Supporting material

Fitness differences associated with *Pgi* SNP genotypes in the Glanville fritillary butterfly (*Melitaea cinxia*)

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Running title: reduced fitness in a butterfly metapopulation

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Appendix S1 ***RNA isolation, cDNA synthesis, and sequencing of Pgi cDNA***

Total RNA was extracted from adult butterfly tissues, typically thorax or abdomen, using Trizol™ reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. cDNA synthesis used 2 µg of total RNA using oligo$(dT)_{20}$ primer and Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Initially we used RNA from one individual to clone and sequence the cDNA copy of *Pgi*. We designed three degenerate PCR primer pairs by aligning *Pgi* sequences of four other insect species (*Anopheles gambiae*, *Bombyx mori*, *Colias eurytheme*, and *Drosophila melanogaster*). The most conserved pair of degenerate primers (forward: 5' GAT STS GGN CCK CTS ATG GT 3', reverse: 5' GAA GAT YTT GTG YTC GTA CA 3') amplified a fragment of 974 bp (excluding primers), which was cloned into pCRII-TOPO (Invitrogen) and sequenced. The sequence aligned between positions 513 and 1486 of the *Colias eurytheme* coding sequence, with 79.9% sequence identity. Based on this partial sequence we designed gene-specific primers, which allowed us to identify the 5' and 3' ends of the *Pgi* mRNA by SMART-RACE (BD Biosciences, gene-specific primers for 5' RACE: 5' TGG CGA CAT CCA AGA ACC AGT TCT TG 3' and 5' GGT TTT GGA CGC TAT GAT GAA 3', gene-specific primer for 3' RACE: 5' GAC CGA GGC TCT CAT GAA AGG CAA AA 3'). Cloned products yielded the remaining coding sequence of the gene, including the entire 3' untranslated region (UTR) up to the poly-A tail and 75 bp of the 5' UTR.

Pgi cDNA sequences were obtained from 33 adult butterflies. These butterflies originated from 21 local populations from the Åland Islands in Finland scattered across the entire metapopulation and they represent a subset of a larger sample used in an earlier allozyme study (Haag *et al.*, 2005). For the PCR amplification of the *Pgi* gene, high-fidelity Phusion DNA polymerase (Finnzymes, Finland) and two primer sets (mPGI-16 and mPGI-17 or seqPGI-21F and mPGI-4) were used. The primer sequences were designed on the synthesized cDNA as described above and are as follows:
mPGI-16: 5'-CCGTGTACTCGAAAACTTTATTTG-3',
mPGI-17: 5'-CGCATCAATAATAATTGGACCAT-3',
seqPGI-21F: 5'-ATGGAGCCTAAAGTGAATTTG-3',
and mPGI-4 5'-GAAGATYTTGTGYTCGTACA-3'.

Additionally, internal primers were designed to obtain several reads by parallel sequencing of the same region with multiple PCR primer pairs. The internal primers sequences are as follows:
mPGI-598R: 5'-TCTCAGGGTTCAATTTCTT-3',
mPGI-634F: 5'-AGACAGCTCTCTTCATCATAG-3',
mPGI-1192R: 5'-CTGGTCCCCTGGTGTAT-3'.

Table S1 List of samples sequenced at the *Pgi*. Sequences results at the three target SNPs, SNP-genotypes, allozyme genotypes, and accession numbers to GenBank are shown.

