Fit2Breed user guide

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Contents

Introduction

Fit2breed was developed in collaboration with Vereniging Het Nederlandse Kooikerhondje (VHNK) to support breeders in breeding healthy pups and to facilitate a sustainable population of Kooikerhondjes for now and in the future. Maintaining genetic diversity in the population and decreasing the incidence of hereditary diseases are important to keep the Kooikerhondjes as healthy as possible. Fit2Breed uses pedigree information, genetic information (SNP-genotype data), results of DNA tests, screening results and health data. The advice and warnings in Fit2Breed are based on evidence based health information and scientific studies and is not based on breeding rules from a specific country. This document is meant to guide the user through the interface of Fit2Breed and help with the interpretation of its results. This document also explains the diseases for which clinical screening and DNA test results are available in the Fit2Breed module for the Kooikerhondje. This document will give a brief explanation of what each disease's signs and symptoms are and how the breeding rules are included in the Fit2Breed algorithm.

This document is organized as follows:

- Section 1 summarizes the instructions for using Fit2Breed
- Section 2.1 explains the three diseases for which DNA testing is validated for the Kooikerhondje
- Section 2.2 focuses on diseases with mandatory clinical screening for breeding
- Section 2.3 shows other diseases registered in the Kooikerhondje
- Further information about the diseases and breeding strategies can be found in Section 3
- A Glossary of terms is available at the end of the document, in Section 4.

If any further questions arise or if you have feedback where we can improve the program, you can contact us at: ecgg@uu.nl. Together we work towards a healthy and sustainable future for the Kooikerhondje!

1 Fit2Breed usage instructions

1.1 The login page

To have access to Fit2Breed, you need to log-in with your personal username and password. Access is only granted to members of breeding clubs from the Kooikerhondje. If your club is collaborating with Fit2Breed, but you haven't received your personal log-in information, please contact us at: ecgg@uu.nl

Figure 1 shows a screenshot of the login page as you enter Fit2Breed. Figure 2 shows the initial interface of Fit2Breed once logged in.

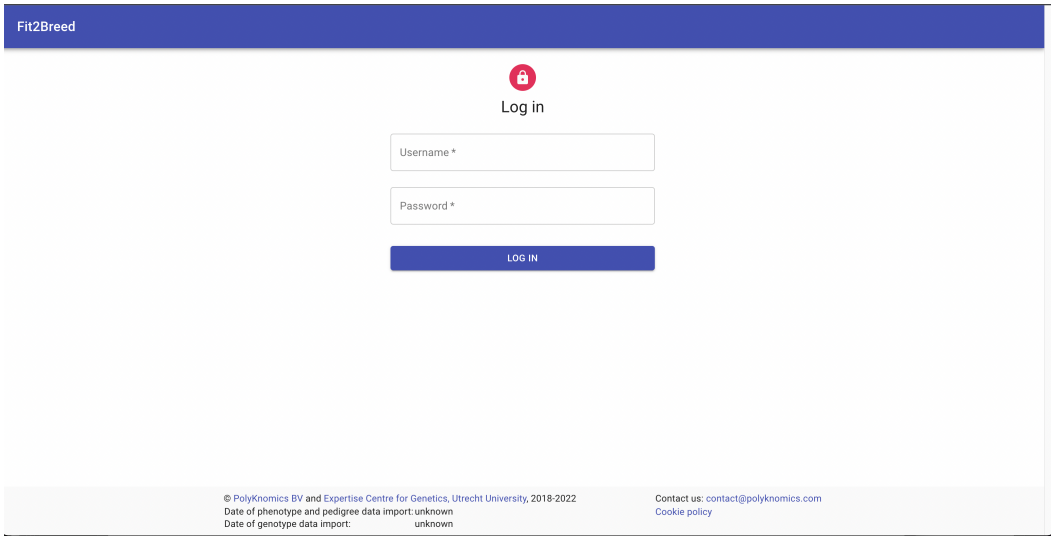

Figure 1: The log-in page for Fit2Breed.

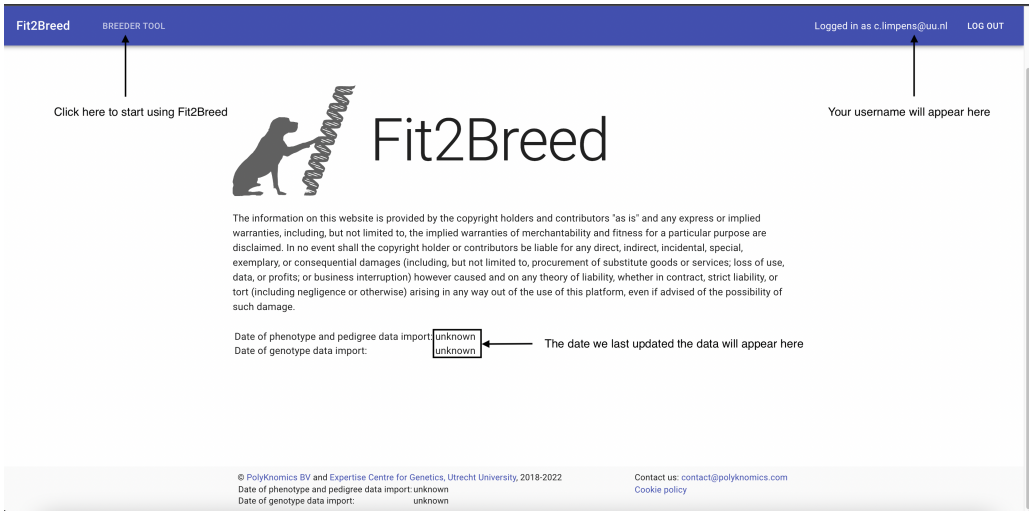

Figure 2: The Fit2Breed start page, once logged in.

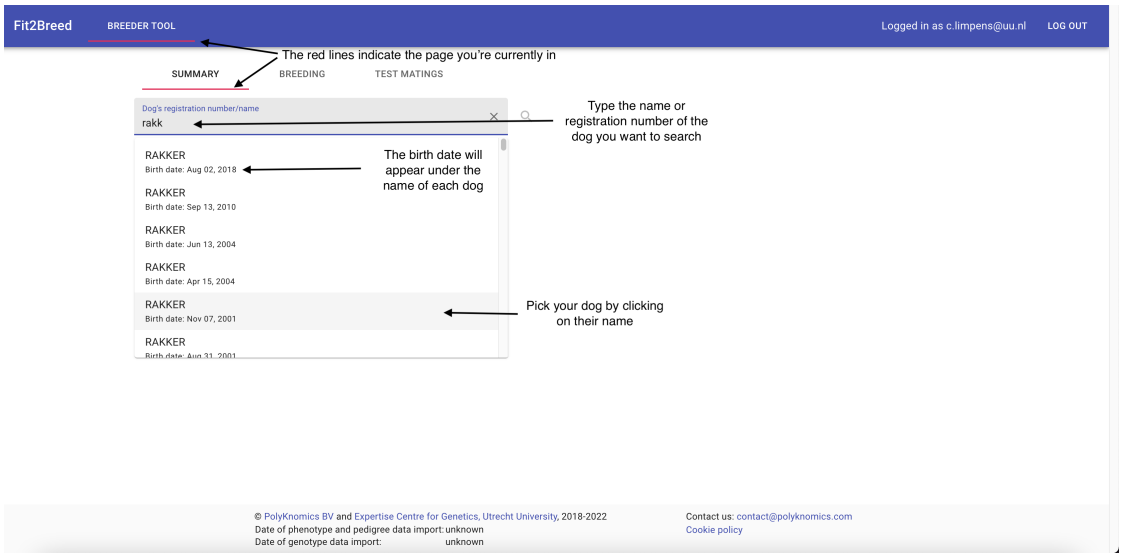

Figure 3: An example of the use of the search bar. You can look for your dog using either registration number or name. If your dog shares a name with other animals, you can identify them by checking their date of birth.

