ESTIMATION OF HERITABILITY OF PATELLAR LUXATION IN FOUR DOG BREEDS IN FINLAND

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Tiivistelmä —Referat—Abstract

Patellaluksaatio (PL) eli polvilumpion sijoiltaanmeno on etenkin pienten koirien yleinen ortopedinen sairaus. Polvilumpio voi luksoitua mediaalisesti tai lateraalisesti. Mediaalinen luksaatio on huomattavasti yleisempi kuin lateraalinen. PL:lla arvellaan olevan perinnöllinen tausta, sillä tietyillä roduilla esiintyvyys on korkea ja oireet alkavat nuorena. PL arvioidaan koirasta nk. Putnamin arvosteluasteikolla palpoimalla ja manipuloimalla polviniveltä. PL arvostellaan välillä 0=terve…4=pysyvä luksaatio.

Tämän tutkimuksen tavoitteena oli määrittää PL-aineiston ja sukupuuaineiston avulla PL-fenotyypin varianssikomponentit, periytymisaste, geneettinen trendi, oikean ja vasemman polven geneettinen korrelaatio sekä japaninpystykorvalle edellisten lisäksi PL:n ja lonkkadysplasian (HD) geneettinen korrelaatio. Tutkimusrodut olivat chihuahua, pomeranian, suomenpystykorva sekä japaninpystykorva.

PL-, HD- ja sukupuuaineistot saatiin Suomen Kennelliitto ry:ltä. Aineistot muokattiin sopivaan muotoon R- ja Microsoft Office Excel-ohjelmistoilla. Sukupuiden laaduntarkastus tehtiin RelaX2-ohjelmistolla. Varianssikomponentit laskettiin DMU-ohjelmistolla REML-menetelmää käyttäen *(Restricted Maximum Likelihood).*

Periytymisasteet (h^2) olivat hyvin matalia tai matalia riippuen mallista ja rodusta. Kun tarkasteltava fenotyyppi oli koiran vasemman ja oikean polven keskiarvo, matalin h^2 oli pomeranianilla h^2=0,03 ja korkein chihuahualla h^2=0,18. Vasemman ja oikean polven geneettinen korrelaatio oli kaikissa roduissa 1, mikä viittaisi siihen, että geneettisesti kyseessä on sama ominaisuus. Japaninpystykorvan PL:n ja HD:n geneettinen korrelaatio oli -0,05. Jalostusarvojen trendi oli myönteinen chihuahualla ja japaninpystykorvalla. Trendi oli negatiivinen pomeranianilla ja neutraali suomenpystykorvalla.

Tämän tutkimuksen tulosten perusteella yksilön omaan tulokseen perustuva jalostusvalinta ei ole ollut tehokasta PL:ta vastustettaessa. Polviterveyttä tulisi edistää koirissa käyttämällä valintatyökaluna jalostusindeksejä, sillä PL:n periytymisaste on matala. Jalostusvalinta voisi tehostua ominaisuuden fenotyypityksen kehittämisen myötä.

Avainsanat —Nyckelord—Keywords

Patellaluksaatio, koira, periytymisaste, geneettinen korrelaatio, EBV

Säilytyspaikka —Förvaringsställe—Wheredeposited

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Tiivistelmä—Referat— Abstract

Patellar luxation is a typical orthopaedic disorder in small sized dogs. Patella can luxate either medially or laterally, medial luxation being by far more common than the lateral luxation. PL is considered hereditary since certain breeds have great susceptibility to get the condition, and the symptoms may occur at young age. PL is diagnosed by following the so-called Putnam's scale where the stifle joint is palpated and manipulated. PL is graded from 0=normal…4=permanent luxation.

The aim of this study was to estimate the variance components and the heritability of PL, to visualize PL's genetic trend and to calculate the genetic correlation between left and right stifle and between PL and hip dysplasia (HD) in Japanese Spitz.

The PL, HD and pedigree data were provided by the Finnish Kennel Club. Data were modified with both R-program and Microsoft Office Excel. The pedigree check was performed with RelaX2 program and variance component analyses were done with DMU program using the restricted maximum likelihood method.

Heritabilities (h^2) were from very low to low depending on the model and breed. When the dependent variable was the mean of left and right patellae of an individual the lowest heritability was in the Pomeranian h^2=0.03 and highest in the Chihuahua h^2=0.18. The genetic correlation of left and right patellae was 1 in all breeds which suggests that they are genetically the same trait. In the Japanese Spitz the genetic correlation between PL and HD was -0.05. The genetic trend of PL was favorable in the Chihuahua and the Japanese Spitz. In the Pomeranian the trend was negative and neutral in the Finnish Spitz.

Based on the results, the selection against PL has not been efficient. In future, patellae health should be controlled by using breeding indexes because the heritability of PL is low. Also, improvements in phenotyping could lead to more accurate selection.

Avainsanat—Nyckelord— Keywords
Patellar luxation, dog, heritability, genetic correlation, EBV

Muita tietoja— Övriga uppgifter —Further information
Supervisors: Pekka Uimari and Lea Mikkola

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ABBREVIATIONS

BLUE	Best linear unbiased estimation
BLUP	Best linear unbiased prediction
EBV	Estimated breeding value
GWAS	Genome wide association study
HD	Hip dysplasia
LPL	Lateral patellar luxation
MPL	Medial patellar luxation
PL	Patellar luxation
QTL	Quantitative trait locus
REML	Restricted maximum likelihood
SNP	Single nucleotide polymorphism

1 INTRODUCTION

Patellar luxation (PL) is one of the many disorders affecting purebred dogs. Mainly small sized dog breeds are affected but some large sized dog breeds may develop PL as well. A typical symptom is pain in the stifle joint which leads to lameness. A few surgical treatments are described to treat PL. PL is considered hereditary because of the vastly varying prevalence between different breeds. It is speculated that for instance weak angulation i.e. straight pelvic limb might predispose a dog to PL. Also, other morphological features are thought to increase the risk of PL.

The patella, the largest canine sesamoid bone, is located between the femur's craniodistal trochlear ridges i.e. in the trochlear groove. The patella is stabilized with strong ligaments and fasciae. Normally the patella can only move dorsoventrally, and its purpose is to transmit the contraction force from the quadriceps muscle to the tibia and lessen the friction between the quadriceps tendon and the stifle joint. In a few breeds, there is a mandatory patellar luxation evaluation for stud dogs. There is one evaluation standard, the so-called Putnam's scale, which is used both in the FCI (Fédération Cynologique Internationale) countries - such as Finland - and in North America.

2 LITERATURE REVIEW

2.1 Patellar luxation

The stifle joint consists in fact of several joints: the femorotibial, the femoropatellar and the tibiofibular joints (Dyce et al. 1996, p. 91-92). In a dog there are also plantar joints between the femur, and the lateral and the medial flabellae, and between the tibia and the popliteal sesamoid bone (Spencer and Tobias 2018, p. 2512-2513). The patella is the body's largest sesamoid bone (Spencer and Tobias 2018, p. 2512) which lays in the trochlear groove and normally can only move dorsoventrally (proxo-distally). Its purpose is to transmit the force produced by the contracting quadriceps muscle to the tibia and to the rest of the distal limb. The quadriceps muscle consists of four muscle units: *m. vastus lateralis*, *m. vastus medialis*, *m. vastus intermedius* and *m. rectus femoris* (Dyce et al. 1996, p. 96). Distally these four muscles combine and form the quadriceps tendon.