Sample ID	Sequence at SNP sites	SNP genotypes	Allozyme genotype	AN Genbank
P22_04	AA AC AG	AA AC AG	FF	EU888455
P30_04	TT AA GG	TT AA GG	DD	EU888461
PP1_04	AT AC AG	AT AC AG	DD	EU888473
PP13_04	AA AA AA	AA AA AA	CC	EU888466
PP22_04	AA CC AA	AA CC AA	FF	EU888465
PP23_04	TT AA GG	TT AA GG	DD	EU888459
PP4_04	AT AC AG	AT AC AG	DF	EU888453
PP44_04 ?	AT AC AG	AT AC GG	FF	EU888472
PP48_04	TT AA GG	TT AA GG	DD	EU888467
S39_04	TT AA GG	TT AA GG	DD	EU888460
S45_04	AA AA AA	AA AA AA	AA	EU888464
S55_04	AT AA AG	AT AA AG	DC	EU888454
SS10_04	AT AC GG	AT AC GG	DG	EU888469
P27_04	AA AC GG	AA AC GG	BH	EU888468
P47_04	TT AA GG	TT AA GG	DA	EU888458
P51_04	AT AC AG	AT AC AG	DF	EU888457
P74_04	AA CC AG	AA CC AG	FF	EU888470
P83_04	TT AA GG	TT AA GG	DD	EU888462
S11_04	TT AA GG	TT AA GG	DD	EU888456
S59_04	AT AC AG	AT AC AG	FA	EU888471
S69_04	AA CC AA	AA CC AA	FD	EU888463
P20_04 *	AA AC AG	AA AC AG	FF	EU888441
S68_04	AA AA AG	AA AA AG	FC	EU888443
S76_04	AT AC AG	AT AC AG	FD	EU888444
SS22_04 ?	AA AC AG	AA AC GG	FF	EU888445
SS30_04	AA AA GG	AA AA GG	DD	EU888447

SS33_04	AT AA GG	AT AA GG	DD	EU888446
PP39_04 *	TT AA AG	TT AA AG	DA	EU888442
SS37_04 *	AA AA GG	AA AA GG	DD	EU888448
P78_04 *	AA AC GG	AA AC GG	FH	EU888451
34_06	TT AA AG	TT AA AG	DC	EU888450
197_06 ?	AT AC GG	AT AC AG	DF	EU888452
SS43_04*	AA AC GG	AA AC GG	HF	EU888449

? Mismatch between sequence and SNP genotype

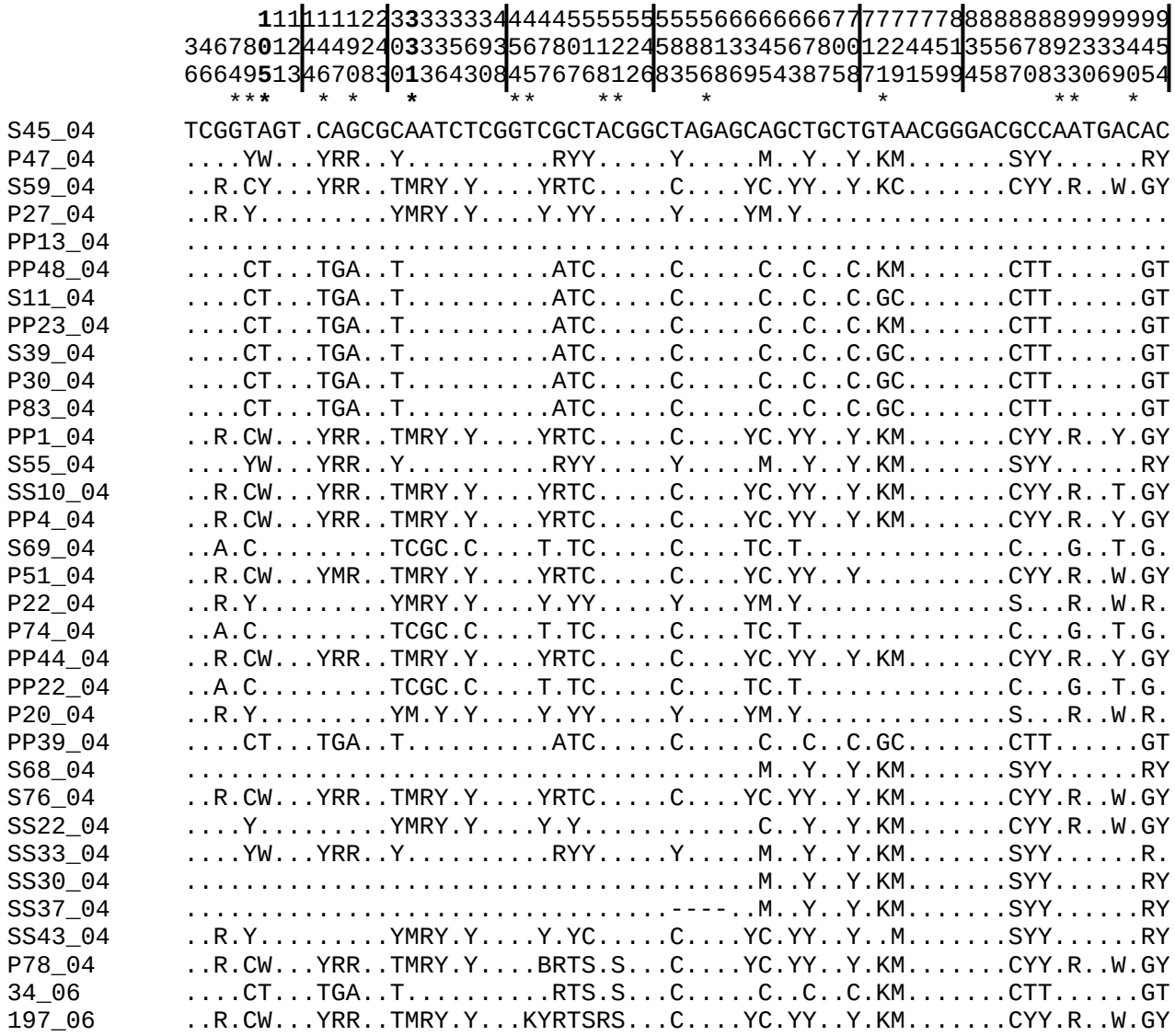
*Partial sequence (10-35bp missing)

