

# The generation of hypo-allergenic mussels

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## Prevalence & Symptoms

Prevalences of self-reported mollusc allergy ranges from about 0.15% (4/2716) in school children in France to about 0.4% (or 20% of all seafood allergic cases) in a household survey of 14,948 individuals in the US [The EFSA Journal 2006]. This allergy is persistent, severe and presents itself with eczema, urticaria, allergic asthma, gastro-intestinal problems, and anaphylaxis.

## Allergens

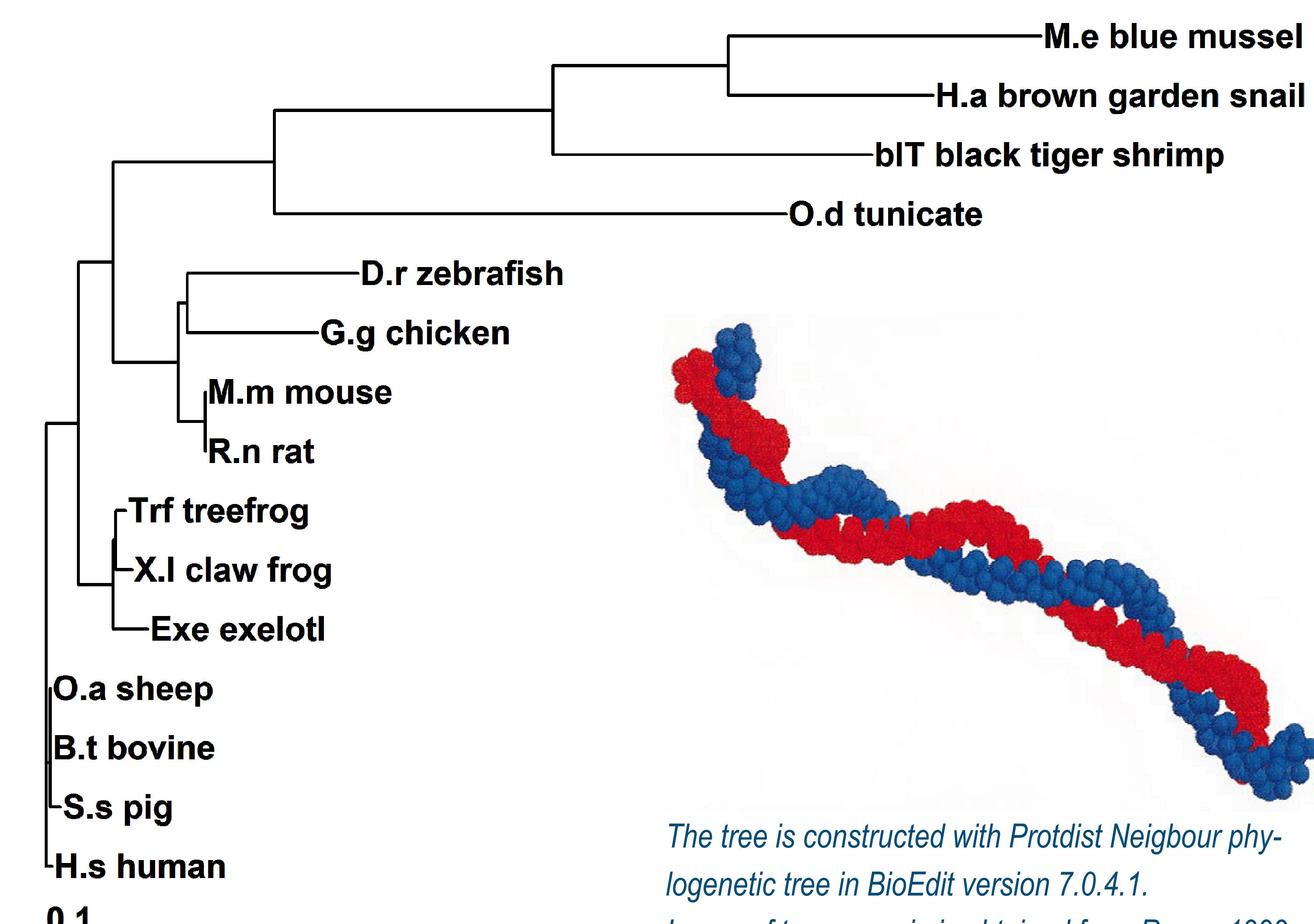
**Tropomyosin** is regarded the most important and most prevalent allergen and is the only well studied mussel allergen. Tropomyosin of several species have been cloned and sequenced and major IgE binding epitopes have been identified. Tropomyosin is highly conserved among different species and therefore is known to be a cross-reactive allergen which is present in muscle and many other cells of the mussel.

