Molecular species identification of predators of endangered species on Okinawa-jima Island

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The ‘Yambaru’, a subtropical rainforest of northern Okinawa-jima Island, Japan, has a diverse and complex biota. It is particularly important habitat for many endangered species, such as the Ryukyu long-haired rat (*Diplothrix legata*), the Okinawa spiny rat (*Tokudaia osimensis*), the Okinawa rail (*Gallirallus okinawae*), the Ryukyu black-breasted leaf turtle (*Geoemyda japonica*), Ishikawa’s frog (*Odorrana ishikawae*), and others. These species are all threatened with extinction, due mainly to habitat destruction, poaching, and negative effect by introduced species.

In the Yambaru, carnivores that prey on these endangered species were once absent. The human-introduced predators, such as the domestic dog (*Canis lupus familiaris*), the domestic cat (*Felis catus*), and the Javan mongoose (*Herpestes javanicus*), seem to be the greatest threat to the endangered fauna of the Yambaru. For example, the wild population of Okinawa rail is in decline (from 1,500–2,100 birds in 1985 to 820–1,300 birds in 2006) due to habitat loss and predation by stray dogs and cats (Ozaki 2008).

In recent years, the mongoose’s distribution has expanded into the Yambaru area (Ozaki et al. 2002). Therefore, predation of the endangered animals in the Yambaru is likely to increase.

To establish an effective predator control program, one must first identify the predator species, because a proper trapping system must be selected for predator eradication. Therefore, the identification of predator species is essential if there were multiple predator species in geographical area of predator eradication activities like a Yambaru area. However, predator identification is difficult because direct observations of predation are rare, and often a predator’s bite marks are not distinctive. We focused instead on predator saliva remaining on the carcass. Saliva has long been used as an important source of DNA in human forensics (Butler 2005). Recently, DNA from saliva has been used to investigate livestock predation (Williams et al. 2003; Sundqvist et al. 2008). Unfortunately, the amount of DNA that can be extracted from predator saliva on prey is extremely small and is mixed with large amounts of prey DNA. Additionally, when the prey is a wild animal, several days usually pass before the carcass is discovered, during which the DNA tends to degrade. For all these reasons, effective predator control requires a DNA-based method of predator identification that is both highly sensitivity and highly species-specific.

Here, we report a technique to identify predators from salivary DNA remaining on prey carcasses. First, we designed species-specific nested primers for the cytochrome *b* gene of dogs, cats and Javan mongooses and developed two PCR methods to identify predators, a species-specific nested PCR and a multiplex nested PCR that included multiple species-specific primer pairs in a single reaction. We applied them to predator species identification using skin, hairs or feathers of prey animals. Specifically, we tested our protocol on carcasses of the Ryukyu long-haired rat, the Okinawa rail, the white-breasted waterhen (*Amauromis phoenicurus*), and the Okinawa woodpecker (*Sapheopipo noguchii*).

Materials and methods

Sample collection and DNA extraction

Pure predator DNA was extracted from muscles of the domestic dog, the domestic cat, and the Javan mongoose using the DNeasy Blood & Tissue Kit (Qiagen). DNA samples were also extracted from the carcasses of the Ryukyu long-haired rat (1 sample), Okinawa rail (14),

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