1.2 Searching for a Dog

Once logged in, you can click on the breeder tool tab. You can search for your dog using the search bar. You can use either your dog's name or pedigree number.

You will see a list of dogs under the search bar, pick the dog that matches the name and birth date of your dog. Figure 3 show an example of a dog with a common name.

1.3 The Summary Tab

The Summary Tab displays all information regarding your dog. Figure 4 shows an overview of the Summary Tab. The Summary Tab contains the following segments:

- The dog's general information
- DNA test results for monogenetic diseases
- DNA test results for complex diseases
- Clinical screening results
- Disease status
- Pedigree chart

- Genetic coefficient of inbreeding plot
- List of offspring

Each of the sections will be explained throughout this document.

1.3.1 General Information

The General Information Section can be found on the top left box of the Summary Tab. Here you will find all pedigree and identifying information of your dog. The general information section will display your dog's:

- Name
- Registration number
- Sex
- Birth date (and current age)
- Number of offspring
- The Mother's registration number
- The Mother's name
- The Father's registration number
- The Father's name
- Dog's breed (if relevant for your version of Fit2Breed)
- Owner's email address
- Date of death (if relevant)

If you wish to look into the parents of your dog, you can click on the Father and Mother's registration numbers to access their Summary Tab. An example of this section of the Summary Tab is displayed in Figure 5.

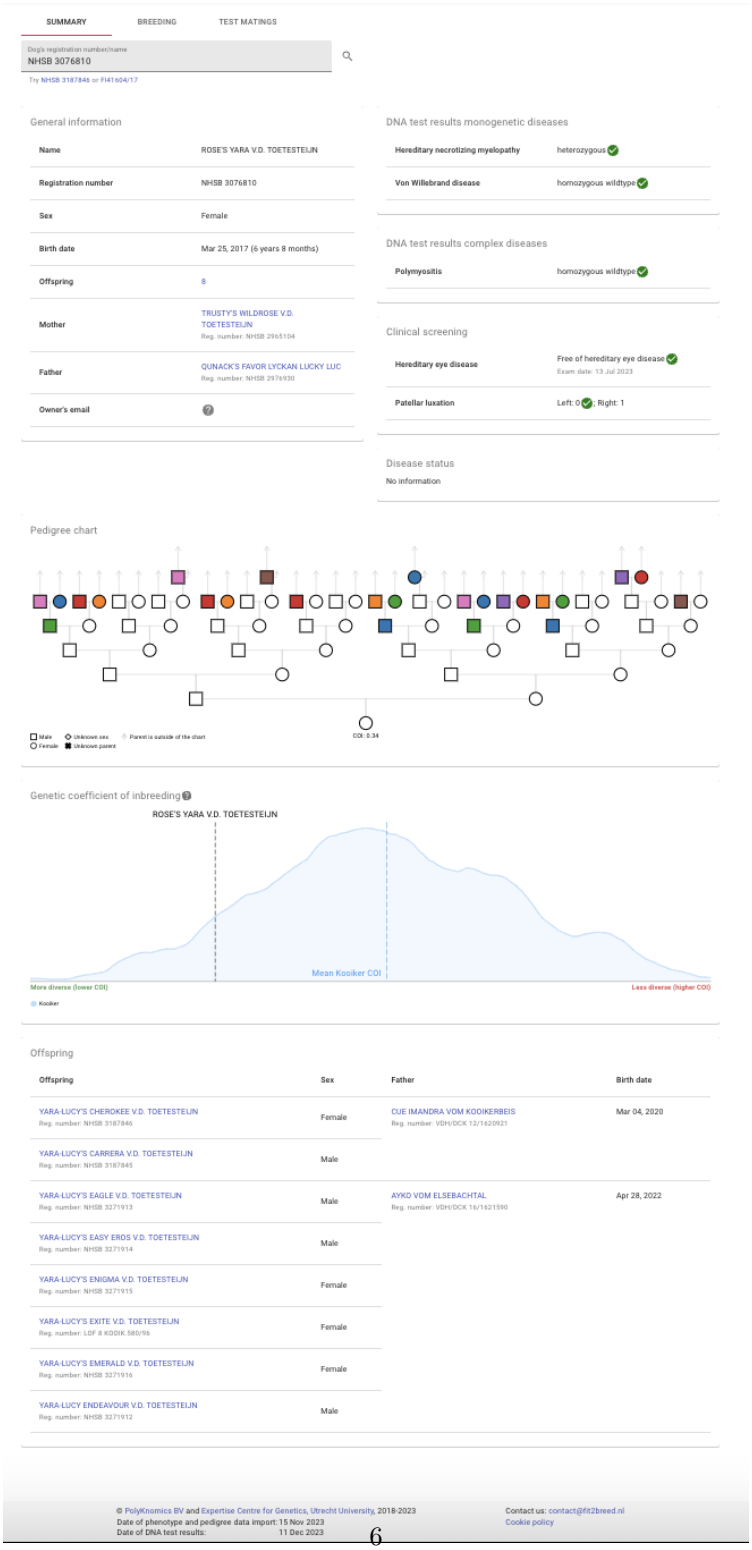

Figure 4: Overview of all the information available in the Summary Tab.