Table S2 List of pseudo-haplotypes as inferred from the unphased data using the program DNAsp (Rozas *et al.*, 2003). For each pseudo-haplotype, the allozyme allele based on the allozyme electrophoresis, the inferred allozyme group and the net charge are shown. The inferred haplotype group is based on the information in Table 2.

Pseudo-haplotype	Allozyme allele	Haplotype group	Net charge
PP39_04_1	A	E-1	3.08
S59_04 _1	A	E-1	3.08
34_06 _1	C	E-2	3.08
P47_04 _1	A	A-1	2.91
PP13_04 _1	C	A-1	2.91
S45_04 _1	A	A-1	2.91
S45_04 _2	A	A-1	2.91
SS22_04_2	F	A-2	2.91
PP13_04 _2	C	C-1	2.91
S55_04 _1	C	C-1	2.91
S68_04 _1	C	C-1	2.91
P27_04 _1	B	B	2.75
P30_04 _1	D	D-1	1.91
P30_04 _2	D	D-1	1.91
P47_04 _2	D	D-1	1.91
P83_04 _1	D	D-1	1.91
P83_04 _2	D	D-1	1.91
PP1_04 _1	D	D-1	1.91
PP23_04 _1	D	D-1	1.91
PP39_04_2	D	D-1	1.91
PP4_04 _1	D	D-1	1.91
PP44_04_1	F	D-1	1.91
PP48_04 _1	D	D-1	1.91
S11_04 _1	D	D-1	1.91
S11_04 _2	D	D-1	1.91
S39_04 _1	D	D-1	1.91
S39_04 _2	D	D-1	1.91
S55_04 _2	D	D-1	1.91
S76_04 _1	D	D-1	1.91
SS10_04 _1	D	D-1	1.91
SS33_04 _1	D	D-1	1.91
34_06 _2	D	D-2	1.91
P51_04 _1	D	D-2	1.91
PP23_04 _2	D	D-2	1.91
PP48_04 _2	D	D-2	1.91
197_06 _1	D	D-3	1.91
P78_04_2	H	D-4	1.91
P20_04_2	F	F-1	1.91
P22_04 _2	F	F-1	1.91