Several bivalves were shown to have at least **2 isoforms** of tropomyosin, but only one form was found in the used mussel species *M. galloprovincialis* [Fujinoki 2006] which has 100% amino acid identity to the edible blue mussel (*M. edulis*) [Taylor 2008].

Several allergens which are not tropomyosin, have been found in other mollusc species but these have not been identified in detail. These allergens were suggested to be **hemocyanin, myosin heavy chain and amylase** [Taylor 2008]. The presence of these non-tropomyosin allergens in mollusc species suggest that other allergens may be present in mussels as well. Serum from patients hypersensitive to shrimp reacted to a second band from the Asian green mussel (*P. viridis*) [Leung 1996].

## Cross-reactivity

Binding of IgE from allergic patients to shrimp-tropomyosin was inhibited by several invertebrate species like shrimp, lobster, crab, blue mussel, octopus, house dust mite and German cockroach [DeWitt 2004]. These results suggest that tropomyosin is cross-reactive.



## Objectives

The first objective is the characterization of **new allergens** and to determine the allergenic activity. Secondly, mussels with proven hypo-allergenic or even non-allergenic activity will be selected to make hypo- or non-allergenic mussels by **genetic selection** using the identified proteins and their genes as targets for the selection process.

## Methods

Mussel protein fractions will be prepared and 2D separated. Sera from allergic patients will be used to detect IgE binding places. If IgE will bind to proteins which are not tropomyosin, the **peptide sequence** will be determined and blasted against the available online gene databases to predict to type of protein. This can be done for several different organs.

