY WORDS: Compatible genes, genetic quality, good genes, inbreeding depression, sexual selection.

Identifying the mechanisms that drive the evolution of directional mating preferences for more ornamented mates, and that maintain such preferences over evolutionary time, remain key aims in evolutionary ecology (Neff and Pitcher 2005; Kokko et al. 2006; Kempenaers 2007; Kotiaho et al. 2008a). Clear knowledge of the nature and operation of these mechanisms is critical to understanding the evolution of costly, ornamental secondary sexual traits (Mead and Arnold 2004; Tomkins et al. 2004; Kokko et al. 2006; Radwan 2008).

Directional mating preferences are relatively easy to understand when preferred mates provide direct fitness benefits, for example through increased resources or parental care (Andersson 1994; Kokko et al. 2003). In contrast, the continued expression of directional preferences is much harder to explain when mate choice provides no direct fitness benefit, or incurs a direct cost. In such cases, the fitness benefits driving preference evolution are presumably solely genetic. Two key questions remain to be definitively answered with regard to this scenario (Kempenaers 2007): are genetic benefits sufficient to explain the evolution of costly mate choice mechanisms, and if so, what is the nature of these genetic benefits?

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Evolution 63-3: 684–694

PROVA 2

PROCEEDINGS B

royalsocietypublishing.org/journal/rspb

Research

Cite this article: Fan R, Reader SM, Sakata JT. 2022 Alarm cues and alarmed conspecifics: neural activity during social learning from different cues in Trinidadian guppies. *Proc. R. Soc. B* **289**: 20220829. https://doi.org/10.1098/rspb.2022.0829

Received: 29 April 2022
Accepted: 15 July 2022

Subject Category:
Neuroscience and cognition

Subject Areas:
behaviour, cognition, neuroscience

Keywords:
social learning, social information, fear conditioning, conditioned threat learning, anti-predator behaviour, pS6

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Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.6124062.

THE ROYAL SOCIETY
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Alarm cues and alarmed conspecifics: neural activity during social learning from different cues in Trinidadian guppies

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Learning to respond appropriately to novel dangers is often essential to survival and success, but carries risks. Learning about novel threats from others (social learning) can reduce these risks. Many species, including the Trinidadian guppy (*Poecilia reticulata*), respond defensively to both conspecific chemical alarm cues and conspecific anti-predator behaviours, and in other fish such social information can lead to a learned aversion to novel threats. However, relatively little is known about the neural substrates underlying social learning and the degree to which different forms of learning share similar neural mechanisms. Here, we explored the neural substrates mediating social learning of novel threats from two different conspecific cues (i.e. social cue-based threat learning). We first demonstrated that guppies rapidly learn about threats paired with either alarm cues or with conspecific threat responses (demonstration). Then, focusing on acquisition rather than recall, we discovered that phospho-S6 expression, a marker of neural activity, was elevated in guppies during learning from alarm cues in the putative homologue of the mammalian lateral septum and the preoptic area. Surprisingly, these changes in neural activity were not observed in fish learning from conspecific demonstration. Together, these results implicate forebrain areas in social learning about threat but raise the possibility that circuits contribute to such learning in a stimulus-specific manner.

1. Introduction

Recognizing and responding appropriately to danger is essential for survival and success in risky environments, with both excessive caution and excessive risk-taking having maladaptive consequences [1]. In variable environments with new risks, animals can directly learn about novel threats like predators when they co-occur with aversive events, such as physical injury or pursuit [2]. Given the risks of direct learning, it is often advantageous to learn to respond to novel threats using social information, where the cues that evoke defensive responses come from conspecifics [3–8]. Animals can form associations between a novel stimulus and conspecific cues that indicate threat such as chemical cues released during predation or defensive responses by experienced conspecifics ('demonstrators' [9–11]). This learning can rapidly lead to defensive responses to the novel stimulus alone [9,12,13]. Furthermore, such processes can result in the spread of novel threat responses through populations.

Considerable attention has been devoted to the adaptive significance of conspecific threat cues [2,5,10]. However, in contrast to the extensive work on the function and neural mechanisms of non-social conditioned threat learning (or 'fear conditioning') in a variety of vertebrates [14,15], much less is known about the neural substrates underlying conspecific-mediated (social) threat learning, particularly in non-mammalian vertebrates (but see [14,16–19]). Revealing the neural circuits underlying ethologically important forms of social learning across species can provide important insights into the mechanisms underlying evolutionary and experience-dependent variation in cognition

PROVA 3

© 2023. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2023) 226, jeb244712. doi:10.1242/jeb.244712

RESEARCH ARTICLE

Frequency-dependent viscosity of salmon ovarian fluid has biophysical implications for sperm–egg interactions