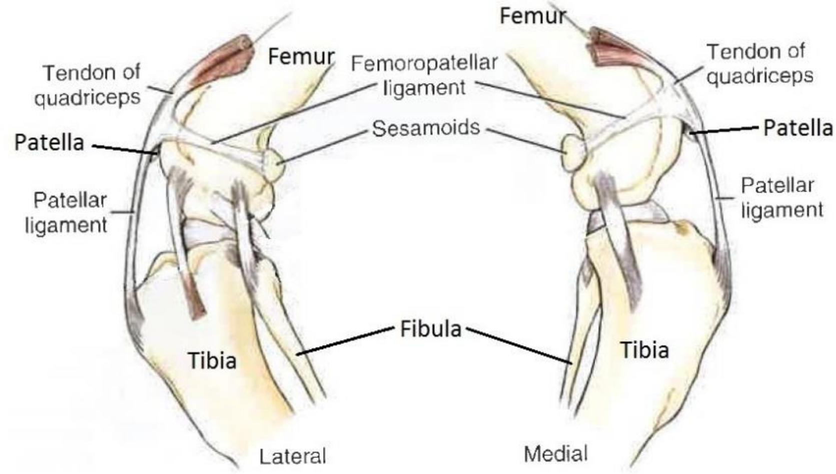

Figure 1. Stifle joint anatomy and ligaments. Picture modified from Evans, H.E. and de Lahunta, A. 2016. Guide to the Dissection of the Dog, 8th edition. Elsevier, St. Louis.p. 74.

The tendon passes cranially the femorotibial joint and the patella and transforms into the straight patellar ligament (patellar tendon). The patellar ligament eventually inserts into the tibial crest cranially (Dyce et al. 1996, p. 96). The patella is extended by bilateral parapatellar cartilages (Hwittick; W. I. 1974, p. 310, Dyce et al. 2010, p. 88; Spencer and Tobias 2018, p. 2512,). The patella is stabilized to the trochlear groove lateromedially by the lateral and medial femoropatellar ligaments which originate in the parapatellar cartilages and insert into the femoral fasciae (Spencer and Tobias 2018, p. 2512 and p. 2515). These ligaments are crucial to preventing PL (Spencer and Tobias 2018, p. 2512). The femoropatellar ligaments, the femoral fasciae and the joint capsule compose the patellar retinaculum (Hwittick, W. I. 1974, p. 310). The most relevant structures of the stifle joint are shown in the Figure 1.

If not caused by a trauma, PL is congenital and considered to be a consequence of conformational defects in the stifle joint and the coxofemoral angle. The trochlear groove can be too shallow which allows the patella to dislocate medially or laterally. There may also be looseness in the attachment ligaments which makes dislocation possible while various forces affect the joint during movement. The patellar ligament attaches to the tibial crest which may be located so that the line from the quadriceps tendon to the tibial crest is not vertically straight. This causes the patella to press either side of the trochlear groove's ridges (Hwittick, W. I. 1974, p. 315). Also, deformations in the limb angulation such as coxa vara and coxa valga (Bound et al., 2009, Kalff et al., 2014), and the rotation of the distal femur are thought to expose the stifle system to harmful forces which can lead to PL (Hwittick, W. I. 1974, p. 315). Coxa vara signifies a decreased angle of the femur and the femoral neck, whereas coxa valga means an increased angle between the two (MOT dictionaries 2019).

Already back in 1963, patellar luxation was described to affect mainly small sized pedigree dogs. The top five breeds especially at risk were found to be the Poodles (Toy, Miniature and Standard), the Yorkshire Terrier, the Cairn Terrier, the Chihuahua and the Pomeranian (Hodgman, S. F. J. 1963). Priester (1972) investigated the risk factors of PL, concluding that females and small dog breeds indeed are more prone to get the condition. The most affected breeds were the Pomeranian, the Yorkshire Terrier, the Chihuahua, the Miniature and Toy Poodles and the Boston Terrier (Priester 1972). Bound et al. (2009) also found females to be at greater risk of developing PL than males. However, Vidoni et al. (2006) and Nganvongpanit et al. (2011) did not find significant differences between sexes in developing PL. In the study of Nganvongpanit et al. (2011), two breeds had a significantly increased (The Poodles, Miniature and Toy) and decreased (the Jack Russell Terrier) risk of PL. Later on, numerous studies have shown that small and miniature dog breeds are at the highest risk of developing PL (Hayes et al., 1994, LaFond et al., 2002, Vidoni et al., 2006, Nganvongpanit et al., 2011).

The patella can either luxate medially or laterally and it is repeatedly observed that the medial patellar luxation (MPL) is by far a more common type of luxation compared to the lateral patellar luxation (LPL) (Gibbons et al., 2006, Vidoni et al., 2006, Alam et al., 2007, Bound et al., 2009, Nganvongpanit et al., 2011). However, large breed dogs seem to have LPL more often than small breed dogs (Hayes et al 1994, Alam et al., 2007, Kalff et al., 2014). It is also reported that both MPL and LPL can occur in the same stifle, even though this is rare (Vidoni et al., 2006).

Some studies have found a connection between PL and concomitant cranial cruciate ligament changes or complete ruptures (Gibbons et al., 2006, Nganvongpanit et al., 2011). The underlying reason for cranial cruciate ligament injuries is speculated to be a cause of abnormally directed forces in subclinical or clinical PL stifles that eventually strain and even rupture the cranial cruciate ligament (Willauer and Vasseur, 1987). For instance, Campbell et al. (2010) discovered that dogs with the worst grade of MPL were more likely to have cranial cruciate ligament ruptures than dogs with milder grades of MPL. The group of dogs having both MPL and cranial cruciate ligament rupture were also older than dogs with only MPL. This finding might suggest that the cranial cruciate ligament problems are indeed developmental and secondary to MPL.

PL is treated either conservatively or surgically. Usually a grade one condition with no lameness is treated conservatively (Spencer and Tobias 2018, p. 2646). Surgery aims to realign the extensor mechanism and to stabilize the patella into the trochlear groove (Spencer and Tobias 2018, p. 2647). Depending on the conformational deformities, one or more techniques of the following are used:

- Trochleoplasty where the trochlear groove is reshaped i.e. deepened
- Tibial crest transposition where the insertion of straight patellar ligament to the tibia is relocated
- Soft tissue reconstruction where the patellar retinaculum is loosened from the side of luxation and tightened with sutures from the opposite side

(Hwittick, W. I. 1974, p. 319, Spencer and Tobias 2018, p. 2647-2656).

2.2 Genetic background of patellar luxation

PL is considered to have a complex genetic background since it is commonly bilateral and appears already in young dogs lacking history of trauma (Hayes et al. 1994). Moreover, some breeds have a significantly higher risk for the condition than others (Hayes et al. 1994, LaFond et al., 2002). The published heritability estimates of PL are from low to intermediate ($0.03 < h^2 < 0.44$) (Lavrijsen et al., 2013, Nilsson et al., 2018, Wangdee et al., 2014, Wangdee et al., 2017, Zanders 2014). Zanders (2014) found in her MSc thesis that in all breeds the veterinarian who evaluated the stifle had highly significant (p-value < 0.0001) effect on PL scores. As there are numerous qualified veterinarians for the official PL evaluation, Zanders (2014) treated the grading veterinarian as a random effect in the statistical models. A model which separated different PL scores (Y = 0-4) gave heritability estimates of 0.25 (±0.023), 0.21 (±0.062), 0.08 (±0.058) and 0.03 (±0.033) for Chihuahua, Bichon Frisé, Pomeranian, and French Bulldog, respectively. Later, Nilsson et al. (2018) published Zanders’ results for the Chihuahua and the Bichon Frisé but changed the PL scores from a linear trait to a binary trait (affected/unaffected). Using the binary data, the estimated heritabilities were 0.22 (±0.02) for Chihuahua and 0.18 (±0.04) for Bichon Frisé.

With a threshold model, as high as 0.44 (±0.04) heritability estimate was obtained for a Thai Pomeranian population (Wangdee et al., 2017). The estimated heritability of PL for the Dutch Kooikerhondjes population was 0.27 (±0.07) (Wangdee et al., 2014) and for the Flat-Coated Retrievers 0.17 (±0.03) (Lavrijsen et al., 2013).

An explanation for the different estimations might be the varying genetic background between populations, the statistical model applied and the validity of PL scoring system. As Zanders (2014) noticed, the effect of the evaluating veterinarian on the scores is important. This raises the question of whether the current scoring protocol is the best for the PL evaluation purposes. Also, in statistical analysis it is not ideal that there are many graders since the human component is always prone to errors. A summary of the published heritabilities is given in Table 1.