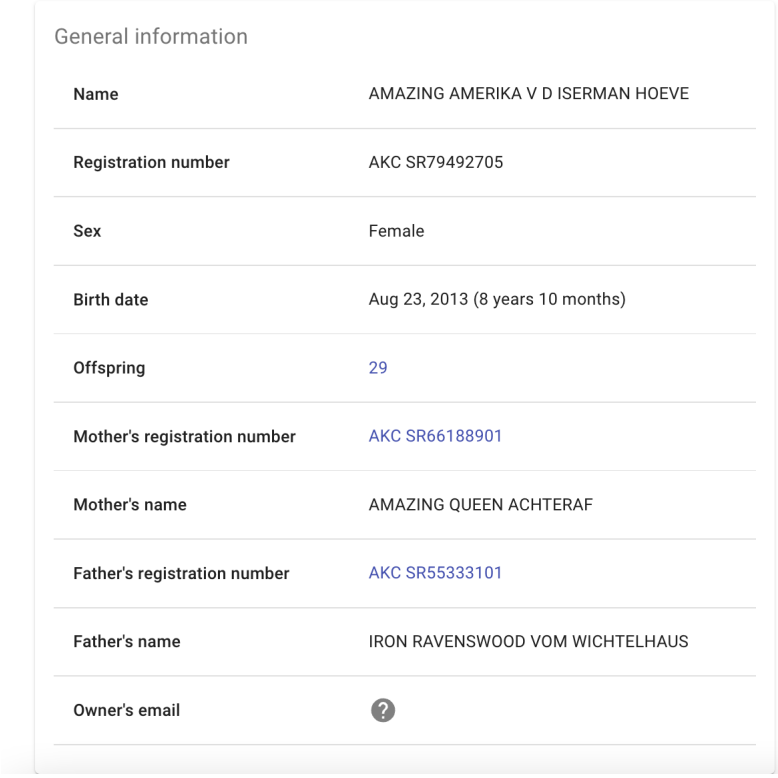

Figure 5: The general information section of a dog in Fit2Breed.

1.3.2 DNA Test Results

The DNA tests relevant for the breed will appear in two sections: test results for monogenetic diseases, and for complex diseases. If there are DNA test results for your dog, you will see their results and interpretation of the results here. For the Kooikerhondje breed, Fit2Breed shows monogenetic test results for Hereditary Necrotizing Myelopathy (ENM), and for von Willebrand disease (vWD); and complex disease test result for Polymyositis (PM). By hovering with your mouse over the disease names you can read a short description on them. There are four possible results for each of the DNA tests:

- Homozygous mutant – homozygous mutant animals carry two copies of the disease gene, and will be affected with the disease if it is monogenetic. It is not advisable to breed with them. The animals will show a "homozygous mutant" test result, and a red exclamation point.
- Homozygous wildtype – homozygous wildtype animals carry two copies of the "normal" gene, and will have a low risk to be affected with the disease. They will show a "homozygous wildtype" test result, and a green checkmark, as shown in Figure 6.
- Heterozygous – heterozygous animals carry one copy of the disease gene, and one normal copy. They will show a "heterozygous" test result as shown in Figure 7.
- Unknown – animals that have not been genotyped yet will have an unknown test result. It is recommended to test the animals before breeding. They will show a gray question mark.

Breeding rules will vary per disease and per genotype. More information regarding breeding rules and each disease is available in section 2.1.

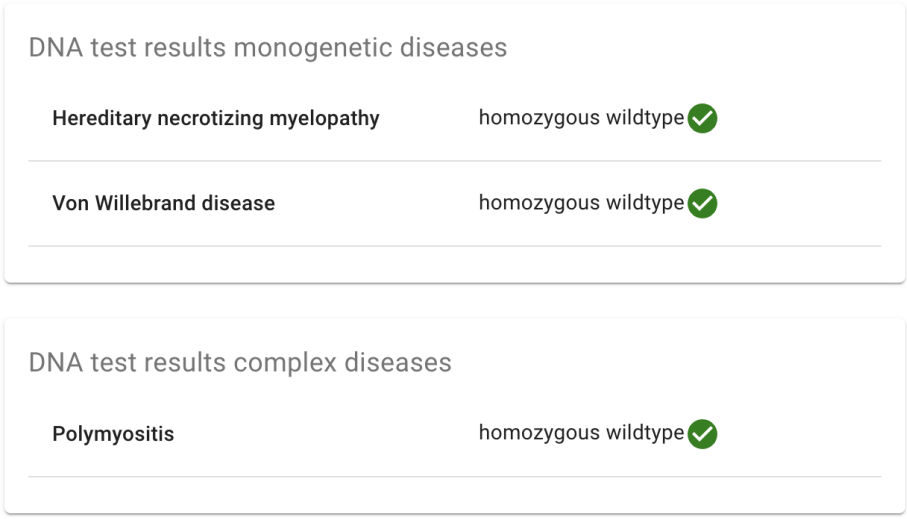

Figure 6: DNA test results for a homozygous wildtype dog.

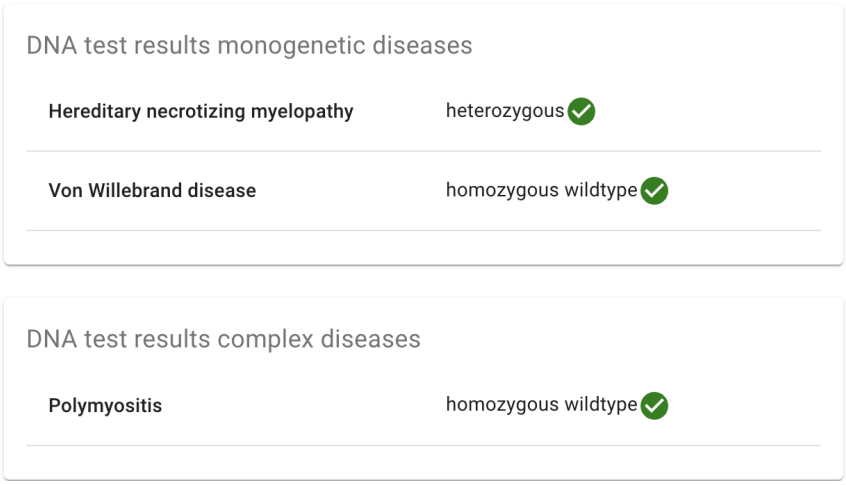

Figure 7: An example of the DNA test result section for a dog that is heterozygous for hereditary necrotizing myelopathy (ENM) and homozygous wildtype for von Willebrand disease (VWD). This animal can be bred with a homozygous wildtype animal for ENM and VWD.

1.3.3 Clinical Screening

Some inheritable diseases cannot be tested with a DNA test. This may be because the diseases are complex in nature, or because the tests haven't been developed yet. For these diseases, clinical screenings may be performed, in which an animal is examined by a specialized veterinarian before breeding. All mandatory clinical screening conditions for a breed will be available in Fit2Breed.

Breeding rules will vary according to the condition. For the Kooikerhondje, Fit2Breed analyzes the results of two conditions:

- Patellar Luxation (PL) – Patellar luxation screening scores will appear with a number (0, 0.5, 1, 2, 3, 4) depending on the grade of the luxation. An example of a dog with grade 0 can be found in Figure 8.
- Hereditary Eye Disease (HED) – screening scores will appear as "Free of hereditary eye disease" or "not free of hereditary eye disease". The date of the last screening test will appear under the screening result. If a dog is diagnosed with a specific eye disease, the diagnosis will appear in the "Disease status" section.

To read more information on these diseases and the screening procedure, please refer to Section 2.2.

