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Source: Journal of Vertebrate Biology, 69(4)

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: <https://doi.org/10.25225/jvb.20039>

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New finding of melanic three-spined sticklebacks *Gasterosteus aculeatus* in the Scottish Hebrides

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► Received 22 April 2020; Accepted 1 June 2020; Published online 3 July 2020

Abstract. Colour traits can be elaborated through sexual selection and have potential to drive reproductive isolation. Male three-spined sticklebacks (*Gasterosteus aculeatus*) express striking visual signals to attract choosy females during courtship, typically expressed as red carotenoid-based pigmentation on their throat and jaw during the breeding season, along with blue eyes and blue/green flanks. The extent and intensity of red colouration in males have been linked to fitness benefits to females, including body condition, parasite resistance, parental ability and nest defence. In some populations in the Pacific Northwest of North America, male three-spined sticklebacks express melanic nuptial colouration. In these populations, male possess black throats instead of red, and have dark or black bodies. Melanic males are associated with waterbodies that are red-shifted due to the presence of tannins, where the ambient light environment is dominated by long wavelengths. Here we report the first discovery outside North America of melanic populations of three-spined sticklebacks on the island of North Uist in the Scottish Hebrides, on the northwest Atlantic coast of Europe. These populations are associated with a hotspot of stickleback morphological diversity and occur in association with red-shifted waterbodies.

Key words: adaptive radiation, Gasterosteidae, intraspecific variation, *machair*, *Pungitius pungitius*, speciation

Introduction

Colour traits can be elaborated through sexual selection and have potential to drive reproductive isolation (Ritchie 2007). As such, the evolution of colour traits has attracted the attention of evolutionary biologists, and a widely investigated model organism for research on this subject has been the three-spined stickleback (*Gasterosteus aculeatus*) (Wootton 1976, 2009, Bell & Foster 1994, Wootton & Smith 2015). The evolutionary history of the three-spined stickleback is one of

repeated, independent invasions of freshwater habitats by marine populations. These invasions are characterised by a reduction in anti-predator “armour”, comprising lateral bony plates, pelvic girdle and spines, and dorsal spines, as well as other aspects of their biology (Bell & Foster 1994). Their wide distribution, tolerance of a wide range of environmental conditions, and striking phenotypic variability makes the three-spined stickleback an ideal model for investigating selective forces underpinning adaptive evolution (Wootton 1976, 2009, Bell & Foster 1994).

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Male three-spined sticklebacks express striking visual signals, which serves to increase mating success during courtship, though there is variation in male nuptial colour among populations (Wootton 1976, Wedekind et al. 1998). Sexually mature male three-spined sticklebacks typically express red carotenoid-based pigmentation on their throat and jaw during the breeding season, along with blue eyes and blue/green flanks. Females show a preference for mating with males that develop extensive red pigmentation on their throat and jaw during the breeding season (Wootton 1984, Bakker & Mundwiler 1994). The extent and intensity of red colouration in males have been linked to fitness benefits to females, including body condition, parasite resistance, parental ability and nest defence (Frischknecht 1993, Barber 2000, Barber et al. 2001). Red colouration may also be linked to success in male-male competition (Baube 1997, Rick & Bakker 2008). Male red nuptial colouration may have evolved as a sensory drive (Smith et al. 2004).

In some populations in the Pacific Northwest of North America, male three-spined sticklebacks express melanic nuptial colouration (McPhail 1969, Moodie 1972, Reimchen 1989, Scott 2001, Bolnick et al. 2016). In these populations, male possess black throats instead of red, and have dark or black bodies (McPhail 1969, Reimchen 1989, Scott 2001). Melanic males are typically allopatric to red males, though in some locations the two colour morphs co-exist in sympatry (Hagen & Moodie 1979). Melanic and red males are genetically distinct (McPhail 1969), though there also appears to be some degree of plasticity in expression of the alternative colour forms (Lewandowski & Boughman 2008). Black is most likely the plesiomorphic state for male nuptial colour in the stickleback family (Gasterosteidae) and is seen in related species, such as the nine-spined stickleback (*Pungitius pungitius*) (McLennan 1996).

Melanic males are associated with waterbodies that are red-shifted due to the presence of tannins, where the ambient light environment is dominated by long wavelengths (Reimchen 1989). A proposed adaptive explanation for the reversal in male colouration to a plesiomorphic state is to enhance colour contrast. Under this model of colour evolution, red colouration is predicted in full spectrum light because it has high contrast with the background, whereas black shows better contrast in long wavelength light. Enhanced contrast of nuptial colouration is predicted to

be favoured both through inter- or intra-sexual selection because males are easier for females or rivals to see (Fuller et al. 2005).

Tannin-stained freshwaters are not unique to the Pacific Northwest of North America and it is unclear why reversal to nuptial melanism is not more widespread in the three-spined stickleback species complex given its global distribution throughout coastal freshwaters in the northern hemisphere. The implication is that the selective pressures required to fix male melanism in populations are exceptionally rare. Alternatively, this trait may be more widespread but not widely recognised.

