Genetic Variation of *Goodyera velutina* (Orchidaceae) Based on Chloroplast DNA Sequences from *trn*L-*trn*F Region

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To investigate the genetic variation of the *G. velutina*, we carried out the single stranded comformation polymorphism (SSCP), restriction enzyme digestion and comparing the nucleotide sequences of *trn*L (Leu) and *trn*F (Phe) intergenic spacer. The *trn*L (Leu) and *trn*F (Phe) intergenic spacer in chloroplast DNA (cpDNA) were cloned from 10 individuals of the *G. velutina* by polymerase chain reaction (PCR). The total number of bases in the *trn*L (Leu) and *trn*F (Phe) intergenic spacer were 489. SSCP analysis of denatured and amplified products was carried out by polyacrylamide (10%) gel electrophoresis followed by silver staining. PCR products from 10 individuals were digested with 4 restriction enzymes, *Bam*H I, *Eco*R I, *Hinf* I, and *Fok* I. The results of the SSCP and restriction enzyme digestion clearly showed two different types, contained same results as those detected in the SSCP analysis. Comparing of the nucleotide sequences between two different taxa, base substitutions in twelve sites were found. These results show that SSCP, restriction enzyme digestion and DNA sequencing were useful in elucidating the interspecific variation in the *G. velutina* population.

F810

PCR-RLFP Analysis of Mitochondrial Cytochrome *B* Gene in Cheju Native Horses

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We analyzed mitochondrial DNA (mtDNA) cytochrome *B* (Cyt*B*) gene in Cheju native horses using polymerase chain reaction (PCR) - restriction fragment length polymorphism (RFLP). *CytB* in mammals flanks in tRNA-Glu and tRNA-Thr in mitochondrial genome and 1140 bases long. To acquire the complete fragment of mtDNA *CytB*, we designed PCR primers in tRNA-Glu (H14158) and in tRNA-Thr (L15368) among *Equus*, *Sus* and *Bos*. The amplified PCR products were digested with 6 restriction endonucleases (*Bam*H I, *Eco*R I, *Rsa* I, *Msp* I, *Hae* III and *Hinf* I). Throughout the electrophoretic patterns, we found two types of fragments digested with *Rsa* I, *Msp* I and *Hae* III in Cyt*B* gene, respectively. Two kinds of *CytB* in Cheju native horses were found, though the frequency of variant was low. In addition, it is necessary to analyze the nucleotide sequences of *CytB* to develop a useful marker for identifying the maternal lineage and investigating the origin of Cheju native horses and relationships among other populations of east Asian native horses.

F811

Molecular Genetic Studies on the Structural and the Evolutionary Relationship of the Late Chorion Locus(Hc) from Wild Silkmoth, *Bombyx mandarina*.

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The chorion proteins(A, B, C, D, E, HcA and HcB) are synthesized by monolayer of follicular epithelial cells and assembled after secretion into egg shell. They are served as a protective layer of egg ; facilitating gas exchange, preventing desiccation. They are composed of 100~150 genes as superfamily in the second chromosome. Chorion gene is the good subjects to study evolutionary and developmental regulation. The 140kb late chorion locus of *B. mori* contains 15-multigene families (HcA, HcB), which are divergently oriented gene pairs. The chorion proteins of *B. mandarina*(wild silkmoth) apparently differ from those of *B. mori* in the structure and morphology. we have studied the chorion late locus of *B. mandarina*, constructed wild silkmoth genomic library, and obtained the 10 Hc clones with hybridization and PCR. pCH417 (one of the obtained clones) was sequenced and characterized. The sequence similarities between *B .mori* and *B. mandarina* are 94%, 93% for HcA, HcB respectively. A major sequence difference is founded is C-arm, which consists of (Cys-Gly-Gly), (Cys-Gly), meaning the variation is the result of frequent expansion and contraction events, but central domain is highly conserved region. We will study the structural and evolutionary relationship of Hc between *B. mori* and *B. mandarina*.

F812

Gene Therapy of Type 1 Diabetes Mellitus: Liposome-mediated DNA Delivery to Murine Skin

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Type 1 diabetes mellitus is caused by severe insulin deficiency secondary to the autoimmune destruction of pancreatic b cells. We constructed plasmid vector coding insulin gene with keratin 14 promotor and the vectors were liposome-mediated injected into murine skin targeting keratinocytes by the jet injection device. Low-level but sustained expression of the insulin from the murine keratinocytes was achieved *in vitro* administration of the plasmid vectc and sequencing revealed it was identical to human preproinsulin sequences combined keratin 14 promotor region. In advance of *in vivo* application, we injected another two kinds of constructed vectors containing *LacZ* instead of insulin gene. Gene expression was shown in skin as early as 1 week after DNA application and has been sustained until 20 weeks. By PCR and southern blot analyses showed the *LacZ* gene integrated into chromosomes as we expected. We have taken different approach to study proper insulin gene delivery and its activity in body. Streptozotocin (STZ) selectively destroyed insulin producing beta islet cells of the pancreas providing a model of type 1 diabetes mellitus with remarkable similarity to that of human IDDM patients. So we applied DNA vectors to 4 groups of mice and blood glucose levels were monitored. Both multiple low-dose and single high-dose injections of STZ were sufficient to induce hyperglycemia, increasing the blood g . cose levels resulting from loss of pancreatic cells and 6 levels of dose DNA vector treatment decreased 24-68% of blood glucose levels in STZ-induced diabetic mice. Microscopic examination of the X-gal stained region of the gene treated skin tissue explained the promoter activity compared with lacki:ıg of the region. And microscopic images of pancreas explained the number of beta cells greatly reduced and almost destroyed at the same time statistical data of vector injection showed sustained normal blood glucose levels. Furthermore keratinocyte-specific gene expression by immunohistochemistry and quantitative analysis of insulin produced from keratinocytes in STZ induced diabetic