Table 1. Published heritability estimates of PL.

Publication	**Breed**	**h^2 (±SE)**
Lavrijsen et al. (2013)	Flat-Coated Retrievers	0.17 (±0.03)
Nilsson et al. (2018)	Chihuahua	0.22 (±0.02)
	Bichon Frisé	0.18 (±0.04)
Wangdee et al. (2014)	Kooikerhondje	0.27 (±0.07)
Wangdee et al. (2017)	Pomeranian	0.44 (±0.04)
Zanders (2014)	Chihuahua	0.25 (±0.023)
	Bichon Frisé	0.21 (±0.062)
	Pomeranian	0.08 (±0.058)
	French Bulldog	0.03 (±0.033)

As a solution to phenotyping errors, some researchers of veterinary medicine have studied whether different radiographic measurements could replace or complement the current scoring. Mortari et al. (2009) suggested that the quadriceps angle could be useful when evaluating the PL grade 3 stifles. On contrary, they noticed that for severe luxation (grade 4) it was problematic to obtain good radiographies. In her master’s thesis, Marttinen investigated the correlation of MPL (grades 1-2) between eight different femoral angles in Lancashire Heelers. Three measurements were found to be statistically significant: FVA (femoral varus angle, p-value = 0.002), IFA (inclination of the femoral head angle, p-value = 0.008) and aLDFA (anatomical lateral distal femoral angle, p-value = 0.028). Sample size in the study was 75 hind limbs.

Recently, also quantitative trait locus (QTL) mapping studies have been conducted for PL (Chase et al., 2009, Chomdej et al., 2014, Lavrijsen et al., 2014, Wangdee et al., 2014, Wangdee et al., 2017) but only a few candidate genes have been named for this complex disease. Chase et al. (2009) used across-breed mapping approach to find QTLs affecting different behavioral and morphological traits. They first found that longevity and size associated with a region on CFA15 (*Canis lupus familiaris* chromosome 15) where one of the candidate genes was *IGF1* (insulin-like growth factor 1). *IGF1* encodes a protein that has a high growth-promoting activity, for instance by regulating glycogen synthesis in osteoblasts and stimulating glucose transport and DNA synthesis (UniProt 2019). As PL incidence correlates with body size, Chase et al. (2009) also tested the correlation of *IGF1* and PL disease frequency finding a significant association (p-value < 0.01). This suggests that selection for body size has influenced the incidence of PL.

Soontornvipart et al. (2013) studied linkage of PL phenotype and five different microsatellite markers that were chosen by their location close to five different collagen genes. No association was found. The same research group also did a GWAS with 1536 SNPs for a sample of n=46 Thai Pomeranians where 37 were cases and 9 controls. One SNP (BICF234J1226) in CFA07 was found promising (Soontornvipart et al. 2013). Another study with a sample of 39 small breed dogs from Thailand did not find an association between CFA07 and PL with a random amplified polymorphic DNA analyses method (Chomdej et al. 2014). On the contrary, this study found a linkage between an ATP synthase gene in CFA36 and PL. In a rather atypical PL breed, Flat-Coated Retriever a GWAS was carried out using two different phenotypes: binary trait (affected / unaffected) and EBVs (Lavrijsen et al. 2014). The strongest association was found on CFA07 with both phenotypes yielding a candidate gene *TNR* (tenascin R). A paralogue of *TNR* in humans is *TNXB* (tenascin XB) in which a mutation is known to cause a connective tissue syndrome. However, Lavrijsen et al. (2014) state that in humans the homologous *TNR* is solely expressed in the brain. Yet the online Expression Atlas (2020) search results suggest that there is evidence of *TNR* expression in other tissues as well.

The most recent gene mapping study was done in 2017 by Wangdee et al. In their GWAS the sample consisted of Pomeranians (48 cases and 48 controls) genotyped with Illumina CanineHD BeadChip (173 K SNPs). Altogether 22 SNPs in 15 different chromosomes were further investigated resulting in four possible candidate genes – two on CFA05 (*SORL1, SC5D*) and two on CFA32 (*BMPR1B, UNC5C*). Two of these were considered most relevant for further studies: The *BMPR1B* encodes bone morphogenetic protein receptor type-1B protein, a member of protein kinase superfamily that's biological processes include chondrocyte development and negative regulation of their proliferation among others (UniProt 2020). The *SC5D* encodes sterol-C5-desaturase enzyme which is part of the cholesterol biosynthetic process (UniProt 2020). Wangdee et al. (2014) also tested the association of the 22 loci to PL in Dutch Kooikerhondjes, Flat-Coated retrievers and Labrador retrievers and Thai Chihuahuas and Toy Poodles. No association was found.

2.3 Breeding against PL

As a rule, the Finnish Kennel Club has stated that the minimum age to officially evaluate PL is 12 months and if a dog is evaluated between 1-3 years of age, the PL score is valid for two years. PL evaluation done to a dog aged 3 years or more is valid permanently. If there is a breeding restriction considering PL in a breeding plan of a given breed, a stud dog's PL evaluation needs to be valid when mating takes place. Putnam's scale is used to evaluate PL (Table 2) (the Finnish Kennel Club, 2012).

Table 2. Putnam's scale for grading PL (Orthopedic Foundation for Animals, 2019).

Grade	**Description**
0	No abnormalities
1	Manually the patella easily luxates at full extension of the stifle joint but returns to the trochlea when released. No crepitation is apparent. The medial, or very occasionally, lateral deviation of the tibial crest (with lateral luxation of the patella) is only minimal, and there is a very slight rotation of the tibia. Flexion and extension of the stifle are in a straight line with no abduction of the hock.
2	There is frequent patellar luxation, which, in some cases, becomes more or less permanent. The limb is sometimes carried, although weight bearing routinely occurs with the stifle remaining slightly flexed. Especially under anesthesia, it is often possible to reduce the luxation by manually turning the tibia laterally, but the patella re-luxates with ease when manual tension of the joint is released. As much as 30 degrees of medial tibial torsion and a slight medial deviation of the tibial crest may exist. When the patella is resting medially the hock is slightly abducted. If the

	condition is bilateral, more weight is shifted onto the forelimbs. Many dogs with this grade live with the condition reasonably well for many years, but the constant luxation of the patella over the medial trochlear ridge of the trochlea causes erosion of the articulating surface of the patella and also the proximal area of the medial lip. This results in crepitation becoming apparent when the patella is luxated manually.
3	The patella is permanently luxated with torsion of the tibia and deviation of the tibial crest of between 30 degrees and 50 degrees from the cranial/caudal plane. Although the luxation is not intermittent, many animals use the limb with the stifle held in a semi-flexed position. The trochlea is very shallow or even flattened.
4	The tibia is medially twisted, and the tibial crest may show further deviation medially with the result that it lies 50 degrees to 90 degrees from the cranial/caudal plane. The patella is permanently luxated. The patella lies just above the medial condyle and space can be palpated between the patellar ligament and the distal end of the femur. The trochlea is absent or even convex. The limb is carried, or the animal moves in a crouched position, with the limb flexed.

In Finland, national breed clubs can rather autonomically set breeding restrictions and recommendations for stud dogs in order to manage hereditary defects in their breeds. In 2018, 25 different breed clubs had set a mandatory PL screening prior to mating. Some of them included a threshold value for PL scores in stud dogs. Clubs that have not done so, must follow Kennel Club’s common rule of grade 3 being the worst score accepted for stud dogs. PL score for an individual dog is marked for instance “0/0” which means that both left and right stifle are healthy.

2.4 Breeds

The breeding rules and recommendations within the studied breeds (Chihuahua, Pomeranian, Finnish Spitz and Japanese Spitz) have varied through time. There was at least a recommendation on the usage of stud dogs concerning PL in each breed at the time of data export (spring 2015). Only in the Finnish Spitz there was a PL score limitation set as a precondition to registration of a litter. Below is a presentation of each breed’s breeding plan for PL at the time of data export with a brief introduction to the breed.