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|     |  |
|-----|--|
| H.s | MDAIKKMQLKLDKENALDRAEQAEDKKAAEDRSKQLEDELVSLQKKLKGTEDELDKYSEALKDAQEKELEAKKATDAEADVASLNRRIQLVVEELD       |
| D.r | .....G.....  |
| G.g | .....E.....  |
| O.a | .....  |
| B.t | .....A.....  |
| M.m | .....  |
| R.n | .....  |
| S.s | .....R.....A.....P.....  |
| M.e | ....VAM.ME.....L.QKLRET.EAKAKI..DYN...SIQ..ND..NTQTQ.Q.V.A.Y.TT..QIAEH.QEIQ..T.K.SML..DIM              |
| H.a | ....IAM.ME.....V.QKLRC.CNKNKV.ED.NN...FAIL.NDF.SIN.Q.L..NT..AS..NAEI.SET.G.Q..L.D.E                    |
| bIT | ....AM..E.D..M..DTL.QQN.E.NN.AEKS.E.VHN..RMQQL.ND..QVQ.S.LK.NIQ.VEKD.ALSN..GE..A..L..D.E               |
| Trf | ....G..K.....A.....  |
| X.I | ....G..K.....A.....  |
| O.d | ....E..V..D..S..NA..EKAGKA.E..Q.A..Q.A..E..NSAK.R..QKV..DE..KA..A..NE..THC..KK..MTM                    |
| Exe | ....V..M..G..K.....A.....S..D..K..S..  |
|     | 10 20 30 40 50 60 70 80 90 100   |
| H.s | RAQERLATALQKLEERAEKAADESERGMKVVISRAQKDEEKMEIQEIQOLKEAKHIAEDADRKYEVARKLVIIIESLERAEERAELSSEGKCAELEELIKTV |
| D.r | .....N..L.....E.....V..GE..T.....N.S..S.....   |
| G.g | .....  |
| O.a | .....  |
| B.t | .....  |
| M.m | .....  |
| R.n | .....  |
| S.s | .....  |
| M.e | KSE..YT..AS..S.....NR..L..NLNCGND..RIDQL..K..T..W..E..K..A..A..T..V..A..L..AA..A..VID..Q..TV..         |
| H.a | ..SE..QS..TE..S.....R..L..SLA..D..RLDGL..A..Y..E..FD..A..A..T..V..A..L..AA..A..IL..V..                 |
| bIT | ..SE..N..TT..A..SQ.....MR..L..N..SLS..R..DAL..N..RFL..E..D..AMV..A..TG..S..IV..RV..                    |
| Trf | .....N..L.....L.....E.....G.....S.....   |
| X.I | ....S.....N..L.....L.....E.....G.....S.....  |
| O.d | SV..K..N..SIV..D..N.....R..A..A..RLKD..TA..SV..E..K..LV..T..V..K..A..TRAN..A..                         |
| Exe | ....N..L.....L.....Q..E.....G.....   |
|     | 110 120 130 140 150 160 170 180 190 200  |
| H.s | TNNLKSLEAQAEKYSQKEDRYEEEIKVLSDLKLEAETRAEFAERSVTKLEKSIDDLDELEYAQAKLKYKAISEELDHALNDMTSI                  |
| D.r | .....K.....T.....A..T..E..K..SHA..EENLDMNQM..BQT..LELNMM   |
| G.g | DQT..A..M..AED..K.....T.....EVAHA..EENLNHMOM..QT..LELNMM   |
| O.a | .....K.....  |
| B.t | .....K.....  |
| M.m | .....K.....EVAHA..EENLSHMOM..QT..LELNMM  |
| R.n | .....K.....EVAHA..EENLSHMOM..QT..LELNMM  |
| S.s | .....K.....  |
| M.e | GA..I..T..QV..NDQA..R..S..T..RD..TNR..D..N..TE..T..S..R..EV..R..LTE..E..D..ATFAELAGY                   |
| H.a | G..M..ISSEQEA..R..S..T..RD..TQR..D..N..SE..T..S..Q..EV..R..L..E..ER..T..D..STFAELAGY                   |
| bIT | G..VSE..AN..R..EA..K..Q..T..TN..A..A..Q..Q..EV..R..VNE..E..S..TD..QTFSELSGY                            |
| Trf | .....K.....T.....T..A.....M  |
| X.I | ....K.....T.....T..A.....  |
| O.d | A..A..TT..AQ..I..VRS..E..D..GE..DH..K..E..ST..E..K..E..I..QTV..DM..NTIHASAL..M                         |
| Exe | ....K.....T.....T..A.....  |
|     | 210 220 230 240 250 260 270 280  |

Tropomyosin sequences from different species are obtained from NCBI and EMBL and are aligned with ClustalW Multiple Alignment in BioEdit version 7.0.4.1. Legend: D.r = *D. rerio*-zebrafish\_TMyo-1alpha\_NP\_957228; G.g = *G. gallus*-chicken\_NP\_990732; O.a = *O. aries*-sheep\_NP\_001119823 ; B.t = *B. taurus*-bovine\_NP\_001013608; M.m = *M. musculus*-mouse\_NP\_077745; R.n = *R. norvegicus*-rat\_NM\_024427; S.s = *S. scrofa*-pig\_NP\_001090952; H.s = *H. sapiens*-human\_P09493; M.e = *M. edulis*-mussel\_U43005-EMBL; bl = *bITigerShrimp*\_AAX37288; Tr = *Treefrog*\_AAZ04161; X.I = *X. laevis*-clawfrog\_Q01173; O.d = *O. dioica*-tunicate\_AAS21359; Ex = *Exelotl*; H.a = *Helix aspersa* -snail\_O97192