Here we report the first discovery of melanic populations of three-spined sticklebacks outside of North America on the island of North Uist in the Scottish Hebrides, on the northwest Atlantic coast of Europe. These melanic populations are associated with a hotspot of stickleback morphological diversity and occur in association with red-shifted waterbodies.

North Uist supports numerous populations of both low plated and spine-, plate- and pelvic-girdle reduced or deficient sticklebacks in freshwater lochs that vary in biotic and abiotic conditions (Giles 1983, Campbell 1985, MacColl et al. 2013, Spence et al. 2013, Klepaker et al. 2016, Smith 2018). The west coast of North Uist is characterised by calcium-rich shell-sand grassland, termed the *machair* that supports rich vegetation and alkaline, biologically productive lochs. In the central and eastern regions, the *machair* gives way to blanket peat bogs with acidic and oligotrophic lochs (Friend 2012, Klepaker et al. 2016). The pH of freshwater lochs on North Uist range from 4.5-7.4, with a gradient of decreasing pH from West to East. The chief fish predator of sticklebacks on North Uist is the brown trout (*Salmo trutta*), which occurs naturally in the majority of lochs. The only other freshwater fishes to occur are eels (*Anguilla anguilla*), which occur infrequently, nine-spined stickleback, and small, localized populations of arctic charr (*Salvelinus alpinus*).

Material and Methods

In June 2018 we collected three-spined sticklebacks from 31 lochs on the Scottish Hebridean island of North Uist. Fish were captured using dip nets at water depths of between approximately 0.05-1.2 m. Collected fish were killed with anaesthetic



Fig. 1. Colour and morphological differences among sticklebacks on North Uist. A) Sexually mature male three-spined stickleback (*Gasterosteus aculeatus*) expressing typical red nuptial colouration. B) Sexually mature melanic three-spined stickleback. C) Sexually mature nine-spined stickleback (*Pungitius pungitius*).

Table 1. Morphological and habitat characteristics of melanic three-spined stickleback populations collected from four freshwater lochs on North Uist, Scotland.

| Population | pH | Elevation (m) | Sample size (both sexes) | Mean (SD) standard length (mm) (both sexes) | Mean (SD) number of lateral plates (both sexes) | Predators present | Secchi depth |
|------------|-----|---------------|--------------------------|---------------------------------------------|-------------------------------------------------|-------------------|--------------|
| Loch 1 | 5.7 | 17 | 19 | 30.9 (5.7) | 0 (0) | yes | < 0.8 m |
| Loch 2 | 6.4 | 12 | 30 | 32.8 (4.6) | 0 (0) | yes | < 0.8 m |
| Loch 3 | 5.8 | 9 | 27 | 32.0 (3.4) | 0 (0) | yes | < 0.8 m |
| Loch 4 | 6.1 | 11 | 45 | 33.0 (4.1) | 0.2 (0.4) | yes | < 0.8 m |

(benzocaine) and fixed in 4% buffered formalin. For each fish a record was made of standard length (measured from the tip of the snout to the origin of the tail) and total number of lateral plates. A pelvic score was assigned to each fish, which comprised a count of the elements of the pelvic structure, with a score of zero indicating complete absence of the pelvic structure and eight the presence of all the pelvic elements (Klepaker et al. 2016). Loch pH was measured in the field using a multi-probe portable water quality meter (Eutech, PCSTestr 35) and transparency was scored as either high, > 0.8 m Secchi depth, or low < 0.8 m. Elevation of lochs above

sea level was estimated from Google Earth using Web-based software (www.freemaptools.com).

Results

In four unnamed lochs we exclusively collected melanic male three-spined sticklebacks. Two lochs were adjacent and just 300 m apart, but the third was approximately 2 km from these sites and the fourth a further 5 km, with lochs supporting red males located between them. Sites were at low elevation, tannin-stained and with low pH (Table 1). While relatively abundant in many lochs on North Uist,



nine-spined sticklebacks were absent from the four lochs that supported melanic males. Of the 31 sites sampled in 2018, only six were tannin-stained with $\text{pH} < 6.6$ and with nine-spined sticklebacks absent. Of these six, four were inhabited by melanic males and the remaining two by the typical phenotype. All lochs with melanic males were inhabited by trout. Sexually mature melanic males were small relative to three-spined stickleback populations across the species range in Europe (Smith et al. 2020), though not atypical for peat lochs in North Uist (Klepaker et al. 2016), and either completely lacked lateral plates or expressed very low numbers of plates (Table 1). In all cases fish lacked a pelvic girdle and spines. They all possessed dorsal spines, though these were reduced in both size and number (Fig. 1). While some retained a small number of orange pigmented chromatophores on their throat, they were predominantly black and markedly different in appearance to red males from adjacent populations. Superficially they resembled nine-spine sticklebacks (Fig. 1).

