Discussion

Our data reveal the levels of introgressive hybridization and migration rate are low between *T. viridipunctatus* and *T. luyeanus* (Table 4, Table 6). No recent migrants were found based on the microsatellites and mitochondrial DNA data. The low migration rate may be an underestimated due to incomplete sampling. However, this study indicated the effective migration between these two species is low, and no sympatric distribution area of *T. viridipunctatus* and *T. luyeanus* was found (Figure 3, Figure4).

In animals, high levels of introgression can be reached within relatively few generations (Goodman *et al*. 1999). Most examples are seem in amphibians (*Plethodon*: 5~15%, Weisrock 2005; *Lyciasalamandra*: 14%, Johannesen 2006), and birds (*Phylloscopus collybita*: 13.97%, Helbig 2001). In reptiles, hybridization seems to be common in turtles (Karl 1995; Seminoft 2003; Spinks 2004; Stuart 2004; Buskirk *et al.* 2005) and parthenogenetic lizards with their parental species (Kearney 2005, Strasburg 2005), but not to be common in diploid, and sexual lizards except genus *Sceloporus* (Arevalo 1993, Reed 1995, Sites 1996, Jonathon 2001).

According to the data, introgression of *T. viridipunctatus* and *T. luyeanus* is bidirectional. Sixteen hybrids have one or more specific alleles from the other species based on microsatellite DNA. This implies that hybrids which carry rare or specific alleles are more easily detected than those carrying alleles with similar frequencies from both species.

Two of the hybrids, number 133 and 224 (Figure 3, Figure4), carry

more than 50% genetic character of the other species in both the 13-loci and 6-loci analyses. This former individual (number 133) could be an offspring of one female hybrid mating with a male *T. luyeanus*, or it also could be a hybrid which exhibits more characters of *T. luyeanus* which migrated to the northern-bank of the river. The latter individual (number 224) could be an offspring of one female hybrid mating with a male *T. viridipunctatus*, or a hybrid which exhibits more characters of *T. viridipunctatus* which migrated to the southern-bank of the river. These results represent the congruity of 13-loci and 6-loci analyses.

From the mitochondrial DNA data, only one hybrid (individual of number 232) from the southern bank carried genetic characters of *T. viridipunctatus* (Figure 4). The data indicated that this hybrid was caused of female introgression, but the STRUCTURE results from the 6-loci microsatellite analysis indicated low proportion of genetic characters of *T. viridipunctatus*. It is presumed that this hybrid had backcrossed with *T. luyeanus* for several generations, and it also indicates that these hybrids may be viable.

This situation is similar to the recent study of African forest and savanna elephants because their mitochondrial DNA markers in same populations, forms two highly divergent clades (Roca *et al.* 2005; Debruyne 2005). However, these did not always correspond to the nuclear genotypes of the elephants that carried them (Roca *et al.* 2001; Comstock *et al.* 2002; Roca *et al.* 2005). Recurrent backcrossing of female hybrid elephants to large savanna males, out-competing the smaller forest or hybrid males, would dilute and replace the forest elephant component of the nuclear genome, but it would retain the

maternally inherited forest-typical mitochondrial DNA haplotypes (Roca *et al.* 2005). Other deleterious effects of hybridization that differentially harm male hybrids might have also played a role, like Haldane's rule (Roca and Obrien 2005).

The unequal migration rates between males and females could first be deduced from male-biased dispersal. In animals, sex-biased dispersal may play a crucial role contributing to the incongruence pattern between mitochondrial and nuclear genome (Goudet 2002, Lampert *et al*. 2003, Moller and Beheregaray 2004, Palo *et al.* 2004, Song 2005). However, lack of ecological research, we do not have sufficient evidence to verify this hypothesis. On the other hand, Haldane's rule provided an alternative explanation. Haldane's rule is one of the most widely applicable paradigms in evolutionary biology, stating that between species crossings, the heterogametic sex will suffer more severely in terms of sterility and inviability (Haldane 1922, Orr 1997). Lacertid lizards belong to ZW type of sex determination (Cree *et al.* 1995), representing that the heterogametic sex is female. According to Haldane's rule, female hybrids in *Takydromus* might represent lower viability or lower fertility. This explains the lower proportion of female introgression than male introgression between these sister species.

R_{st} within *T. viridipunctatus* populations are between 0.0089-0.3707 (Table 8) and 0.0299-0.3354 within *T. luyeanus* populations (Table 9). Most R_{st} within *T. viridipunctatus* populations and *T. luyeanus* populations are significantly large ($p<0.05$). The high differentiation between subpopulations may be due to the complicated topography of Taiwan; some sampling sites are in restricted habitats due to geographic

barriers while the others are in open areas. Geographic boundaries may be the reason for high polymorphic genotypes and high R_{st} values of genus *Takydromus*. More specifically, the Liwu River might play an important role in influencing the low levels of introgressive hybridization, the low migration rate, and high differentiation between *T. viridipunctatus* and *T. luyeanus*.

The genetic data of mitochondrial DNA and nuclear genome show that these two species are distinct species. Low level of gene flow can be detected after their secondary contact. The result can be viewed as the process by which one species complex diverges genetically to the stage at their subsequent populations merge would not be possible. The migration rates of both species are low; it is presumed that the reproductive isolation almost completed. I suggested that there are certain selection forces working on both species. Adaptation and competition, even the sex characters, such as recognizably colors and the body sizes of male lizards, would be the possible reasons in reproductive isolation between these two species (Rolan-Alvarez 1999, Barton 2001, Helbig 2001, Smith 2003, Emily 2004).