## Sequence Identity Matrix (BioEdit version 7.0.4.1)

| Seq-> | D.r   | G.g   | O.a   | B.t   | M.m   | R.n   | S.s   | H.s   | M.e   | bl    | Tr    | X.I   | O.d   | Ex    | H.a   |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| D.r   | ID    | 0.792 | 0.82  | 0.992 | 0.992 | 0.915 | 0.915 | 0.985 | 0.51  | 0.559 | 0.936 | 0.94  | 0.598 | 0.929 | 0.556 |
| G.g   | 0.792 | ID    | 0.813 | 0.795 | 0.795 | 0.859 | 0.859 | 0.792 | 0.471 | 0.556 | 0.809 | 0.799 | 0.556 | 0.795 | 0.503 |
| O.a   | 0.82  | 0.813 | ID    | 0.82  | 0.82  | 0.901 | 0.901 | 0.813 | 0.496 | 0.549 | 0.816 | 0.82  | 0.556 | 0.82  | 0.51  |
| B.t   | 0.992 | 0.795 | 0.82  | ID    | 1     | 0.915 | 0.915 | 0.992 | 0.51  | 0.559 | 0.936 | 0.94  | 0.602 | 0.929 | 0.556 |
| M.m   | 0.992 | 0.795 | 0.82  | 1     | ID    | 0.915 | 0.915 | 0.992 | 0.51  | 0.559 | 0.936 | 0.94  | 0.602 | 0.929 | 0.556 |
| R.n   | 0.915 | 0.859 | 0.901 | 0.915 | 0.915 | ID    | 1     | 0.908 | 0.489 | 0.552 | 0.866 | 0.862 | 0.577 | 0.859 | 0.538 |
| S.s   | 0.915 | 0.859 | 0.901 | 0.915 | 0.915 | 1     | ID    | 0.908 | 0.489 | 0.552 | 0.866 | 0.862 | 0.577 | 0.859 | 0.538 |
| H.s   | 0.985 | 0.792 | 0.813 | 0.992 | 0.992 | 0.908 | 0.908 | 0.929 | 0.507 | 0.556 | 0.929 | 0.933 | 0.598 | 0.922 | 0.552 |
| M.e   | 0.51  | 0.471 | 0.496 | 0.51  | 0.51  | 0.489 | 0.489 | 0.507 | ID    | 0.57  | 0.514 | 0.517 | 0.429 | 0.51  | 0.704 |
| bl    | 0.559 | 0.556 | 0.549 | 0.559 | 0.559 | 0.552 | 0.552 | 0.556 | 0.57  | ID    | 0.566 | 0.577 | 0.471 | 0.57  | 0.637 |
| Tr    | 0.936 | 0.809 | 0.816 | 0.936 | 0.936 | 0.866 | 0.866 | 0.929 | 0.514 | 0.566 | ID    | 0.975 | 0.598 | 0.961 | 0.552 |
| X.I   | 0.94  | 0.799 | 0.82  | 0.94  | 0.94  | 0.862 | 0.862 | 0.933 | 0.517 | 0.577 | 0.975 | ID    | 0.591 | 0.964 | 0.559 |
| O.d   | 0.598 | 0.556 | 0.556 | 0.602 | 0.602 | 0.577 | 0.577 | 0.598 | 0.429 | 0.471 | 0.598 | 0.591 | ID    | 0.58  | 0.457 |
| Ex    | 0.929 | 0.795 | 0.82  | 0.929 | 0.929 | 0.859 | 0.859 | 0.922 | 0.51  | 0.57  | 0.961 | 0.964 | 0.58  | ID    | 0.549 |
| H.a   | 0.556 | 0.503 | 0.51  | 0.556 | 0.556 | 0.538 | 0.538 | 0.552 | 0.704 | 0.637 | 0.552 | 0.559 | 0.457 | ID    | 0.549 |

## Methods continued

The availability of oligonucleotide microarrays and RNA profiling techniques together with cloning and sequencing of new allergens provides us with the unique opportunity to the rational design and genetic selection of hypo-allergenic and allergen-free mussels.

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