Addendum
Response to Examiner’s clarification

I am very thankful to the reviewer for raising points of clarification and increasing the understandability of the thesis.

Chapter 2

The first concern with the accuracy of alignments, as repeat sequences are known to yield alignments that are erroneous. Thus, when considering different repeat regions among families, the conclusions might be subjective of the actual alignments, and might differ based on a different alignments obtained by different set of penalty parameters. The second concern is about evolutionary pressure (positive) based solely on ka/ks ratio. It is well known that such evolutionary interpretations need to be drawn from different evidences, and ka/ks ratio is only one of them. The larger question would be what drives this positive selection?

In response to the first comment regarding the accuracy of the alignment of the repeats in Chapter2, I would like to clarify that in the MSA depicted in Fig. 2.3 and Fig. 2A3.1-2A3.7, the portions of the PGIP sequences that aligned with the regions recognized by Pfam as Leucine-rich Repeat (LRR) and also aligned with the consensus sequence pattern of the repeat in the other PGIPs of the family, were thus doubly confirmed as repeats for further studies in the chapter. Regarding construction of MSA, reviewer’s concern that the change of penalty parameter might result in different alignment is thoroughly merited. However, I would like to mention that a Gap opening penalty (GOP) of 10 and Gap extension penalty (GEP) of 0.2 has been used in the study. Since the sequences are too closely related to each other, selection of GOP as high as 10 and as the sequences are comparable in length, choice of GEP as low as 0.2 were justified (to obtain the best alignment) / (for the optimization of the alignment).

As pointed out by the reviewer, there are several methods other than Ka/Ks for estimating evolutionary pressure. Here, Ka/Ks have been estimated and positive selection pressure was found rare among PGIPs. The proteins were found evolutionarily conserved as synonymous codon substitutions were found to dominate in PGIPs. In these defense proteins, positive selection appears to be constrained by the need of maintaining function: most amino acid sites are subject to strong purifying selection and only a small fraction is potentially targeted by adaptive evolution, likely because of their role in the interaction with the pathogen-derived ligands.
Chapter 3

Two points of concerns are- the number of decimal accuracy in potential energy values reported in table 3.2 is unwarranted. Similarly, the accessible surface areas in table 3.4 and 3.5 are also reported to accuracies which are beyond statistical significance. Secondly, on the docking figures make it appear as if some of the docked pair might have severe short contacts. Candidate might like to clarify during the viva voce examination if indeed there are short contacts (if yes, these should be eliminated from analysis), or not. A minor concern is regarding the depth in which the secondary structures of the models are described, after all these are only homology models!

The output of GROMACS was pasted as it is in both the above mentioned tables and so the number of decimal figures shown is definitely insignificant as has been rightly pointed out by the examiner.

In response to the reviewer’s next concern from the 3rd chapter, I would like to clarify that there are few short contacts in the PGIP-PG docked structures. The structures were predicted by GRAMM through the best surface match between the molecules by correlation technique using FFT. Method based on such shape complementarity can successfully predict the structures of molecular complexes even with few short contacts when the docked molecules are large, (26-31) since the occurrence of the few unfavourable contacts can be compensated by a large number of favourable ones. There are only three, two and six short contacts compensated by the large number of favourable contacts in \(PvPGIP2-FpPG\), \(PvPGIP1-FpPG\) and \(GmPGIP3-FpPG\), respectively.

In response to the minor concern of the reviewer regarding the depth of the secondary structure of the homology models, I would like to mention that Table 3.3 only contains the data obtained from DSSP as representation of the secondary structures between the three molecules without any other interpretation.
Chapter 4

Candidate might have wished, however, to represent the angle graphs in (dihedral angles for example), such that -180° to +180° were closer, so that the fluctuations seen in Figure 4.5, 4.8 etc. did not appear as dramatic.

In response to the suggestion of the reviewer, the dihedral angle graphs in 4th chapter were redrawn between -180° to +180°. Fluctuations appeared similar to the original figures. Fig. 4.8 as redrawn between -180° to +180° is shown below as an example.

**Fig.** Chi1 angle of (A) Q224 in WT and K224 in DM (B) K225 (C) F201 in WT and DM as a function of time
References