2.4.1 Chihuahua

The Chihuahua is known to be the smallest purebred dog that exists. According to the breed standard, a Chihuahua should weigh less than three kilograms. It is solely a companion dog originally from Mexico. There are two coat types of Chihuahuas, a long

coated and a smooth coated version. In the breed standard, the following is said about the pelvic limbs: “Hind legs well-muscled with long bones, vertical and parallel to each other with good angulation at hip, knee and hock joints, in harmony with angulation of forequarters” (Breed standard, FCI, 2015).
An official patellae examination is mandatory for breeding dogs in Finland even though no limitations on PL scores are set. The Finnish Chihuahua Breed Club recommends only to breed healthy (0/0) dogs. However, with “heavy reasons” it is acceptable to mate a dog whose summed patella luxation score is max. 2 with a healthy partner. This means breeding 1/1 or 0/2 or 2/0 scored dog to a 0/0 dog (Jalostuksen tavoiteohjelma, 2015).

Figure 2. A sample of phenotypic variation in the Chihuahua. ©Titta Lähdesmäki

2.4.2 Pomeranian

The Pomeranian is a miniature sized companion and watch dog originating from Central European ancient spitz type dogs that lived there already in the Stone Age. According to the present FCI breed standard (2015) “The stifle joint is strong with only moderate angulation and is turned neither in nor out in movement.”

The Finnish Pomeranian Breed Club recommends to officially examine dog’s stifles at the age of > 12 months. A dog with a score 1 or 2 can be used for breeding if “it is of high quality regarding other requirements”. However, the mating dogs’ combined patella score should not be more than 2. Breed club also recommends taking into account the patella scores of breeding dogs’ parents and siblings because of the complex inheritance (Jalostusohjesääntö, 2017).

Figure 3. The Pomeranian. ©Pixaday

2.4.3 Finnish Spitz

The Finnish Spitz is an old hunting breed that marks and stops game by barking. The first breed standard was established already in 1892 and the Finnish Spitz is now the national dog breed of Finland. The Finnish Spitz is a medium sized red colored dog that barks mainly at game birds but also at moose and sometimes at bear (Breed standard, FCI, 2015).

In order to register a litter to the Finnish Kennel Club's register, the parent dogs have to have PL score 0-1. This is an unconditional precondition. PL score 1 dogs can have scores 1/0, 0/1 or 1/1. However, this rule does not apply to a dog's first litter (Jalostuksen

Figure 4. The Finnish Spitz. ©Päivi Ruotanen

tavoiteohjelma, 2016). According to the club's chairperson (Thommy Svevar, Suomen Pystykorvajärjestö, email to the author on October 13th, 2016) the first litter's exception is a compromise: unchecked stud dogs are a risk to breed's PL health, but in this system more different dogs are used for breeding at least once. As a result, the breeding population has probably maintained wider which is beneficial from the genetic diversity perspective.

2.4.4 Japanese Spitz

The Japanese Spitz is a small white-coated companion dog. According to the breed standard, the Japanese Spitz originates from an imported white German Spitz in the 1920's. Later, imported white spitz dogs from Canada, USA and China were introduced to the early Japanese Spitz breed (Breed standard, FCI, 2015).

The Finnish Japanese Spitz Breed Club recommends not to mate a dog with worse than score 1 stifles. However, if a 1/1, 0/1 or 1/0 scored dog is used for breeding the mating partner should be healthy (0/0) (Jalostuksen tavoiteohjelma, 2016).

Figure 5. The Japanese Spitz © Pekka Uimari

3 AIMS OF THE STUDY

The first aim of this study was to estimate the variance components and the heritability of PL for all the breeds included in the study. The second aim was to estimate breeding values for each animal with an animal model to observe the genetic trends of PL. The third aim was to estimate the genetic correlations of PL between left and right stifle and between HD and PL in the Japanese Spitz.

4 MATERIAL AND METHODS

Both the phenotypic and pedigree data was provided by the Finnish Kennel Club. The data export from the breeding database KoiraNet was performed in January 2015. The original file format was .csv. All data was modified with both Microsoft Office Excel (2016 version) and R-program (R version 3.2.2 (2015-08-14)). Pedigree analyses were performed with RelaX2 (Strandén and Vuori 2006) and variance component and breeding value estimation with the restricted maximum likelihood (REML) method applied in the DMU program package (Madsen and Jensen 2013).

4.1 Pedigree data

The original pedigree files included name of the dog, registration IDs for the dog and its parents, birth year and the breed code. The original, pruned and final number of dogs in the pedigree files are given in Table 3. The pedigree error check was run with the pedigree analysis program RelaX2. Errors such as duplicates, or the same animal marked both as dam and sire were removed. These criteria lead to “pruned n” in the Table 3. The other criterion for the final pedigrees was that only animals who contributed to the variance component estimation within four generations were kept in the pedigree data. Also, only the largest population for each breed was accepted. This means that all the animals in the accepted pedigree had a known relation to each other. These criteria lead to the “final n” given in the Table 3. The pedigrees of short- and long-coated Chihuahuas were merged because the breeding population is the same.

The registration of the Finnish Spitz and the Chihuahua was established already in the 1950's which explains the sizes of the original pedigrees. Also, the first Pomeranians were registered in the Finnish Kennel Club's register in the 1950's but the breed's popularity has not reached the numbers of the Finnish Spitz and the Chihuahua. The Finnish Spitz was an extremely popular breed with nearly 2000 registrations annually for almost the whole decade of 1990's. Since 1999, registrations have dropped below 1000 puppies per year and continue to decrease dramatically. Quite the opposite has happened to the popularity of the Chihuahua which has increased steadily breaking the limit of one thousand registrations in 2007. The Pomeranian's annual registration numbers have been 100-200 for nearly 30 years now. In Finland, the Japanese Spitz is the youngest breed of these four. The first registrations date back to the 1970's. The Japanese Spitz's annual registration number has varied from 160 to 300 over the past 30 years.

Table 3. Number of animals in pedigrees before and after quality control.

Breed	**Original n**	**Pruned n**	**Final n**
Chihuahua	26007	17855	8152
Pomeranian	8065	5977	2088
Finnish Spitz	48342	42421	5921
Japanese Spitz	7923	6347	1576

4.2 Phenotypic data

The phenotypic data included ID of the dog, its litter ID, ID of the veterinarian that performed the PL evaluation, sex of the dog (male=1, female=2), age in months when the PL evaluation was done, birth year and month, the postal code and ID of the breeders if known, size of the litter the animal was born to, the left and right patella score (either 0, 1, 2, 3, 4, "no statement" or "operated"), and finally the mean of the previous two. Additionally, there was information about the coat types (1=long or 2=short) for the Chihuahua.

The age limit for the official patellar luxation examination is 12 months, however, in most of the breeds there were some evaluations done for younger dogs. Those records were kept in the data because if a young dog is showing symptoms of PL, the cause of PL is more likely genetic than environmental, and the phenotype is severe. Many dogs were tested more than once in their lifetime. In these cases, only the worst grade was kept because PL is considered congenital. The LPL and MPL scores were combined into one numeric PL score. A few PL scores stated “operated”. For further analyses these dogs were removed from the data since there was no knowledge of the true reason for operating the stifle (could be trauma) and the small number of cases. There were also random “no statement” records, i.e. no information was available on what was the reason to this kind of a record. Therefore also “no statement” records were removed. The mean PL score for each animal was calculated. The structure of the phenotypic data is given in Tables 4 – 7.

Table 4. Descriptive parameters for the data where the variable was mean of the left and the right stifles’ PL scores.

Breed	**n**	**Average**	**Standard deviation**	**Min value**	**Max value**
Chihuahua	5800	0.29	0.61	0	4
Pomeranian	780	0.32	0.61	0	3
Finnish Spitz	3278	0.05	0.24	0	4
Japanese Spitz	875	0.19	0.45	0	4

Table 5. Distribution of mean of the left and right PL scores.

