Detection Of Perivitelline Membrane Bound Sperm gDNA Profiles Using Quail-Specific Microsatellites

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Microsatellites are small tandem repeat DNA sequences interspersed throughout an organism's genome. The number of microsatellite repeats can differ, allowing for genetic identification of individuals. This technique has been used for human identification in crime investigations and paternity determination. Likewise, microsatellite analysis can also be utilized for animal identification. For example, dog and horse parentage confirmation is critical to breed management, captive breeding facilities can use microsatellites to establish pedigrees for optimal genetic diversity pairings, and wildlife studies identification of individuals can help estimate emigration and immigration, and in zoos, microsatellites have been utilized to determine paternity in animals managed in colonies.

Microsatellites have previously been used in studies to determine paternity, estimate extrapair copulations (EPCs), and cryptic mate choice/sperm storage gland function in birds. However, these studies are based on the paternity of the offspring. However, if an EPC event does not result in offspring, the EPC is not "counted," and the actual rate of EPC events (as opposed to extrapair paternity) estimate is skewed.

We previously presented a method to isolate gDNA from the perivitelline membrane to confirm mating, in addition to fluorescent microscopy identification of sperm heads embedded in the PVM). However, no one, to our knowledge, has explored the possibility of using PVM-bound sperm to genetically identify sperm donors. Such information would be invaluable to basic avian reproductive physiology through more accurate assessments of female cryptic choice, sperm-storage gland sperm selection, or EPC events.

We identified four quail-specific microsatellite primers exhibiting allele diversity in our quail research population, allowing us to differentiate between two male and two female quail from our research colony. One male was allowed to mate with the females, and eggs were collected for PVM removal and gDNA isolation, followed by microsatellite PCR amplification. We successfully isolated gDNA (5-10 ng/□L, 250-500 ng) at a high enough concentration to attain microsatellite profiles for each egg. All the eggs exhibited microsatellite amplification, allowing us to build a DNA profile for each egg. However, due to too many common alleles, only one microsatellite was able to confirm male-only gDNA by excluding both females in a third of the eggs and excluding the non-breeding male in all the eggs.

This project could significantly impact basic avian reproductive research and captive management, allowing zoo and conservation managers to estimate EPC rates within a species or population. This is important because studbooks are based on actual pairings, which, depending on the EPC, may not accurately reflect true paternity. Knowing the estimated EPC will allow facilities to decide if chick paternity testing costs are warranted for studbook accuracy. We will use this method to investigate cryptic female choice, including sperm-storage gland sperm selection.