P51_04 _2	F	F-1	1.91
P74_04 _1	F	F-1	1.91
P74_04 _2	F	F-1	1.91
P78_04_1	F	F-1	1.91
PP1_04 _2	D	F-1	1.91
PP22_04 _1	F	F-1	1.91
PP22_04 _2	F	F-1	1.91
PP4_04 _2	F	F-1	1.91
PP44_04_2	F	F-1	1.91
S59_04 _2	F	F-1	1.91
S69_04 _1	D	F-1	1.91
S69_04 _2	F	F-1	1.91
S76_04 _2	F	F-1	1.91
P27_04 _2	H	H	1.75
SS43_04_2	H	H	1.75
P20_04_1	F	O-1	1.75
P22_04 _1	F	O-1	1.75
SS30_04 _2	D	O-1	1.75
SS33_04 _2	D	O-1	1.75
SS37_04_2	D	O-1	1.75
SS43_04_1	F	O-1	1.75
S68_04 _2	F	O-2	1.75
SS30_04 _1	D	O-2	1.75
SS37_04_1	D	O-2	1.75
197_06 _2	F	G-1	0.75
SS10_04 _2	G	G-1	0.75
SS22_04_1	F	G-2	0.75

Figure S1 Synonymous and non-synonymous segregating sites across 12 exons at *Pgi*. Each row is an individual from the Finnish metapopulation, with segregating variation indicated by nucleotide positions at the top of the column. Dots represent nucleotides that are identical to the first entry in the column. Non-synonymous polymorphisms are identified by an asterisk (*). The three charge-changing amino acids used in the SNP design (Table 1) are highlighted in bold phase. Vertical lines delimit exons boundaries.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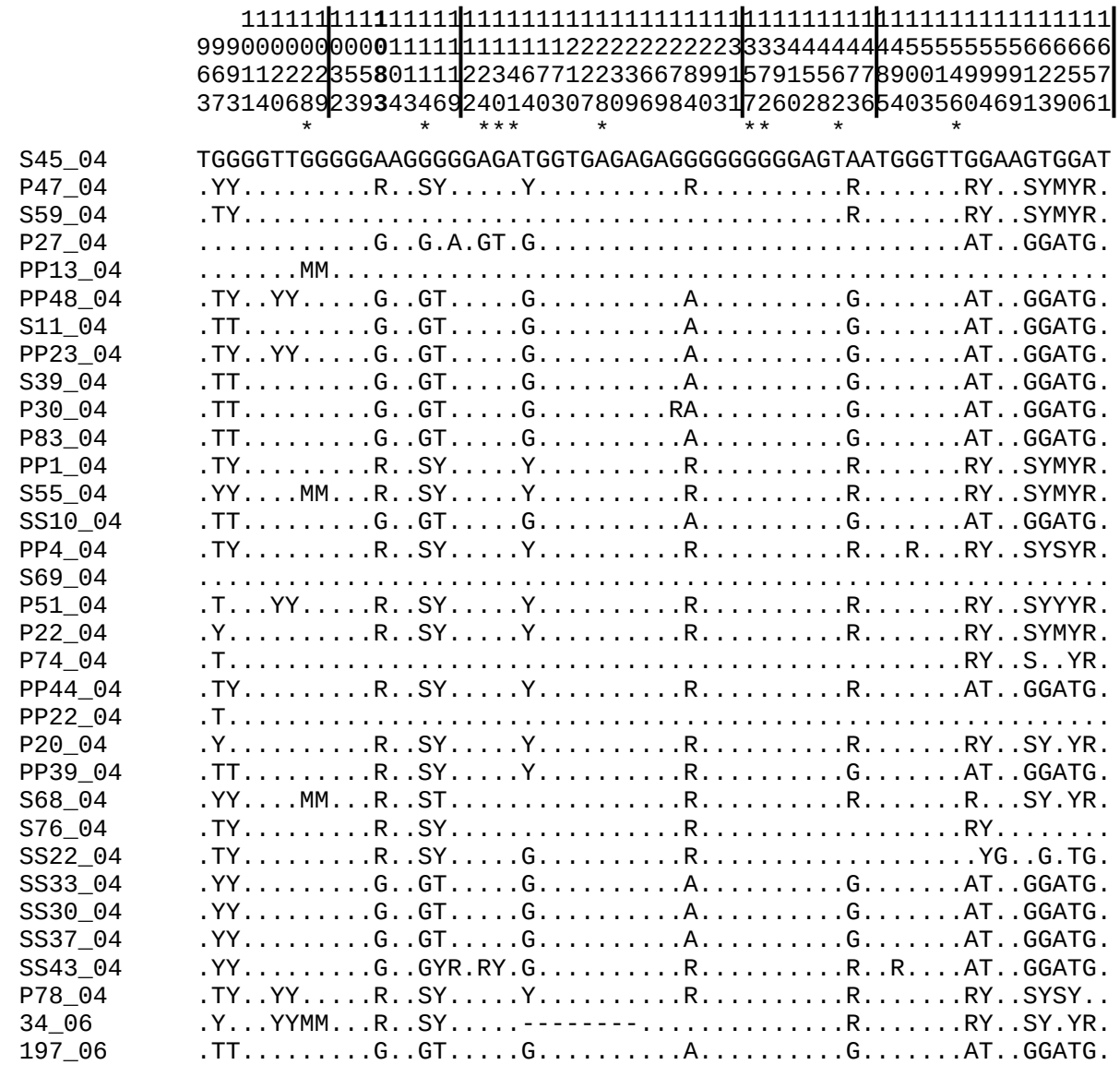
TCGGTAGT.CAGCGCAATCTCGGTCGCTACGGCTAGAGCAGCTGCTGTAACGGGACGCCAATGACAC
S45_04 ....YW...YRR..Y.....RYY.....Y.....M..Y..Y.KM.......SYY......RY
P47_04 ..R.CY...YRR..TMRY.Y....YRTC.....C....YC.YY..Y.KC.......CYY.R..W.GY
S59_04 ..R.Y.....YMRY.Y....Y.YY.....Y....YM.Y.....
P27_04 ....CT...TGA..T.....ATC.....C.....C..C..C.KM.......CTT......GT
PP13_04 ....CT...TGA..T.....ATC.....C.....C..C..C.GC.......CTT......GT
PP48_04 ....CT...TGA..T.....ATC.....C.....C..C..C.KM.......CTT......GT
S11_04 ....CT...TGA..T.....ATC.....C.....C..C..C.KM.......CTT......GT