Three-spined sticklebacks are not threatened and receive no special protection in the UK. However, their special status on North Uist is increasingly recognised (Smith 2018). While scientific study of these populations is to be encouraged, their unrestricted and undocumented collection is a potential threat since some North Uist lochs support populations that appear entirely morphologically unique and demographically small and, as such, risk depletion or even extinction through indiscriminate collecting. Hence, until we better understand the status of these melanic populations we do not intend to publish their exact locations.

Discussion

Similar phenotypes are predicted to evolve under equivalent environmental conditions through parallel evolution (Haldane 1932, Bolnick et al. 2018). Because they have repeatedly invaded and adapted to new freshwater environments, the three-spined stickleback offers a powerful system for understanding the agents of selection in nature that drive parallel evolution (Wootton 2009, Smith et al. 2020).

The Pacific Northwest of North America supports an unusually high morphological diversity of three-spined sticklebacks (Wootton 1976, Bell & Foster 1994). However, a similar adaptive radiation

of sticklebacks occurs over a much smaller area on the Hebridean island of North Uist (Klepaker et al. 2016). Melanic three-spined sticklebacks were believed to be unique to the Pacific Northwest, however, comparable environmental conditions on the northwest coast of Scotland appears to have independently resulted in the evolution of a similar phenotype.

Divergent selection on sexually-selected traits in different environments has the potential to drive speciation (Ritchie 2007, Tinghitella et al. 2018). In the case of three-spined sticklebacks, because male nuptial colouration appears to be underpinned by the light environment, partly as a by-product of adaptations by male and female visual systems, signal evolution can drive reproductive isolation and, thereby, speciation (Boughman 2001). The three-spined stickleback offers a valuable system for tackling questions relating to how selection operates in different light environments, and the implications for the evolution of colour variation, visual systems and male conspicuousness.

Given the unusual conditions under which melanistic populations were encountered, with fish of reduced plate morph and small size, in combination with low pH, tannin-stained water and absence of nine-spined sticklebacks, an additional explanation for the evolution of melanic sticklebacks might relate to access to carotenoids, primarily astaxanthin and tunaxanthin/lutein, which provide the basis to the red nuptial pigmentation of red-coloured male sticklebacks (Wedekind et al. 1998). Fish are unable to synthesise carotenoids *de novo* and these pigments are primarily obtained in the diet. The potential absence of a dietary source of carotenoids in some North Uist lochs, therefore, may be an alternative or additional contribution to the appearance of melanic phenotypes. If the case, a prediction is that degree of melanism will show significant phenotypic plasticity, which has proven the case in some North American populations (Lewandowski & Boughman 2008). Further investigation of melanistic populations on North Uist will shed light on the degree to which this colour form is an adaptive and inherited trait, or a plastic response to unusual environmental conditions.

The nine-spined stickleback is widespread on North Uist and potentially competes with three-spined sticklebacks for resources (MacColl et al. 2013, Smith et al. 2020). It was notable that melanic

three-spined sticklebacks were only encountered at sites where nine-spined sticklebacks were absent. While it is premature to draw definitive conclusions from this observation given the small sample size, the potential role of competition by nine-spined sticklebacks in inhibiting the evolution of melanic three-spined sticklebacks warrants investigation.

In North America melanic populations have been shown to possess both fewer and more gill rakers than red males (McPhail 1969, Moodie 1972), lower salinity tolerance as adults and eggs (McPhail 1969), and better larval predator avoidance (McPhail 1969). In addition, some melanic populations have a different body shape to those with normal colouration (Jenck et al. 2020), both fewer and more lateral plates, and the same and larger body size (Moodie 1972, Jenck et al. 2020). In North Uist, we found melanic males to be smaller and with fewer lateral plates than red-coloured males in adjacent populations. Thus, the only consistent characteristic of all melanic populations, both in North America and North Uist, is an association with red-shifted tannin stained water. Further investigation of populations on North Uist will focus on their behaviour and morphology, as well as the ecological features of the sites in which they occur.

At present the distribution of melanic three-spined sticklebacks appears limited to one region of North Uist, though further research will clarify whether they are more widespread. It is also unclear whether melanic forms in North Uist share ecological and behavioural characteristics with those in North America, which show some variation in traits, for example some populations are unusually large (Moodie 1972). The ecological and evolutionary significance of melanic phenotypes will be the focus of ongoing research.

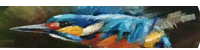
Acknowledgements

We are grateful to Iain Barber, Alex Kent and Martin Reichard for comments, and to two anonymous reviewers and Matej Poláčik for constructive reviewing. Research was supported by a POLONEZ Fellowship from the National Science Centre, Poland (2015/19/P/NZ8/03582) awarded to CS. The project received funding from the EU Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 665778. Author contributions: Sample collection was undertaken by all authors. C. Smith drafted the manuscript, which was revised and approved by all the authors.



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