Breed	**Chihuahua**		**Pomeranian**		**Finnish Spitz**		**Japanese Spitz**	
Mean PL score	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
0	4297	74.1	548	70.3	3099	94.5	687	78.4
0.5	565	9.74	92	11.8	86	2.62	96	10.9
1.0	474	8.17	77	9.87	63	1.92	66	7.63
1.5	188	3.24	26	3.33	15	0.46	16	1.82
2.0	157	2.71	23	2.95	11	0.34	3	0.34
2.5	50	0.86	2	0.26	3	0.00	2	0.34
3.0	51	0.88	12	1.54	0	0.00	3	0.34
3.5	10	0.17	0	0.00	0	0.00	1	0.11
4.0	8	0.14	0	0.00	1	0.00	1	0.11
Total	5800	100	780	100	3278	100	875	100

Table 6. Number of PL records by sex. The percentage indicates the proportion of animals that had PL score >0.

	Chihuahua		Pomeranian		Finnish Spitz		Japanese Spitz	
Sex	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Male	1924	24	273	24	1213	3	366	17
Female	3876	27	507	33	2065	7	509	25
Total	5800		780		3278		875	

Table 7. Mean of the mean PL scores by birth year and the number of records.

	Chihuahua		**Pomeranian**		**Finnish Spitz**		**Japanese Spitz**	
Birth year	**Mean PL score**	**n**	**Mean PL score**	**n**	**Mean PL score**	**n**	**Mean PL score**	**n**
1978	-	-	0.0	1	0.0	1	-	-
1979	-	-	-	-	0.0	2	-	-
1980	-	-	-	-	0.0	1	-	-
1981	0.0	2	-	-	0.1	5	-	-
1982	0.0	2	-	-	0.1	15	-	-
1983	-	-	-	-	0.0	13	-	-
1984	0.0	2	-	-	0.0	18	-	-
1985	0.3	3	-	-	0.0	16	0.0	1
1986	0.0	2	0.5	1	0.0	38	-	-
1987	0.1	12	1.0	1	0.1	75	0.2	3
1988	0.2	15	0.5	4	0.0	91	0.1	4
1989	0.3	26	0.0	5	0.0	85	0.3	10
1990	0.3	45	1.0	2	0.0	114	0.1	19
1991	0.2	55	0.3	7	0.0	118	0.3	17
1992	0.2	76	0.1	7	0.0	112	0.2	25
1993	0.2	65	0.5	6	0.0	95	0.1	21
1994	0.2	112	0.1	7	0.0	119	0.3	27
1995	0.1	111	0.1	7	0.0	86	0.3	24
1996	0.3	96	0.3	14	0.0	89	0.3	22
1997	0.2	123	0.5	12	0.0	88	0.2	21
1998	0.2	139	0.2	19	0.0	115	0.2	30
1999	0.2	157	0.1	21	0.0	83	0.3	33
2000	0.2	133	0.3	24	0.1	108	0.0	26
2001	0.3	132	0.3	27	0.0	110	0.1	39
2002	0.3	185	0.3	42	0.0	127	0.2	32
2003	0.3	186	0.2	41	0.0	141	0.1	34
2004	0.3	234	0.3	48	0.1	167	0.1	48
2005	0.3	352	0.1	52	0.0	159	0.1	44
2006	0.3	360	0.3	54	0.0	162	0.2	68
2007	0.4	471	0.4	58	0.1	189	0.2	35
2008	0.3	479	0.3	49	0.1	199	0.2	57
2009	0.4	492	0.4	55	0.1	188	0.1	66
2010	0.4	497	0.4	77	0.1	123	0.3	61
2011	0.3	467	0.4	55	0.1	95	0.2	41
2012	0.3	488	0.2	62	0.1	93	0.1	37
2013	0.2	281	0.7	22	0.1	38	0.4	30
Sum	-	5800	-	780	-	3278	-	875

4.1.1 Chihuahua

The PL observations were available from 1990 to 2014 including 6610 MPL and 30 LPL scores for the short-coated Chihuahua. For the long-coated Chihuahua there were 7019 MPL and 30 LPL scores. In the final pruned phenotypic data, there were altogether 5800 observations for the Chihuahua (combined). For the statistical analyses the data from short- and long-coated Chihuahuas were merged. This was reasonable since the breeding populations of both coat types are the same. Age at the time of the patella examination was converted to classes (n=12) according to the Table 8.

Table 8. Classification of the age at the time of the patella examination, Chihuahua

Age when examined [Month]	n	Numeric format in data file
<12	32	1
12-17	3518	2
18-23	948	3
24-29	427	4
30-35	220	5
36-41	217	6
42-47	139	7
48-53	87	8
54-59	56	9
60-65	50	10
66-71	29	11
>72	77	12

4.1.2 Pomeranian

The PL observations were available observations from 1990 to 2014 including 1608 MPL and 20 LPL scores. In the final pruned phenotypic data, there were altogether 780 observations for Pomeranians. Age at the time of the patella examination was converted to classes (n=8) according to the Table 9.

Table 9. Classification of the age at the time of the patella examination, Pomeranian

Age when examined [Month]	n	Numeric format in data file
<12	15	1
12-17	377	2
18-23	183	3
24-29	65	4
30-35	41	5
36-41	28	6
42-47	17	7
>48	54	8

4.1.3 Finnish Spitz

The PL observations were available from 1989 to 2014 including 7146 MPL and 34 LPL scores. Two records were “operated”, and one was “no statement”. In the final pruned phenotypic data, there were altogether 3278 observations for the Finnish Spitz. Age at the time of the patellae examination was converted to classes (n=12) according to the Table 10.

Table 10. Classification of the age at the time of the patella examination, Finnish Spitz

Age when examined [Month]	n	Numeric format in data file
<12	70	1
12-17	508	2
18-23	427	3
24-29	356	4
30-35	338	5
36-41	333	6
42-47	261	7
48-53	210	8
54-59	152	9
60-65	131	10
66-71	109	11
>72	383	12

4.1.4 Japanese Spitz

The PL observations were available from 1991 to 2014 including 1751 MPL and 70 LPL scores. In the final pruned phenotypic data, there were altogether 875 observations for

Japanese Spitz. Age at the time of the patellae examination was converted to classes (n=11) according to the Table 11.

Table 11. Classification of the age at the time of the patella examination, Japanese Spitz

Age when examined [Month]	n	Numeric format in data file
<12	7	1
12-17	448	2
18-23	158	3
24-29	95	4
30-35	45	5
36-41	44	6
42-47	20	7
48-53	12	8
54-59	13	9
60-65	12	10
>66	21	11

For the Japanese Spitz there were also an HD examination data available for the estimation of genetic correlation between HD and PL. The data originally included 775 observations from 380 different animals. The HD data included columns of the veterinarian that evaluated the x-ray images at the Finnish Kennel Club, the examination year and month, age in months when the examination was done (both direct months and months converted to classes, Table 12), sex of the dog, birth year and month, and mean score of the left and right hip grades. Hip scores were converted from alphabetical into numeric format. The same weighting method was used as the Finnish Kennel Club used for HD breeding index calculations: grade E (the worst grade) weighted by +0.5 (Table 13). Originally there were four different veterinarians who had scored the hip X-ray pictures. For two of them there were only a few observations. These were removed in order to unify the data. For each dog the left and right limbs' HD scores were combined into one mean score. The distribution of the mean HD scores is given in Figure 6.

Table 12. Classification of the age at the time of the patella examination, Japanese Spitz

Age when examined [Month]	n	Numeric format in data file
12-17	146	1
18-23	93	2
24-29	33	3
30-35	29	4
36-41	18	5
42-47	10	6
>48	25	7

Table 13. The numerical values used for the original alphabetical hip scores.