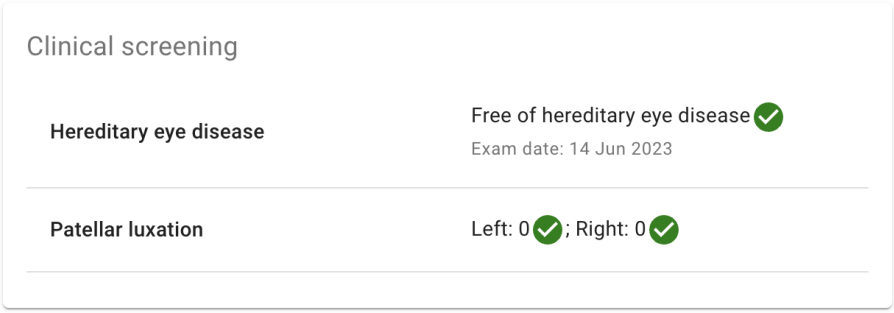

Figure 8: Example of the Clinical screening section on Fit2Breed. This animal is free of hereditary eye disease and patellar luxation.

1.3.4 Disease Status

If an animal was diagnosed with a disease, these will appear in the Disease Status box. The information about the disease status is given to Fit2Breed by the VHNK. Examples of diagnoses that can be present are: epilepsy, kidney problems, behavioral problems, paralysis, skeletal deformities etc. Figure 9 shows an example of the Disease status section in Fit2Breed.

All sections in the Summary Tab should be considered when making a breeding decision. Figure 10 shows how it would look like for a dog fit for breeding.

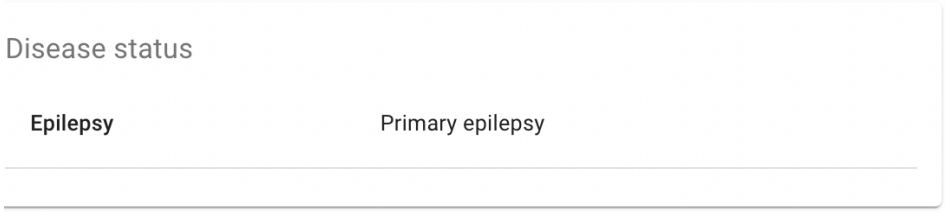

Figure 9: Disease status section in Fit2Breed. Dogs with a registered diagnosis will have it stated in this section. By hovering over the bold title of the disease, you can read a short description of it.

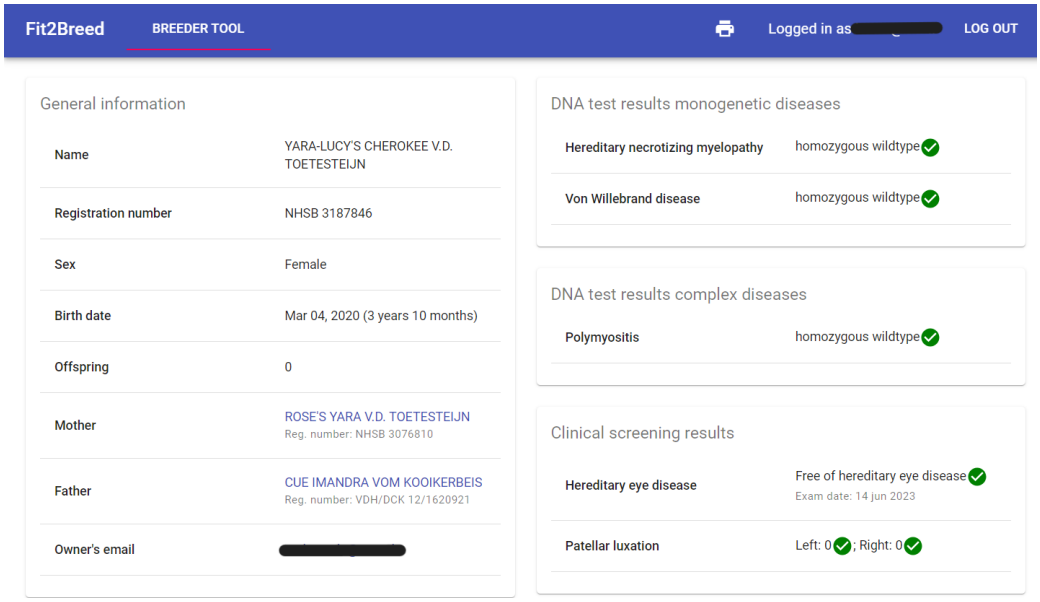

Figure 10: This figure shows an example of all disease related sections for a dog.

1.3.5 Pedigree Chart

The Pedigree Chart will show your dog's pedigree in a schematic way. By default, the chart will contain 5 full generations. If a dog from these 5 generations appears further back in the pedigree, the chart will become bigger and includes more generations. If a dog only has a couple of known generations registered, then the pedigree will appear truncated with the last known parent at the top of the pedigree.

You can know the sex of each dog by looking at the shape representing it in the plot. Males will be represented by squares, and females by circles. If a dog's sex is unknown, they will be represented by diamonds.

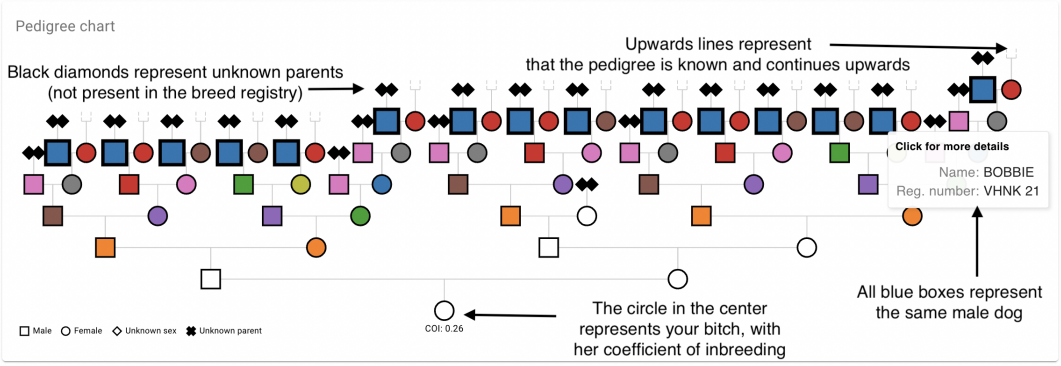

Figure 11: Pedigree plot. This plot shows the pedigree of a bitch. Since some animals get used several times through the pedigree, they appear in the same color. The pedigree also extends past the five generations because of these repeated animals. Black diamonds represent animals that were not present in the pedigree registry.

Since dogs can potentially share ancestors, the pedigree charts on Fit2Breed use colors to find these shared ancestors. If a dog appears multiple times in the pedigree, it will appear with the same color. To see more information on a dog from this pedigree, you can click on it and access its Summary Tab.