Marco Graziano[1,2,*], Swomitra Palit[3], Anand Yethiraj[3], Simone Immler[2], Matthew J. G. Gage[2,‡] and Craig F. Purchase[1]

ABSTRACT

Gamete-level sexual selection of externally fertilising species is usually achieved by modifying sperm behaviour with mechanisms that alter the chemical environment in which gametes perform. In fish, this can be accomplished through the ovarian fluid, a substance released with the eggs at spawning. While the biochemical effects of ovarian fluid in relation to sperm energetics have been investigated, the influence of the physical environment in which sperm compete remains poorly explored. Our objective was therefore to gain insights on the physical structure of this fluid and potential impacts on reproduction. Using soft-matter physics approaches of steady-state and oscillatory viscosity measurements, we subjected wild Atlantic salmon ovarian fluids to variable shear stresses and frequencies resembling those exerted by sperm swimming through the fluid near eggs. We show that this fluid, which in its relaxed state is a gel-like substance, displays a non-Newtonian viscoelastic and shear-thinning profile, where the viscosity decreases with increasing shear rates. We concurrently find that this fluid obeys the Cox–Merz rule below 7.6 Hz and infringes it above this level, thus indicating a shear-thickening phase where viscosity increases provided it is probed gently enough. This suggests the presence of a unique frequency-dependent structural network with relevant implications for sperm energetics and fertilisation dynamics.

This article has an associated ECR Spotlight interview with Marco Graziano.

KEY WORDS: Cryptic female choice, Mate choice, Non-newtonian fluids, Ovarian fluid, Sperm competition, Viscoelasticity

INTRODUCTION

The micro-conditions of fertilisation are poorly understood in the majority of animal species (Cosson, 2015; Eisenbach and Giojalas, 2006; Kholodnyy et al., 2020). Following ejaculation, sperm find and fertilise eggs, but this usually takes place in the presence of post-mating sexual selection arising from sperm competition with rival males (Birkhead and Pizzari, 2002; Parker, 2020) and cryptic female choice that biases paternity (Firman et al., 2017). We now know that polyandry (female mating with multiple males in a given breeding episode) is widespread and common in nature (Taylor et al., 2014) and that post-mating sexual selection plays a crucial role in governing reproductive fitness (Simmons, 2005). It is likely to be responsible for the tremendous diversity in sperm morphology (Ramón et al., 2014; Pitnick et al., 2008) and female reproductive tract morphological complexity (Kelly and Moore, 2016; Sloan and Simmons, 2019). Although many studies have revealed the importance of post-mating sexual selection for dictating variance in individual fertilisation success (Gasparini and Pilastro, 2011; Kekalainen and Evans, 2018; Lüpold et al., 2012), we still understand little about the exact mechanisms that control the outcome of such sexual selection and, ultimately, individual fertilisation success (Birkhead and Pizzari, 2002). In this context, we aimed to explore whether the physical properties of salmon ovarian fluid could support the basis for a physically controlled post-mating choice. Changes in the rheological attributes of the fluid under varying physical stresses might furnish an evolutionary basis for sexual selection in fish, as in other animal models. Specifically, in this paper we analysed the rheological properties of salmon ovarian fluid to explore their role in modulating sperm behaviour and swimming efficiency, looking to highlight a possible novel mechanism of post-mating sexual selection in external fertilisers. Females might be able to sort among sperm from different partners and within a partner, through the governing capacities exerted by their ovarian fluid to favour certain sperm phenotypes, thus influencing their success and evolution.

In terms of female control over paternity, internal fertilisation clearly offers greater direct opportunity to manage sperm and the fertilisation process, compared with external fertilisation. In internal fertilisers, sperm are deposited within the female reproductive tract and then move from the insemination site either directly towards the egg for fertilisation, or indirectly via short- or long-term storage. Sperm can move under their own flagellar propulsion, or be moved by female tract mechanisms, but we rarely understand which sex is controlling sperm dispersal, and how, where and when this occurs throughout the reproductive process. Several female mechanisms could control sperm transfer, progress and activity; from mechanical contractions and hydrostatic pressures in the female tract, to sorting sperm from different males in designated organs and through completely ejecting ejaculates or exerting spermicidal actions (Firman et al., 2017). Biochemical complexity in which these dynamics take place is also important, with evidence that the female tract can be either supportive or, at times, hostile to certain male gametes (Firman et al., 2017; Wolfner, 2011). Ostensibly, much remains to be discovered about this reproductive diversity, with recent *in vivo* research using GFP-tagged sperm revealing high

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Received 22 June 2022; Accepted 2 December 2022