Abstract. While analyzing wild mosquitoes caught in Paju, South Korea, we identified one egg-laying hybrid female between Anopheles kleini and Anopheles sinensis. Additional evidence was obtained by identifying several F1 progeny and conducting self-crossing between them. Sequencing of mitochondrial cytochrome c oxidase subunit 1 sequence suggested that the maternal origin of the female should be An. sinensis. Additionally, observation of only two distinct genotypes of F1 progeny (double band, hybrid type, and single-band An. sinensis type) more closely resembled a situation of natural back-crossing between a hybrid female and male An. sinensis. Results of self-crossing between F1 progeny was impaired and yielded abnormally low larval hatchings (3.7%). Overall, the observation of 1 female from 658 caught-wild mosquitoes indicated possible natural hybridization among members of the Hyrcanus group from South Korea.

Members of the Hyrcanus group of mosquitoes recently gained renewed attention in numerous entomologic and epidemiologic studies because some members have been associated with the recent re-emergence of malaria in eastern Asia, including South Korea and China.1-3 Although nearly 30 anopheline species are members of the Hyrcanus group, only six species are indigenous to South Korea.4 These include Anopheles sinensis, An. lesteri, An. pullus, An. sineroides, An. kleini, and An. belenrae.5-7

For sexually reproducing organisms, there are barriers that prevent interbreeding of two or more closely related species that are actually or potentially sympatric, thereby rendering the species reproductively isolated. However, evidence regarding the existence of natural hybrids gives rise to the opinion that these barriers could be comparatively weak in closely related species. In rare cases, when these barriers are broken, hybrids are produced. In particular, there are a few reports that indicate the existence of natural hybrids in sympatric populations of Anopheles mosquitoes. To date, such hybrids are believed to exist in the An. gambiae complex, especially between An. gambiae and An. arabiensis.8-11

In the past, hybrid mosquitoes were identified from the study of polytene chromosomes and crossing experiments,8,9 but in recent years, molecular markers have detected rare hybrids.11 We describe a single gravid female with sufficient evidence to suggest that it is a potential hybrid between An. sinensis and An. kleini.

Light-trap collections were conducted during 2004–2008 in Manu-ri, Majeong-ri, and Samok-ri in Paju County, South Korea. Collected females were allowed to lay eggs inside in Manu-ri, Majeong-ri, and Samok-ri in Paju County, South Korea. E-mail: mingisik@inha.ac.kr

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female should be a normal An. sinensis male. This is precisely the same situation as in the back cross between a hybrid female and a normal male.

The result of the crossing experiment between F1 progeny was analyzed in three steps. First, larval hatching was observed. In the first and second batches, hatching of larvae was not observed by 6–7 days post oviposition, and relatively low larval emergence (10.6%) was seen in the remaining third batch (Table 1). Second, the status of embryogenic development was confirmed, in which dissections of 310 eggs representing the first two batches were performed. There was no sign of embryo development within any of the dissected eggs (0% embryonation rate). Third, during spermathecae examinations of seven females showed that only one contained sperm.

On the basis of data from PCR identification of F1 progenies (Figure 2), our study showed self-crossing between F1 progeny of the hybrid female that were produced from the natural back crossing between the hybrid female and male An. sinensis. Most F1 progeny were not fertile because they yielded abnormal crossing results (Table 1). However, other status of F1 progeny could have been obtained if they were backcrossed with normal partners. However, such an experiment was not possible at the time the wild female was caught.

This is the first report of a natural hybrid among the members of the Hyrcanus group of mosquitoes. Observation of only 1 hybrid from 657 anopheline females suggests that natural hybridization is a rare incidence, similar to that in the An. gambiae complex.11 The hybrid female, which had been back-crossed with a normal An. sinensis, showed normal fertility and the F1 progeny she produced appeared sterile in self-crossing experiments. Therefore, further study will be required to verify the fertility of F1 progenies from laboratory back crosses. Given that progeny of hybrids can survive and are fertile, hybridization might be an important mechanism for gene introgression from one species to another among the members of Hyrcanus group in nature.

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