At the bottom of the pedigree, you will see your dog and its calculated coefficient of inbreeding (COI). Fit2Breed uses the complete dog pedigree to calculate the value that is depicted here. Figure 11 explains each part of the pedigree plot.

1.3.6 Genetic coefficient of inbreeding Plot

The genetic coefficient of inbreeding plot shows how your dog's coefficient of inbreeding is compared to the breed population. Runs of homozygosity using the SNP data of your dog are used to calculate the genetic coefficient of inbreeding. A dog with a higher coefficient of inbreeding will be less genetically diverse. Each dog will inherit one copy of its DNA from its mother, and one from its father. The coefficient of inbreeding is the percentage of the DNA that has the same copies inherited from the mother and the father. Figure 13 shows a schematic representation of the inheritance of a homozygous chromosome in a dog. Figure 12 shows the genetic coefficient of inbreeding plot as it appears in the Fit2Breed Summary Tab. Not all Kooikerhondjes have genotype information available currently. The genetic coefficient of inbreeding and relation of the dog to the mean genetic coefficient of inbreeding may differ from the coefficient of inbreeding based on pedigree information. Besides, pedigree information may not be complete or accurate.

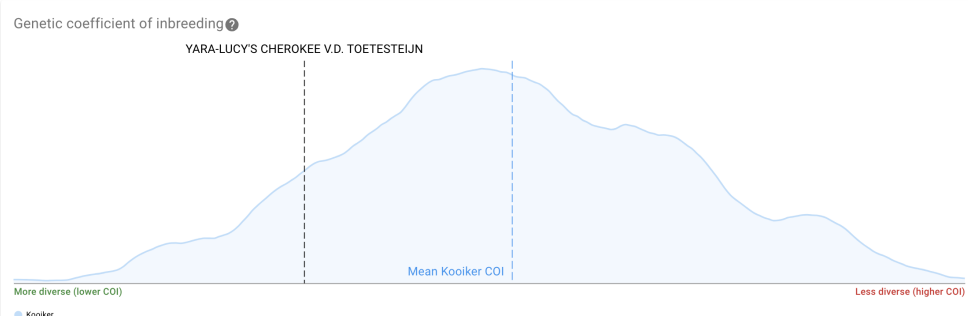

Figure 12: Genetic coefficient of inbreeding plot. This plot shows a dashed line representing where your dog fits with respect to the kooikerhondje population for which genotype information is available (blue graph).

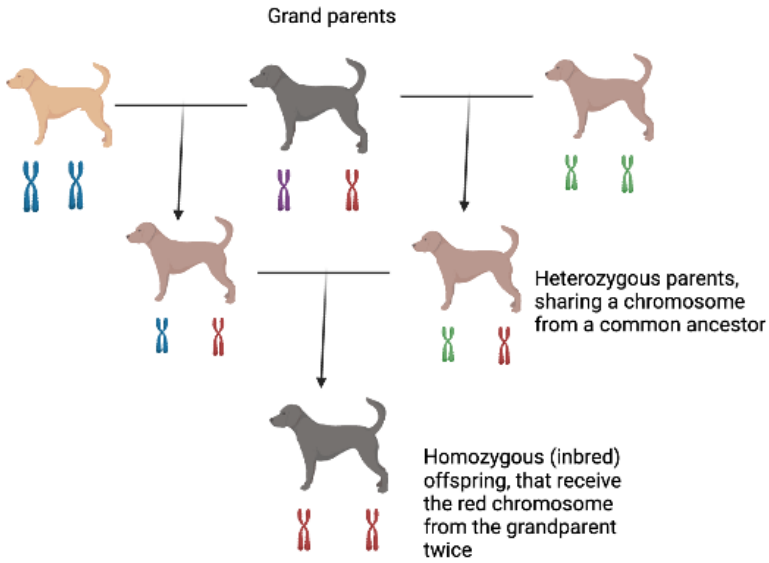

Figure 13: Inheritance of a chromosome in the offspring.

1.3.7 Offspring

The last section in the Summary Tab is the offspring list. This list includes the registration number, sex, and birth date of the offspring of the dog in the Summary Tab. If you click on a registration number, you will be directed to that dog's Summary Tab. Figure 14 shows the offspring list for an example dog. In the right column you will find the partner of the parent that produced the offspring. In this way, each litter is indicated separately.

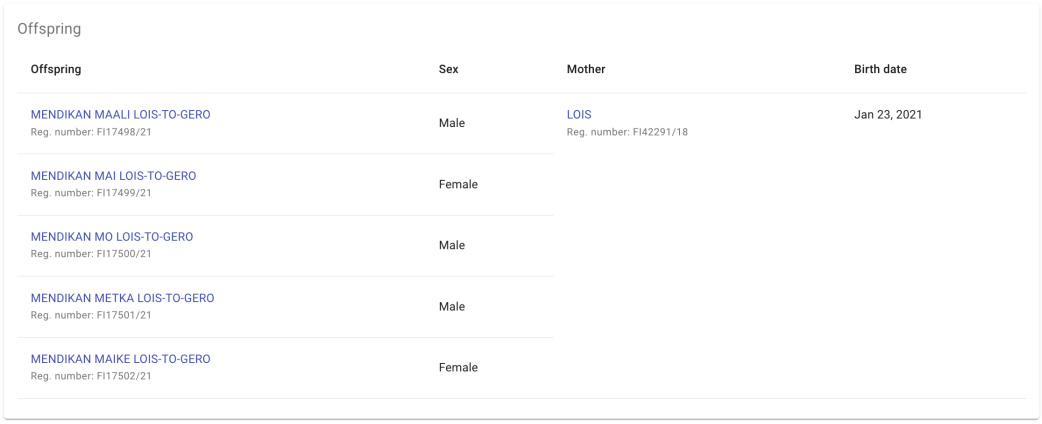

Figure 14: List of offspring for a dog.

1.4 The Breeding Tab

The Breeding Tab is where a breeder can find a suitable match for their bitch. The Breeding Tab starts with a search bar, where you can type the name or registration number of your bitch to start looking for matches. If your bitch is not suitable for breeding (for example, if she has already had too many litters in her life), a warning will appear, and you will not be able to see possible matches for her. Figure 15 shows the search bar in the Breeding Tab.

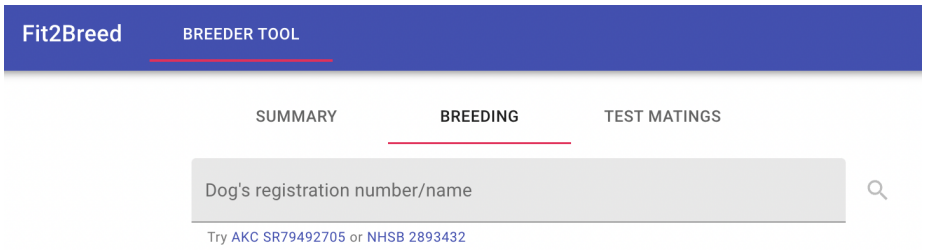

Figure 15: The search bar from the breeding tool. You can look for your bitch using this search bar. This search will only work for females, male dogs will get an error.