Grade	Numeric value in data file
A = normal	1
B = borderline	2
C = mild	3
D = moderate	4
E = severe	5.5

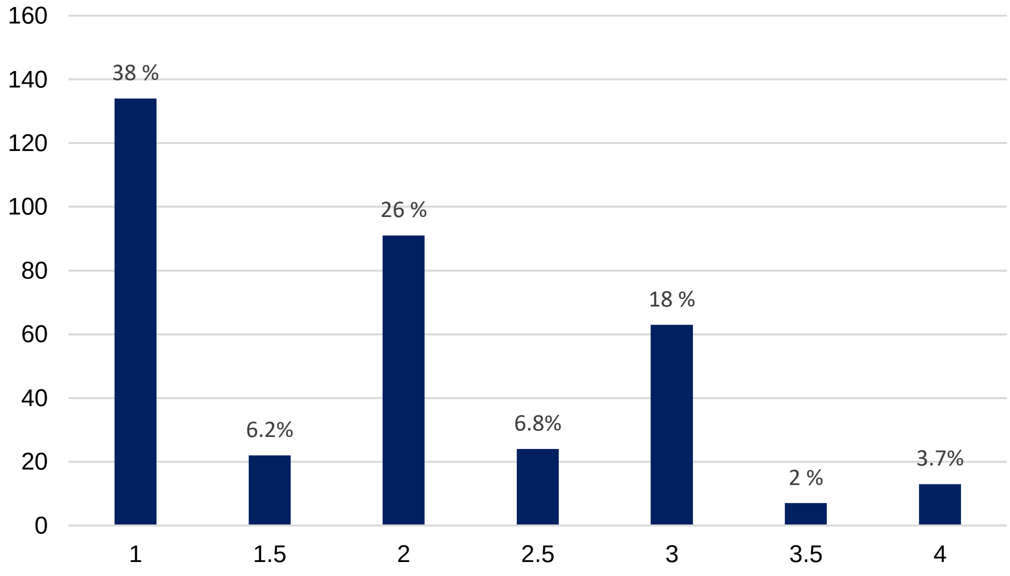

Figure 6. Distribution of the mean HD scores.

4.2 Statistical model

The fixed effects including sex, evaluation year, age of the dog when the evaluation took place, birth year and birth month, were tested with a backward selection method in R. In backward selection, the primary linear model includes all fixed effects, which are dropped one by one after testing their significance leaving only significant variables. The significance level of 0.05 was used. The veterinarians who evaluated the patellae was considered as a random effect because there were hundreds of qualified veterinarians in the PL data each having a relatively small number of patellae evaluated. On the contrary, in the Japanese Spitz's hip data, there were only two different veterinarians which is why these veterinarians were tested as fixed effects (not significant).

The ANOVA-tables (analyses of variance) of the significant variables for the Chihuahua, the Pomeranian, the Finnish Spitz and the Japanese Spitz (PL and HD) are presented in Table 14, Table 15, Table 16, Table 17, and Table 18 respectively. The definitions for the variables are given below the tables.

Table 14. The ANOVA-table for the variables in the final model without random effects for the Chihuahua.

Variable[a]	**df**	**SS**	**MS**	**F**	**P-value**
sex	1	2.7	2.72	7.59	0.006 (**)
year	24	34.8	1.45	4.05	1.15e-10 (***)
age	11	68.4	6.22	17.4	<2e-16 (***)
byear	31	34.6	1.12	3.12	1.33e-08 (***)
residuals	5730	2050	0.358		

Table 15. The ANOVA-table for the variables in the final model without random effects for the Pomeranian.

Variable[a]	**df**	**SS**	**MS**	**F**	**P-value**
sex	1	2.52	2.52	6.96	0.009 (**)
age	7	9.65	1.38	3.81	0.0005 (***)
residuals	771	279	0.362		

Table 16. The ANOVA-table for the variables in the final model for PL without random effects for the Finnish Spitz.

Variable[a]	**df**	**SS**	**MS**	**F**	**P-value**
sex	1	0.25	0.25	4.42	0.04 (*)
residuals	3270	1889	0.06		

Table 17. The ANOVA-table for the variables in the final model for PL without random effects for the Japanese Spitz.

Variable[a]	**df**	**SS**	**MS**	**F**	**P-value**
sex	1	1.81	1.81	9.00	0.003 (**)
age	10	4.78	0.48	2.38	0.009 (**)
residuals	866	173	0.20		

Table 18. The ANOVA-table for the variables in the final model for HD without random effects for the Japanese Spitz.

Variable[a]	**df**	**SS**	**MS**	**F**	**P-value**
age	6	12.2	2.03	2.80	0.01 (*)
residuals	319	231	0.72		

Signif. codes: 0: ‘***’, 0.001: ‘**’, 0.01: ‘*’, 0.05: ‘.’, 0.1: ‘ ’ and 1

Where

df	= degrees of freedom
SS	= sum of squares
MS	= mean square
F	= F-value
sex	= sex of the animal
byear	= animal’s birth year
age	= animal’s age in months (categorized) in the PL (HD) evaluation
year	= the year of the evaluation

Summary of the mixed models is given in the Table 19. Variables sex_i, $year_j$, age_k, and $byear_l$ were treated as fixed effects, a_n is a breeding value of animal n, $\mathbf{a} \sim N(\mathbf{0},\mathbf{A}\sigma^2_a)$, vet_m is a random effect of a veterinarian m, $\mathbf{vet} \sim N(\mathbf{0},\mathbf{I}\sigma^2_{vet})$, and e_{ijklmn} is the residual, $\mathbf{e} \sim$

$N(\mathbf{0},\mathbf{I}\sigma^2_e)$. **I** is a diagonal matrix and **A** is the relationship matrix. Variance components σ^2_a, σ^2_v, and σ^2_e correspond to the effect of breeding values, veterinarians, and residuals, respectively.

Table 19. Summary of the mixed models for each studied breed.

Breed	y = mean PL score	y = right PL score	y = left PL score	y = Mean HD score
Chihuahua	$y_{ijklmn} = \mu + sex_i + year_j + age_k + byear_l + vet_m + a_n + e_{ijklmn}$	$y_{ijklmn} = \mu + sex_i + year_j + age_k + byear_l + vet_m + a_n + e_{ijklmn}$	$y_{ijklmn} = \mu + sex_i + year_j + age_k + byear_l + vet_m + a_n + e_{ijklmn}$	-
Pomeranian	$y_{ijklmn} = \mu + sex_i + age_k + vet_m + a_n + e_{ijklmn}$	$y_{ijklmn} = \mu + sex_i + age_k + vet_m + a_n + e_{ijklmn}$	$y_{ijklmn} = \mu + sex_i + age_k + vet_m + a_n + e_{ijklmn}$	-
Finnish Spitz	$y_{ijklmn} = \mu + sex_i + vet_j + a_n + e_{ijklmn}$	$y_{ijklmn} = \mu + sex_i + vet_j + a_n + e_{ijklmn}$	$y_{ijklmn} = \mu + sex_i + vet_j + a_n + e_{ijklmn}$	-
Japanese Spitz	$y_{ijklmn} = \mu + sex_i + age_k + vet_m + a_n + e_{ijklmn}$	$y_{ijklmn} = \mu + sex_i + age_k + vet_m + a_n + e_{ijklmn}$	$y_{ijklmn} = \mu + sex_i + age_k + vet_m + a_n + e_{ijklmn}$	$y_{ijklmn} = \mu + age_k + a_n + e_{ijklmn}$

Where

y = dependent variable (see column header)

μ = overall mean

sex = sex of the animal

byear = animal's birth year

age = animal's age in months (categorized)

year = the year of the evaluation

vet = veterinary ID

a = EBV of the animal

e = residual

5 RESULTS

5.1 Heritability estimates

The heritability (h^2) indicates proportion of the additive genetic variation from the total phenotypic (observed) variation within a population. The random effect of veterinarian was not included in the formula because it is not an animal-based source of variation. The general formula for heritability is:

$$h^2 = \frac{\sigma_a^2}{\sigma_p^2} \tag{1}$$

Where:

σ_a^2 = additive genetic variance

σ_p^2 = phenotypic variance

For both PL and HD heritability the following variance components were used:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2} \tag{2}$$

Where

σ_a^2 = additive genetic variance

σ_e^2 = residual variance

The estimates of variance components and heritabilities and heritability standard errors are given in the Table 20.

Table 20. The variance components and the heritability estimates for different traits.