PP23_04 ....CT...TGA..T.....ATC.....C.....C..C..C.GC.......CTT......GT
S39_04 ....CT...TGA..T.....ATC.....C.....C..C..C.GC.......CTT......GT
P30_04 ....CT...TGA..T.....ATC.....C.....C..C..C.GC.......CTT......GT
P83_04 ..R.CW...YRR..TMRY.Y....YRTC.....C....YC.YY..Y.KM.......CYY.R..Y.GY
PP1_04 ....YW...YRR..Y.....RYY.....Y.....M..Y..Y.KM.......SYY......RY
S55_04 ..R.CW...YRR..TMRY.Y....YRTC.....C....YC.YY..Y.KM.......CYY.R..T.GY
SS10_04 ..R.CW...YRR..TMRY.Y....YRTC.....C....YC.YY..Y.KM.......CYY.R..Y.GY
PP4_04 ..A.C.....TCGC.C....T.TC.....C....TC.T.....C...G..T.G.
S69_04 ..R.CW...YMR..TMRY.Y....YRTC.....C....YC.YY..Y.....CYY.R..W.GY
P51_04 ..R.Y.....YMRY.Y....Y.YY.....Y....YM.Y.....S...R..W.R.
P22_04 ..A.C.....TCGC.C....T.TC.....C....TC.T.....C...G..T.G.
P74_04 ..R.CW...YRR..TMRY.Y....YRTC.....C....YC.YY..Y.KM.......CYY.R..Y.GY
PP44_04 ..A.C.....TCGC.C....T.TC.....C....TC.T.....C...G..T.G.
PP22_04 ..R.Y.....YM.Y.Y....Y.YY.....Y....YM.Y.....S...R..W.R.
P20_04 ....CT...TGA..T.....ATC.....C.....C..C..C.GC.......CTT......GT
PP39_04 ....Y.....YMRY.Y....Y.Y.....C..Y..Y.KM.......CYY.R..W.GY
S68_04 ....YW...YRR..Y.....RYY.....Y.....M..Y..Y.KM.......SYY......R.
S76_04 ..R.CW...YRR..TMRY.Y....YRTC.....C....YC.YY..Y.KM.......CYY.R..W.GY
SS22_04 ....CT...TGA..T.....RTS.S...C.....C..C..C.KM.......CTT......GT
SS33_04 ..R.Y.....YMRY.Y....Y.YC.....C....YC.YY..Y..M.......SYY......RY
SS30_04 ..R.CW...YRR..TMRY.Y....BRTS.S...C....YC.YY..Y.KM.......CYY.R..W.GY
SS37_04 ....CT...TGA..T.....RTS.S...C.....C..C..C.KM.......CTT......GT
SS43_04 ..R.CW...YRR..TMRY.Y...KYRTSRS...C....YC.YY..Y.KM.......CYY.R..W.GY
P78_04
34_06