If your bitch is suitable for breeding, there are two options to find her match: Pedigree and Genetic Matchmaking. If your bitch is genotyped you will see both Pedigree and Genetic matchmaking as options in the software. These will be explained in the following sections.

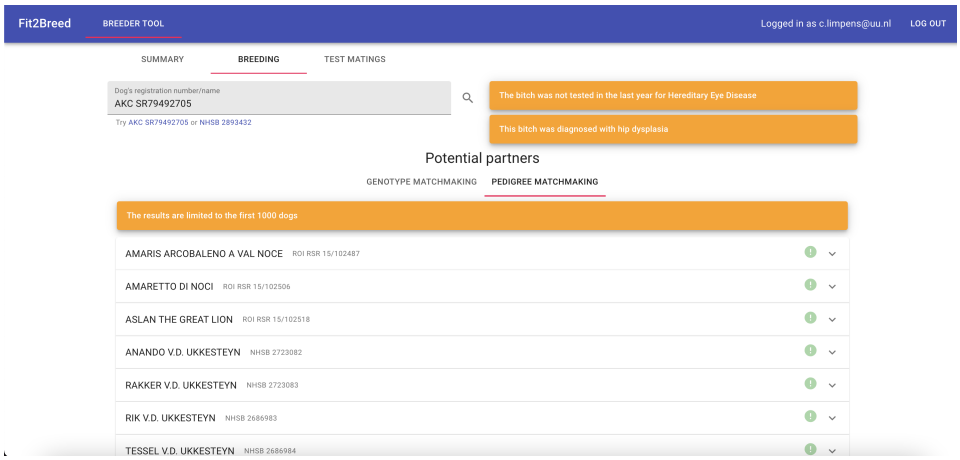

Figure 16: An example of the Pedigree Matchmaking list for a bitch.

1.4.1 The Pedigree Matchmaking Tab

By clicking on the "Pedigree Matchmaking" tab, you will see the list of males that are possibly suitable for breeding with your bitch. Dogs with a dark green check mark have been screened for all relevant genetic and clinical diseases. Dogs with a light green exclamation mark would still make a good match with your bitch with regard to kinship, however they would still need to get a clinical or genetic test done before breeding. These dogs are particularly interesting for the population, since making use of more male breeding dogs and thereby expanding the breeding pool is important for a healthy maintenance of the Kooikerhondjes population.

Dogs in the potential partner list are organized by their kinship with the bitch. Pedigree kinship indicates how related two dogs are from each other based on the available pedigree information. A high kinship value means that the dogs are closely related, a low kinship value means that the dogs are distantly related or unrelated. Dogs at the top of the list have a lower kinship value with the bitch, and they're organized in ascending order. It is recommended to pick dogs that are on top of the list, because they are the most distantly related to bitch as possible.

Figure 16 shows the way the dog list appears in Fit2Breed. If a dog seems particularly interesting, you can click on its name to see the DNA test and clinical screening information for it. You can also click on the "Test mating" button to see more about the predicted offspring. Figure 17 shows the tab that opens once you click on an interesting dog.

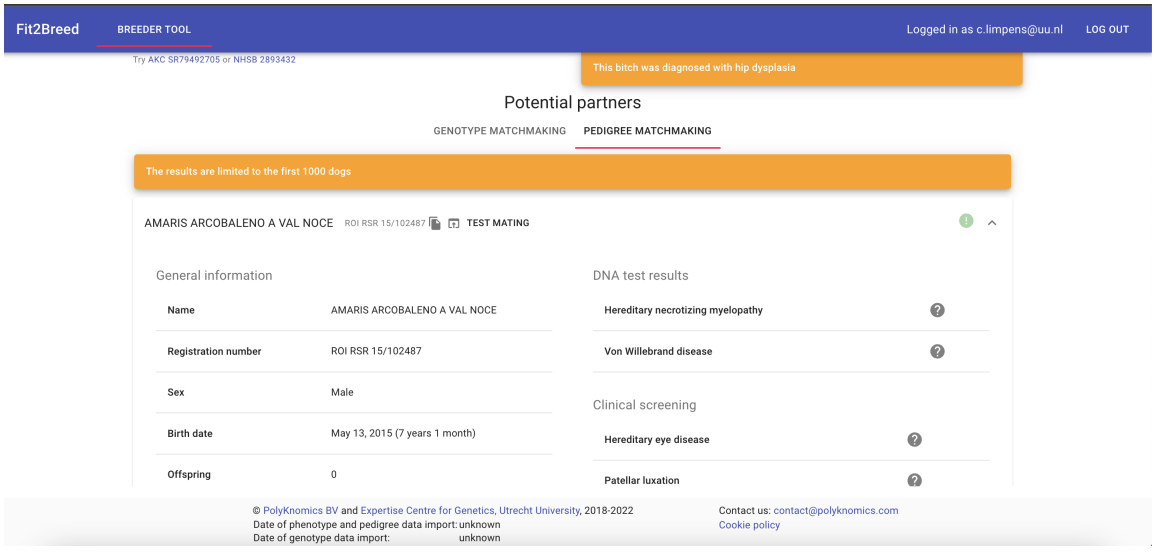

Figure 17: When clicking on a dog, you can see all relevant breeding information from it. To see more about the predicted offspring values, you can click on the "Test Mating" button.

1.4.2 The Genotype Matchmaking Tab

If your bitch has been genotyped, she will be available for genotype matchmaking. Genotype matchmaking works the same way as pedigree matchmaking, however, the kinship value used to rank the male dogs is calculated by comparing the genetic profile of the male dog with the genetic profile of the bitch.

The Fit2Breed interface for the Genotype Matchmaking Tab looks the same as the Pedigree Matchmaking tab, as shown in Figure 16.

It is important to note that a given male dog will never appear in both lists. If a dog is genotyped, it will exclusively appear in the genotype matchmaking list, and not in the pedigree matchmaking one. This does not necessarily mean that male dogs appearing in the genotype matchmaking list are less related to the bitch than the male dogs in the pedigree matchmaking list. However, the kinship calculated based on genotype is more reliable than the one calculated based on pedigree information.

1.5 The Test Mating Tab

You can go to the Test Mating Tab by clicking on the "Test Mating" button on a male dog in the breeding list, or by clicking on the "Test Mating" tab. This will take you to the test mating interface, where both dogs can be combined for a virtual matchmaking. If you click on a dog in the breeding list, both the female and male dog names will be automatically set to your bitch, and the dog you clicked on. If you want to test another dog you can do so by using the search bar. If two male dogs or two female dogs are set, test mating will give out an error. Figure 18 shows the two dog search bars and basic information as displayed in the Test Mating Tab.