	σ^2_a	σ^2_{vet}	σ^2_e	h^2	S.E.
CHIHUAHUA					
Left PL	0.06	0.02	0.41	0.12	0.02
Light PL	0.06	0.02	0.36	0.14	0.02
Mean PL	0.06	0.02	0.28	0.18	0.02
POMERANIAN					
Left PL	0.01	0.04	0.46	0.02	0.05
Light PL	0.02	0.06	0.35	0.05	0.05
Mean PL	0.01	0.05	0.32	0.03	0.05
FINNISH SPITZ					
Left PL	0.01	0.03	0.06	0.14	0.02
Light PL	0.00	0.01	0.07	0.03	0.02
Mean PL	0.01	0.02	0.05	0.17	0.02
JAPANESE SPITZ					
Left PL	0.02	0.02	0.24	0.08	0.05
Light PL	0.01	0.04	0.19	0.05	0.04
Mean PL	0.01	0.02	0.17	0.06	0.04
Mean HD	0.27	-	1.19	0.18	0.09

5.2 Genetic correlations

The genetic correlations between left and right sided PL scores ($r_{left,right}$) were calculated. When r_g = -1 the selection to increase one trait leads to same amount of decrease in the other trait. Whereas, if r_g = 1 selecting one trait leads to a similar genetic change in the other trait and the two traits are therefore outcomes of the same genes. The following formula was used:

$$r_g = \frac{\sigma_{gLR}}{\sigma_{gL} \cdot \sigma_{gR}} \qquad (3)$$

Where

σ_{gLR} = genetic covariance of left and right PL scores

σ_{gL} = standard deviation of left PL scores (additive genetic)

σ_{gR} = standard deviation of right PL scores (additive genetic)

Table 21. Genetic correlation (r_g), standard error (S.E.) and phenotypic correlation (r_p) between left and right sided PL.

Breed	$\mathbf{r_g}$	**S.E.**	$\mathbf{r_p}$
Chihuahua	1.00	0.02	0.57
Pomeranian	1.00	0.88	0.62
Finnish Spitz	1.00	0.08	0.56
Japanese Spitz	-	-	-

The genetic correlations, the standard errors and the phenotypic correlations are given in Table 21. The results for the Japanese Spitz could not be obtained because of convergence problems. As the genetic correlation in the Chihuahua, the Pomeranian and the Finnish Spitz is 1, the left and right patellae health is most likely one trait with a common genetic background. The observation that bilateral PL is more common than unilateral PL supports this conclusion.

The genetic correlations for mean HD and mean PL scores in the Japanese Spitz was calculated using formula (4). A low negative genetic correlation of -0.05 (±0.46) between PL and HD was observed. The phenotypic correlation was 0.20

$$r_g = \frac{\sigma_{gHD,gPL}}{\sigma_{gHD} \cdot \sigma_{gPL}} \qquad (4)$$

Where

$\sigma_{gHD,gPL}$ = genetic covariance of mean HD and PL scores

σ_{gHD} = standard deviation of mean HD scores (additive genetic)

σ_{gPL} = standard deviation of mean PL scores (additive genetic)

5.3 EBVs and genetic trends

An estimated breeding value (EBV) was obtained for animal that were included in variance component estimation (both animals with observations and their ancestors). In order to control the reliability of EBVs only the animals that had their stifles checked were chosen for further inspection. As the phenotype is graded from 0 to 4, the smaller the EBV corresponds to better patellae health. The minimum, median, mean, standard deviation and maximum value of EBVs in each breed are given in Table 22.

Table 22. Descriptive parameters of EBV of PL based on animals with own observations.

	Min.	**Median**	**Mean**	**Standard deviation**	**Max.**
Chihuahua	-0.39	-0.08	-0.06	0.14	0.76
Pomeranian	-0.08	-0.01	-0.00	0.03	0.15
Finnish Spitz	-0.09	-0.02	-0.01	0.04	0.35
Japanese Spitz	-0.06	0.02	0.02	0.04	0.21

To visualize the genetic trend in patellae health, the mean EBVs were calculated by their birth years. At the chronological beginning of the data there were only a few observations per year (Table 7) which caused vast variation to the age group’s mean EBV scores. For this reason, the years where the number of observations was <10, were excluded. Figures 7, 8, 9, and 10 show genetic trends for the Chihuahua, the Pomeranian, the Finnish Spitz, and the Japanese Spitz, respectively. The trend is beneficial in the Chihuahua and Japanese Spitz. The genetic trend in the Finnish Spitz is close to neutral and in the Pomeranian the development is going towards worse patellae health.

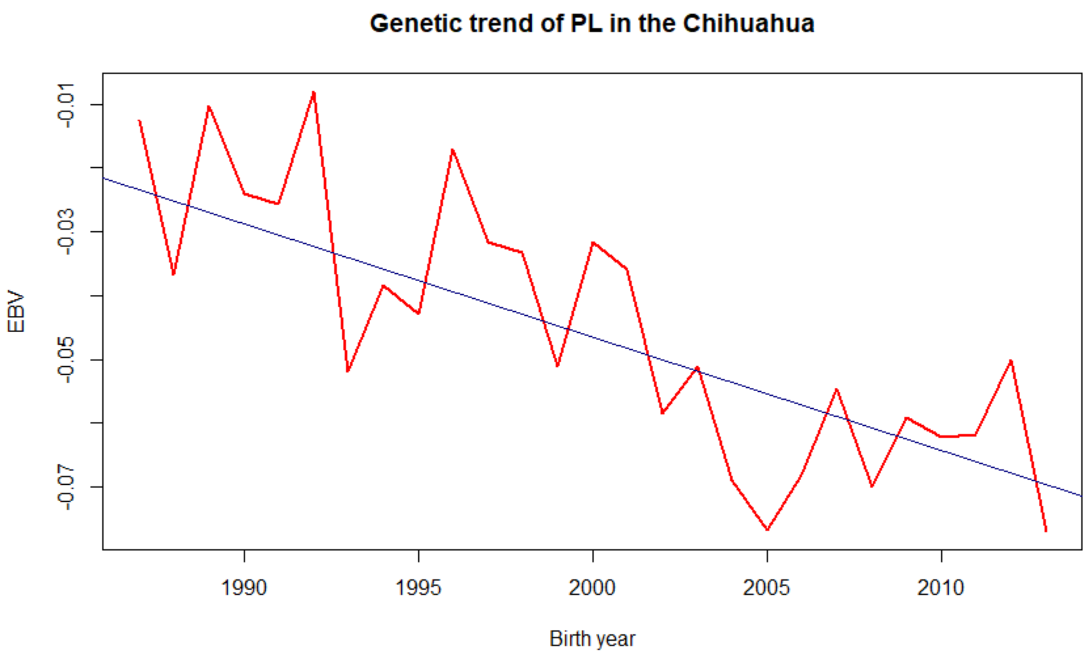

Figure 7. The genetic trend of PL in the Chihuahua.

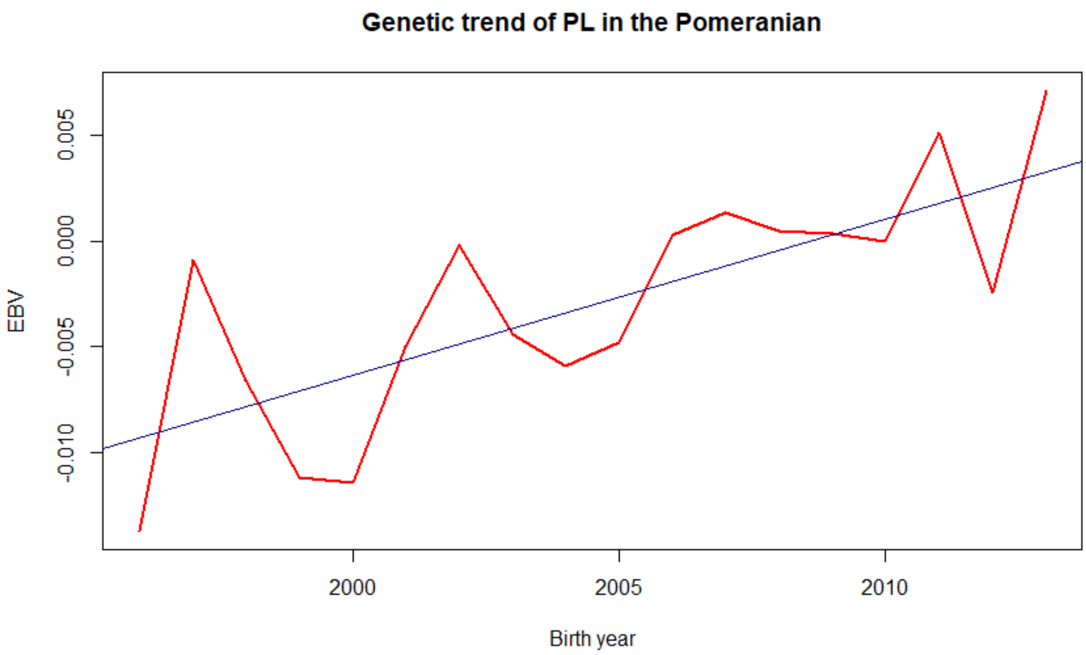

Figure 8. The genetic trend of PL in the Pomeranian.

