RESEARCH ARTICLE

Parentage of Overlapping Offspring of an Arboreal-Breeding Frog with No Nest Defense: Implications for Nest Site Selection and Reproductive Strategy

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Abstract

Overlapping offspring occurs when eggs are laid in a nest containing offspring from earlier reproduction. Earlier studies showed that the parentage is not always obvious due to difficulties in field observation and/or alternative breeding tactics. To unveil the parentage between overlapping offspring and parents is critical in understanding oviposition site selection and the reproductive strategies of parents. Amplectant pairs of an arboreal-breeding frog, *Kurixalus eiffingeri* , lay eggs in tadpole-occupied nests where offspring of different life stages (embryos and tadpoles) coexist. We used five microsatellite DNA markers to assess the parentage between parents and overlapping offspring. We also tested the hypothesis that the male or female frog would breed in the same breeding site because of the scarcity of nest sites. Results showed varied parentage patterns, which may differ from the phenomenon of overlapping egg clutches reported earlier. Parentage analyses showed that only 58 and 25% of the tadpole-occupied stumps were reused by the same male and female respectively, partially confirming our prediction. Re-nesting by the same individual was more common in males than females, which is most likely related to the cost of tadpole feeding and/or feeding schemes of females. On the other hand, results of parentage analyses showed that about 42 and 75% of male and female respectively bred in tadpole-occupied stumps where tadpoles were genetically unrelated. Results of a nest-choice experiment revealed that 40% of frogs chose tadpole-occupied bamboo cups when we presented identical stumps, without or with tadpoles, suggesting that the habitat saturation hypothesis does not fully explain why frogs used the tadpole-occupied stumps. Several possible benefits of overlapping offspring with different life stages were proposed. Our study highlights the importance of integrating molecular data with field observations to better understand the reproductive biology and nest site selection of anuran amphibians.

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Introduction

Overlapping offspring is an interesting phenomenon that occurs more commonly than originally thought, but the patterns, causes, and ecological and evolutionary consequences are not fully understood.

Section Two

When sites are reused for reproduction, the sites may be empty (if previous offspring have left) or may contain offspring from earlier reproduction.

If offspring from previous reproduction remain, this results in two overlapping cohorts.

Overlapping offspring are commonly found in many oviparous animals such as insects [1 , 2], fishes [3 , 4], amphibians [5 – 8] and birds [9 – 12].

Section Three

Often, it is assumed that the overlapping offspring are produced by the same parents; however, the parentage of offspring is not always obvious due to difficulties in field observation and/or alternative breeding tactics [13 – 15].

Section Four

For example, when an intruding male fish takes over an egg-filled nest, it attends the eggs but also breeds with other females, resulting in overlapping offspring with multiple parentage [13 , 16]. Similarly, in a salamander (*Hemidactylium scutatum*), joint nesting, where several females lay eggs at a site, can also result in complex parentage [17 – 19].

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