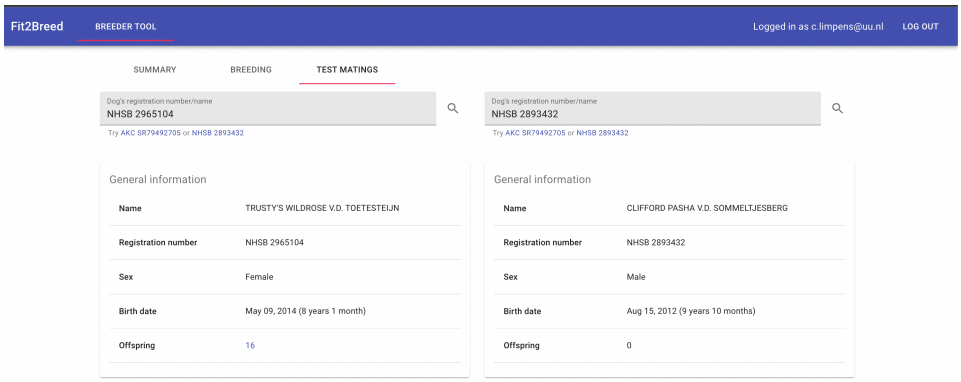

Figure 18: Test Mating Tab search bars and parents basic information.

Under the parent dog information, the section "Predictions for potential offspring" shows the disease result predictions for the offspring of the tested dogs. If there are any warnings for the mating of the dogs, they will appear on

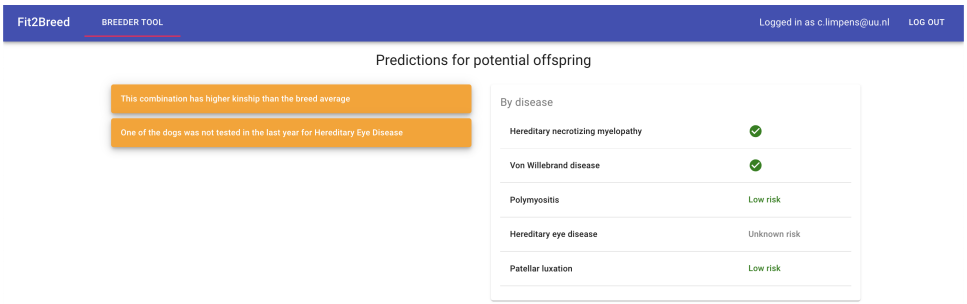

Figure 19: The disease predictions for potential offspring shows for each of the mandatory screening diseases the predicted result for the offspring.

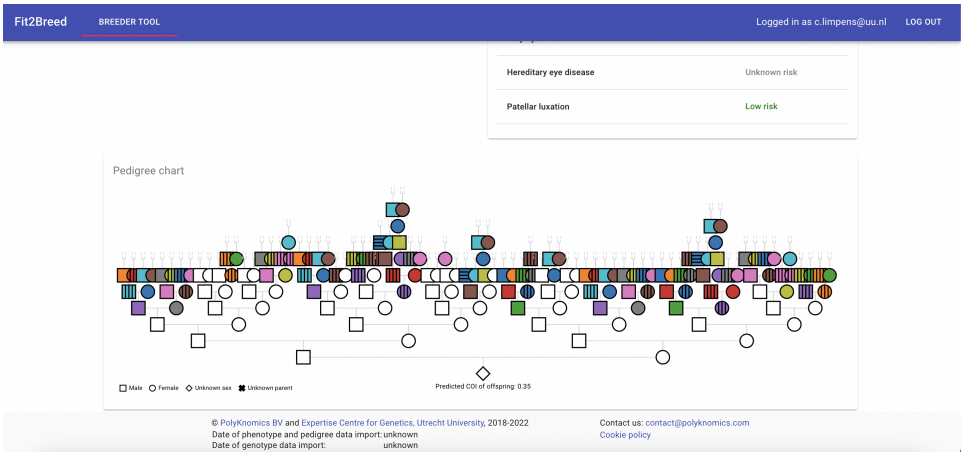

Figure 20: Pedigree chart for the predicted offspring. This pedigree shows the 5 generations pedigree for both parents.

the left side of the page. The predictions for each disease will appear on the right side of the page. Predictions based on DNA test results will appear with a green checkmark, a yellow exclamation point, or a red exclamation point, depending on the risk for the offspring. Some combinations are not recommended even when there is a low risk for disease in the offspring. This is to prevent the disease genes from spreading to the next generation. For clinical screening diseases, the risk of the offspring will be calculated as “low”, “high” or “unknown”. It is recommended to test one or both parents before breeding when the risk is “unknown”. Figure 19 shows this section of the test mating tabs.

At the bottom of the page, there is a pedigree chart similar to the one in the Summary Tab. The pedigree chart works the same way as the one in the Summary Tab. Figure 20 shows an example of this chart.

2 Disease description and inheritance

2.1 Diseases for which a DNA test is available

This section will explain the three diseases for which a validated DNA test is available for the Kooikerhondje. These diseases are divided into monogenetic diseases and complex diseases. Two monogenetic tests are currently available for the Kooikerhondje: Hereditary Necrotizing Myelopathy (ENM) and von Willebrand Disease (vWD). A complex disease is currently being investigated in the breed in the Expertise Center of Genetics. This disease is Polymyositis, and multiple genetic factors are involved in its inheritance.

2.1.1 Monogenetic diseases

Monogenetic diseases are caused by variations (called mutations) in a single gene. This means that if we know the genotype a dog has in that particular gene, we will know whether or not it will develop the disease.

von Willebrand disease

Von Willebrand disease occurs when there is an insufficiency of the von Willebrand blood clotting factor. This disease has an additive genetic effect. This means that every extra copy of the mutation increases the severity of the disease. Heterozygous dogs have one copy of the disease allele, and may present with a mild form of the disease. Homozygous mutant dogs have two copies and will have a more severe form of the disease. The mutation is located on an autosomal chromosome, that's why the frequency of disease in males and females is roughly the same. Dogs with this disease have difficulty with blood clotting and can present with excessive bleeding, which could become life threatening. This disease can be found in a dog by immunological testing of von Willebrand factor in blood, as well as with a DNA test. For the Kooikerhondje measuring von Willebrand factor and DNA testing was used effectively in the breeding policy of Kooikerhondjes bred with a pedigree. As a result, the mutation that causes this disease is now almost non-existent in the pedigree population of Kooikerhondjes. Within Fit2breed it is only permitted to breed with animals that are free of the von Willebrand disease mutation.

Hereditary Necrotizing Myelopathy

Hereditary necrotizing myelopathy (ENM) is a degenerative disease affecting the spinal cord. The first signs appear between the ages of 3 to 12 months. A dog with ENM will present with paralysis on his back limbs, an abnormal gait, unsteady placing of their feet, excessively worn nails and toes and loss of coordination. This disease progresses into an inability to walk. This progress can last a few weeks. There is no available treatment for the disease, affected dogs are euthanized at a young age. This disease has an autosomal recessive

mode of inheritance. This means that the disease is not linked with the sex of the animal, and animals could be carriers without presenting the disease. For this reason, seemingly healthy parents could have offspring with the disease.