197_06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373140689239343469240140307809698403172602823654035604691390611
* * *** * ** * *
S45_04 TGGGGTTGGGGGAAGGGGGAGATGGTGAGAGAGGGGGGGGGAGTAATGGGTTGGAAGTGGAT
P47_04 .YY.....R..SY.....Y.....R.....R.....RY..SYMYR.
S59_04 .TY.....R.....RY..SYMYR.
P27_04 .....G..G.A.GT.G.....AT..GGATG.
PP13_04 .....MM.....
PP48_04 .TY..YY.....G..GT.....G.....A.....G.....AT..GGATG.
S11_04 .TT.....G..GT.....G.....A.....G.....AT..GGATG.
PP23_04 .TY..YY.....G..GT.....G.....A.....G.....AT..GGATG.
S39_04 .TT.....G..GT.....G.....A.....G.....AT..GGATG.
P30_04 .TT.....G..GT.....G.....RA.....G.....AT..GGATG.
P83_04 .TT.....G..GT.....G.....A.....G.....AT..GGATG.
PP1_04 .TY.....R..SY.....Y.....R.....R.....RY..SYMYR.
S55_04 .YY....MM...R..SY.....Y.....R.....R.....RY..SYMYR.
SS10_04 .TT.....G..GT.....G.....A.....G.....AT..GGATG.
PP4_04 .TY.....R..SY.....Y.....R.....R...R...RY..SYSYR.
S69_04 .....
P51_04 .T...YY.....R..SY.....Y.....R.....R.....RY..SYYYR.
P22_04 .Y.....R..SY.....Y.....R.....R.....RY..SYMYR.
P74_04 .T.....RY..S..YR.
PP44_04 .TY.....R..SY.....Y.....R.....R.....AT..GGATG.
PP22_04 .T.....
P20_04 .Y.....R..SY.....Y.....R.....R.....RY..SY.YR.
PP39_04 .TT.....R..SY.....Y.....R.....G.....AT..GGATG.
S68_04 .YY....MM...R..ST.....R.....R.....R...SY.YR.
S76_04 .TY.....R..SY.....R.....RY.....
SS22_04 .TY.....R..SY.....G.....R.....YG..G.TG.
SS33_04 .YY.....G..GT.....G.....A.....G.....AT..GGATG.
SS30_04 .YY.....G..GT.....G.....A.....G.....AT..GGATG.
SS37_04 .YY.....G..GT.....G.....A.....G.....AT..GGATG.
SS43_04 .YY.....G..GYR.RY.G.....R.....R..R....AT..GGATG.
P78_04 .TY..YY.....R..SY.....Y.....R.....R.....RY..SYSY..
34_06 .Y...YYMM...R..SY.....R.....RY..SY.YR.
197_06 .TT.....G..GT.....G.....A.....G.....AT..GGATG.