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Repeatability in nest construction by male three-spined sticklebacks

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Structures built by animals may convey useful information about the builder that may be used by conspecifics in quality assessment. In fish, nest construction has been suggested to reflect qualities of individual builders, but little is known about how consistent individual differences are over time. If nest construction does reliably reflect builder quality, then we expect consistent variation between individuals in this extended phenotypic trait. We test this hypothesis in male three-spined sticklebacks, *Gasterosteus aculeatus*, by measuring the repeatability of nest characteristics. We encouraged males, caught from four populations in mid-Wales, U.K., to complete three consecutive nests under standardized laboratory conditions. We quantified a number of structural components and design characteristics of nests and estimated repeatability (r) of these traits. Within populations, the number of threads used, the area of the nest and the mass of substrate deposited on top of the nest were all repeatable within males ($0.39 < r < 0.51$), showing that individual male three-spined sticklebacks differed consistently in the size and composition of the nests they produced. Our data support the hypothesis that nest characteristics may reveal important information about the quality of individual males, and that they may, at least in part, be under genetic control. We discuss these findings in the context of the evolution of nest characteristics in sticklebacks and other species.

Keywords: evolution; *Gasterosteus aculeatus*; nest building; repeatability; stickleback; structure

Nests are constructed by animals from all vertebrate phyla (von Frisch 1975), and their primary function is to provide protection for developing offspring from the physical and biological environment (Hansell 2005). However, in nest-building species, variation in the structure and the location of the nest can affect not only offspring survival (Bult & Lynch 1997; Spencer 2002; Vinyoles et al. 2002; Warner & Andrews 2002; Burton 2006; Raventos 2006), but also mate acquisition (Johnson & Searcy 1993; Takahashi & Kohda 2002; Östlund-Nilsson & Holmlund 2003; Eckerle & Thompson 2006). Nest structure and design can, therefore, be regarded as extended phenotypic traits (see Dawkins 1999) that are shaped by both natural and sexual selection.

Observed variation in nest characteristics may be influenced by both genetic and environmental factors. For example, the size of the nest built by individual male penduline tits, *Remiz pendulinus* Olphe-Galliard 1891, is consistent across successive nesting attempts throughout a breeding season, despite temporal changes in female preferences, suggesting that aspects of construction may be under a certain degree of genetic control (Hoi et al. 1996; Schleicher et al. 1996). On the other hand, long-tailed tits, *Aegithalos caudatus* (Lin. 1758), reduce the mass of feathers incorporated into their nests when temperatures increase, suggesting that they are able to gauge the thermal environment within the nest and adjust nest-building behaviour accordingly (McGowan et al. 2004).

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To understand how extended traits such as nest building evolve, we need insight into both the heritability of and the selection pressures acting on such traits (Endler 1986). One approach to identifying probable genetic variation in nest-building behaviour is to encourage individual builders to complete multiple nests under fixed environmental conditions and measure the consistency, or 'repeatability', of the nest structure across nesting attempts. Repeatability (r) in this context is defined as the proportion of observed total phenotypic variation that is explained by differences between individuals (Falconer & Mackay 1996). Repeatability therefore indicates the amount of variation in a trait measured on more than two occasions that is a result of differences between individuals. If there is a high degree of variability in the trait over time within individuals, for example between individuals, repeatability values are low. Conversely, where there is a high level of variability between individuals, compared with within individuals, repeatability values are high.

Species that build multiple nests successively within one season offer an ideal opportunity to study the repeatability of nest construction, with consistency of nest location and form across building attempts potentially indicating heritable variation in the trait (Schleicher et al. 1996; Kamel & Mrosovsky 2004, 2005). Repeatability values gained from such studies are clearly not a proxy for heritable differences, because they may also reflect individual differences in responses to environmental conditions. However, because between-individual variation in a trait results from the combined influences of environmental and genetic components, repeatability does set an upper limit to the heritability (h^2) of a trait (Boake 1989; Falconer & Mackay 1996). Determining repeatability therefore represents an important first step towards investigating the potential for genetic variation in nest construction.

During the breeding season, male three-spined sticklebacks, *Gasterosteus aculeatus* (Lin. 1758), compete for territories before constructing a nest of filamentous algal and plant material (van Iersel 1953; Wootton 1976), which acts as a focal point for courtship and spawning and provides shelter for developing eggs and offspring (Wootton 1976). In sticklebacks, there are indications that variation in nest construction reflects male quality (Barber et al. 2001) and is used by females in mate choice (Östlund-Nilsson 2001; Östlund-Nilsson & Holmlund 2003). For example, characteristics of nests built by male 15-spined stickleback, *Spinachia spinachia*, influence the quality of protection the nest provides the offspring, and also provide information on quality of the subsequent paternal care (Östlund-Nilsson 2000, 2001). Therefore, by basing mate choice on nest characteristics, females gain direct benefits through improved offspring survival.

Although male three-spined sticklebacks can build multiple nests within a single breeding season (Wootton 1976), it is not known whether nests built consecutively by individual males are similar in their structure and design. The aim of this study was to measure the level of repeatability in the nest characteristics of individual male three-spined sticklebacks within populations. We replicated this experiment for four populations to obtain a general idea of repeatability within the average population.

METHODS

Fish Collection and Husbandry

Adult sticklebacks were collected using a 2-m seine (mesh size 5×5 mm) and hand nets (mesh size 1×1 mm) during March 2005 from four populations in mid-Wales (U.K.); two lakes, Llyn Frongoch (52°21′46″N 3°52′26″W) and Llyn-yr-Oerfa (52°24′05″N 3°52′19″W), and two rivers, the Afon Rheidol (52°24′16″N 4°02′49″W) and the Afon Ystwyth (52°23′55″N 4°05′08″W). Previous studies had shown that, when building under common conditions identical to those described in the present study, there were no significant population-level differences in nest composition or structure (B. J. Rushbrook & I. Barber, unpublished data). The four populations were therefore used in this study because they were local and readily sampled, rather than representing an interest in, or an expectation of, habitat-specific patterns of nesting behaviour. On transfer to the laboratory, fish were placed in mixed-sex groups in population-specific aquaria ($750 \times 200 \times 380$ mm). Conditions within the laboratory encouraged reproductive development (16:8 h light:dark photoperiod; temperature: $\overline{X} \pm \mathrm{SD} = 17.7 \pm 0.2$ °C). Throughout the experiment, fish were fed daily, ad libitum, on a mixture of chironomid larvae and *Daphnia* sp.

Nest Building

In late March, five males from each population were blotted, weighed (0.001 g) and measured (standard length to 1 mm), and introduced into individual nesting aquaria ($200 \times 350 \times 200$ mm). Each aquarium was provided with a sponge airlift biofilter to maintain water quality, a plastic plant for cover, and a gravel substratum, the front third of which was covered by a layer of sand. Each male was provided with a bundle of 200, 7-cm-long black polyester threads as nesting material. Preliminary studies demonstrated that males from each of the four populations readily constructed nests under these standardized conditions (B. J. Rushbrook, & I. Barber, unpublished data).

Visual access to gravid females (presented in glass jars) was provided for two consecutive 10-min periods each day to encourage nesting activity. Heavily gravid females were kept in stock throughout the study, and four that appeared to be in spawning condition were selected from the stock tanks each day for presentations. Each day, every male received successive presentations of two of the four females, chosen at random. These procedures negated the possibility that any male was presented consistently with the same female, and countered the possibility that females affected male nest building. Nesting aquaria were inspected visually on completion of female presentations, and the date of nest initiation (defined by the appearance of glued nesting threads