Since the availability of a DNA test, dogs have been selected for it, which caused the allelic frequency of the disease to decrease. Keeping up with its selection will help with keeping this number as low as possible. In Fit2breed, combinations of free animals with each other or with a carrier are permitted and result in healthy offspring.

2.1.2 Complex genetic diseases

For complex genetic diseases there are multiple mutations as well as environmental factors that play a part in the development of the disease.

Polymyositis

Dogs with polymyositis show an abnormal movement, general weakness and muscle stiffness. This can be seen in the dog's gait and general locomotion. It can also present signs related to swallowing problems. General clinical signs include lethargy, weight loss, breathing difficulty and exercise intolerance. To diagnose the disease, apart from the clinical symptoms, a blood analysis is used, where elevated CPK can indicate the presence of the disease. CPK stands for Creatine phosphokinase, and it is an enzyme found in the skeletal muscle. A definitive diagnosis is made base on histological examination of a muscle biopsy.

Based only on the animals with an available DNA test, the allele is estimated to be present in 22% of dogs in the population. However, since this disease is genetically complex, the presence of the allele does not necessarily indicate the dog will always develop the disease. A dog with two Polymyositis mutant alleles (a homozygous mutant dog) has a probability of 10% of developing the disease. A heterozygous dog (presenting one polymyositis allele and one normal allele) has a probability of less than 1% of developing the disease. During the research at the Expertise Centre, we also found polymyositis in 2 dogs without the mutation. Further research is currently underway to investigate other mutations that may contribute to the risk of developing polymyositis in the Kooikerhondje. Fit2Breed's test-mating module indicates whether a certain combination of breeding animals has a high or low risk for puppies of developing Polymyositis.

2.2 Clinical screening

2.2.1 Hereditary eye disease

Hereditary eye diseases in the Kooikerhondje are a diverse group of diseases affecting one or both of the dog's eyes. A dog can have more than one hereditary eye disease simultaneously.

The eye diseases dogs get tested for include: Persistent Hyperplastic Tunica Vasculosis Lentis/Persistent Hyperplastic Primary Vitreum (PHTVL/PHPV),

Cornea dystrophia, Membrana Pupillaris persistens (MPP), Distichiasis, Ectopic cilia, Congenital cataract, Non-congenital cataract, Ligamentum pectinatum abnormality, Cherry eye, Entropion/trichiasis, Retina dysplasia, Microphtalmia, Ectropion, Macroblepharon, Progressive retinal atrophy (PRA), Hypoplasia/micropapilla.

It is recommended that dogs are screened for hereditary eye disorders prior to breeding and every 12-24 months thereafter by an eye examination performed by an ECVO specialist.

2.2.2 Patellar luxation

A patellar luxation (PL) occurs when the knee cap slides out of the groove

When a dog gets screened for patellar luxation, it gets assigned a grade. A dog with grade 0 is free of Patellar luxation. A dog with grade 1 has a knee cap that can be manually luxated, but is otherwise inside the knee's groove. Grade 2 dogs have a knee cap that can spontaneously luxate, but can return to the groove on its own. Grade 3 knees are permanently luxated, but can be returned manually into the groove, and grade 4 dogs have a permanently luxated patella that cannot be manually returned to the groove. A grade 0,5 (free but loose) is assigned in the Netherlands when the patella can be moved slightly.

Patella luxation is relatively common in pure bred dogs, as it is encountered in 1.3% of the animals the general dog population. In the case of the Kooikerhondje, around 16% of the tested population has patellar luxation grade 1 or higher.

There is no DNA test available for patellar luxation, and it is considered a complex disease. It is highly inheritable, with a heritability of 30%.

In the case of patellar luxation, free dogs (dogs with PL grade 0) can freely be bred with, however it is still possible that dogs with patellar luxation are born out of a combination of free dogs. This risk is low, compared to breeding dogs with a patellar luxation grade 1 or higher. Animals that had patellar luxation surgery during their life should not be used for breeding purposes.

2.3 Other inheritable diseases present in the breed

If a Kooikerhondje is diagnosed with another potentially inheritable disease, this information will also be available in Fit2Breed. Diseases recorded in Fit2Breed include:

- Hip dysplasia
- Elbow dysplasia
- Kinked tail
- Skeletal abnormalities
- Dental abnormalities

- Kidney problems
- Autoimmune disease
- Epilepsy
- Hearing problems
- Behavioral problems
- Other health problems

Dogs diagnosed with diseases that affect the health or well-being of the dog will not be advised to breed with. Dogs with a first line family member (parent, full-sibling or offspring) that is diagnosed with epilepsy, kidney problems or miscellaneous health problems, will receive a warning or a error for breeding and will not appear in the male breeding list.

3 Recommended links

If you want to know more about the inheritable diseases affecting the Kooikerhondje, you can click on the following links:

Links on research regarding clinical screening diseases and DNA test diseases

- A study on Hereditary Necrotizing Myelopathy in the Dutch Kooikerhondje population
- Study on genetics of Patellar Luxation in the Kooikerhondje
- Scientific article on ENM in the Kooikerhondje

Guides on screening and breed diseases

- Utrecht University site on Polymyositis in the Kooikerhondje (Dutch, English version also available)
- Kooikerhondje breed club site (Dutch)
- Dr. Paul Mandigers site on the Kooikerhondje (Dutch)
- ECVO website, for more information on Hereditary Eye Disease screening

Interesting books and videos

- The book "Breed predispositions to disease in cats and dogs", has a section dedicated to the Kooikerhondje
- Video of a Kooikerhondje diagnosed with polymyositis

Where to get your dog genotyped for Fit2Breed

- Order Embark SNP genotyping at Utrecht University
- Order DNA test for the Kooikerhondje at Utrecht University

4 Glossary of Terms

allele

Alternative forms of a gene. 19, 20, 23

allelic frequency

Percentage of the population alleles that have a particular form. 20

coefficient of inbreeding

Probability of a gene being homozygous due to inbreeding. 1, 5, 12, 13

DNA test result

The genotype of a dog for a disease related gene. 2, 8, 23

genotype

The genetic background of a dog. The collection of genes each dog inherits from its parents. In Fit2Breed we use data from commercial genetic analysis panels (such as MyDogDNA, Wisdom Panel and Embark), and DNA test results. 8, 17, 23

heterozygous

A gene with two different alleles. 8, 9, 19, 20

homozygous

A gene with two identical alleles. 23

homozygous mutant

A gene with two copies of the disease (or mutant) allele. 8, 19, 20

homozygous wildtype

A gene with two copies of the healthy (or wildtype) allele. 8, 9

kinship

Relatedness between two individuals. 16, 17

phenotype

The traits you can observe on the dogs. In Fit2Breed we use the disease data and diagnoses available in ZooEasy. 23

recessive

A "silent" allele that is only expressed in the phenotype when homozygous. 19