Development and evaluation of real-time PCR targets for the detection of insect in feed

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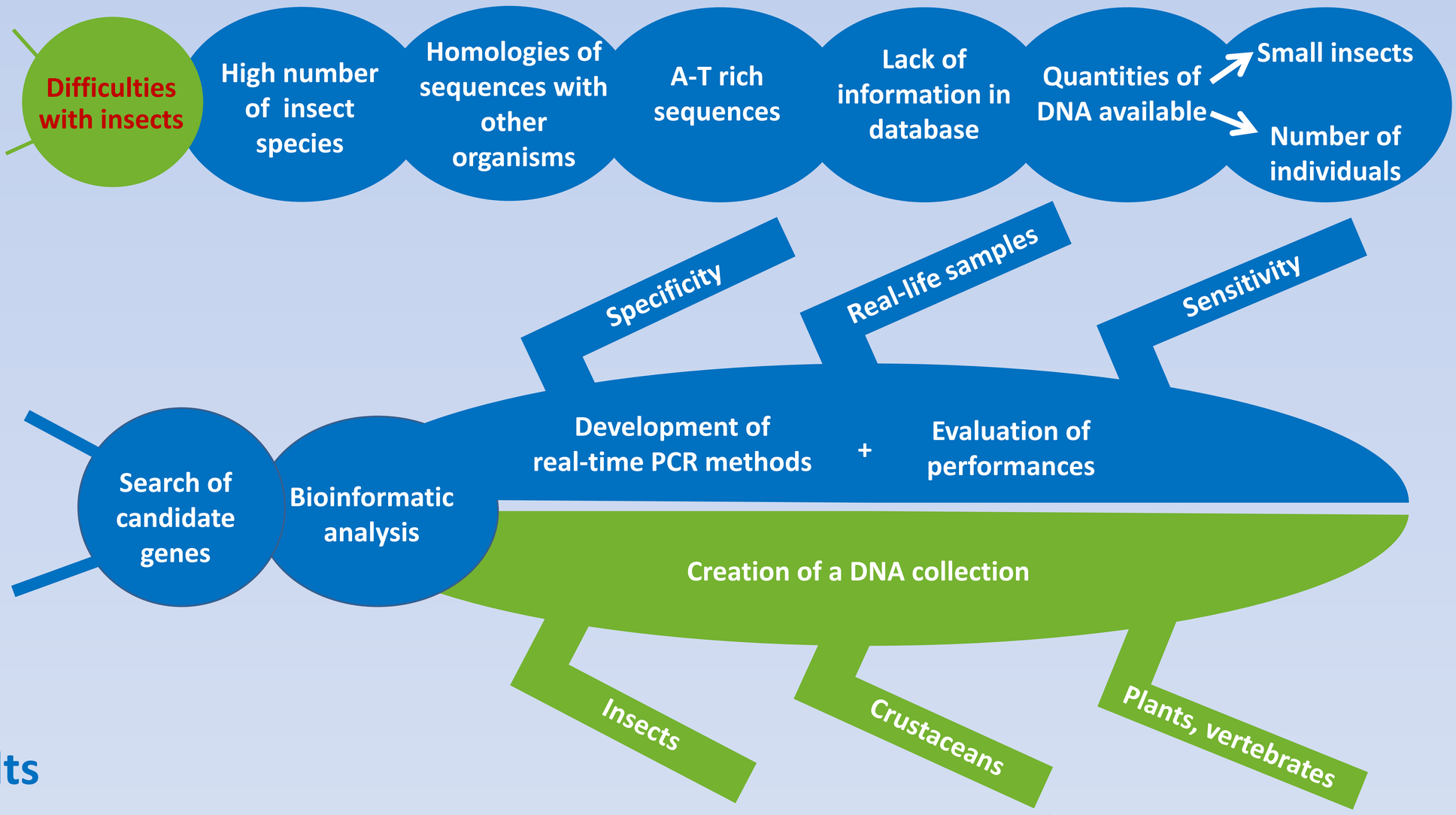
Context

Insects are rich in proteins and could be an alternative source of proteins to feed animals. Numerous companies started the production of insects at small or larger scale as feed for chicken and fish. Most of the business models for feed production are based on the black soldier fly (*Hermetia illucens*) or the mealworm (*Tenebrio molitor*). In Europe, these processed animal proteins are not yet authorized and products are commercialized outside Europe or eventually used as pet food. For further authorization in Europe, many questions must be clarified concerning the presence of antinutritional compounds, the risk associated to pathogens, to residues (pesticides, antibiotics, heavy metals) and to allergens.

To authorize such products on the market, methods to detect if a product really contains insects and to authenticate insect products are also mandatory.

Development of methods

Targets focused on insects (target common to all insects) and targets specific to particular insect species are required. Real-time PCR methods are developed at CRA-W in this way. At this stage, methods were only considered for qualitative purposes.



Results

It was not possible to develop a single **target common to all insects**. A target (based on a multicopy region) was able to amplify DNA of the insects except insects from the Diptera order. A second target more specific to the Diptera order was then designed to propose a **duplex real-time PCR** for the detection of insect species. This duplex was able to detect the DNA of the 37 insect species tested. No positive signals were observed with the 9 vertebrate species tested. Unfortunately, late signals were obtained with wheat and tomato.

Two targets were proposed for the detection of ***Tenebrio molitor***. The first one was based on the *wingless* gene and did not show aspecificities with the non target DNA tested. The second one was based on the *cadherin* gene and late signals were observed with 3 insect species (against 37 non target insect species, 9 vertebrates, 6 crustaceans and 7 plants). Positive signals were also obtained on industrial flours of *Tenebrio molitor*. The limit of detection of these two targets was estimated under 20 copies through the AFNOR XP V03-020-2 standard approach, reaching the recommended performance criteria.

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