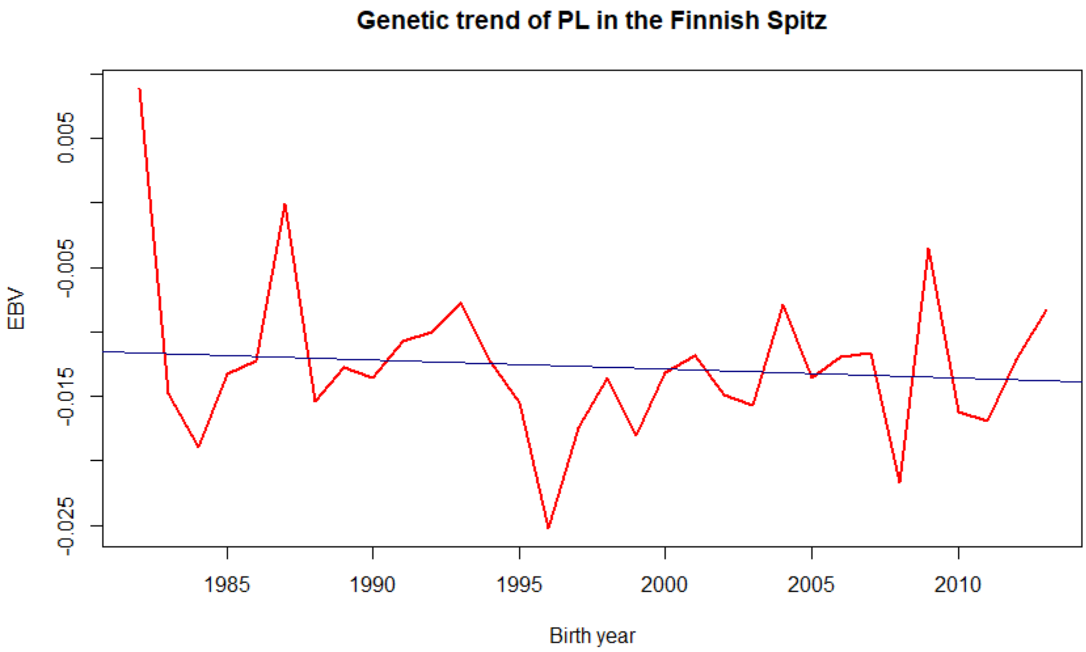

Figure 9. The genetic trend of PL in the Finnish Spitz.

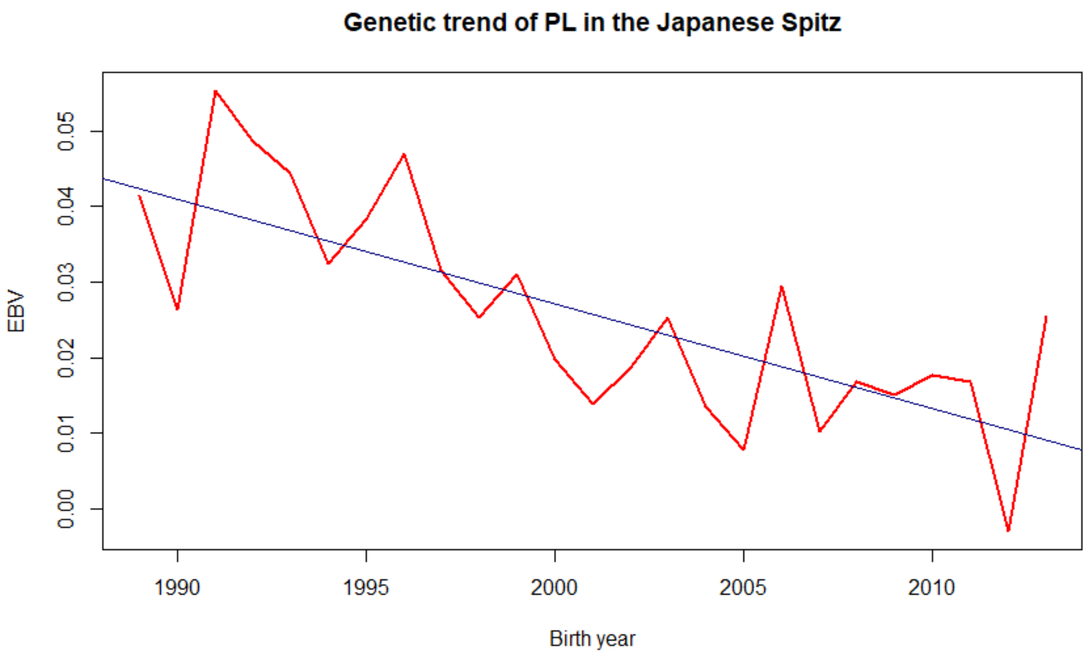

Figure 10. The genetic trend of PL in the Japanese Spitz.

5.4 Effect of sex

On average the males had better EBVs of patella health than females in all studied breeds. However, only in the Pomeranian the difference was statistically significant. Also, in the phenotypic data it was seen that a larger proportion of females were affected with PL than in males (Table 6). The effect of the sex to the mean PL score of left and right stifle are given in the Table 23.

Table 23. The effect of sex.

Breed	Gender	n	BLUE	S.E.	t	P-value
Chihuahua	Male	1923	-0.05	0.02	-0.49	0.35
	Female	3876	0.00	0.00		
Pomeranian	Male	272	0.56	0.09	4.44	0.00
	Female	507	0.96	0.09		
Finnish Spitz	Male	1213	0.10	0.03	0.67	0.32
	Female	2065	0.12	0.03		
Japanese Spitz	Male	366	0.23	0.10	0.8	0.29
	Female	508	0.31	0.10		

6 DISCUSSION

In a breeding program one must set breeding goals, collect phenotypic information, estimate breeding values, disseminate the genetic gain and finally follow up and evaluate the progress (Oldenbroek and van der Waaij 2014, p. 41-43). For that reason, it is important to know the heritabilities of the traits that are under selection. In general, the breeding of dogs differs a great deal from production animal breeding practices. While the maintenance of production animals' breeding programs is centralized to only a few breeding organizations or companies, the responsibility of dog breeding is spread to many individual breeders. In Finland, the national breed clubs collect health, mental, and trial information on the breed and based on this data make breeding recommendations and even regulations. However, individual breeders have great freedom of interpreting the breed standard and putting their own view of ideal dog into action. A common force affecting the selection of stud dogs and development of a breed is success in dog conformation shows. Conformation titles can be valued higher than patellae health. Moreover, the economic value of different traits is hard or impossible to define for dogs, thus there are no financial pressure to develop certain traits in dogs, at least not to same extent as in production animal breeding.

Putnam's PL scoring protocol was introduced to the kennel community in the 1960's. First PL observations date back to the late 1970's in the data of this study. By now, there is a vast awareness of patellar health problems among pedigree dog breeders. One could think that tens of years of patellae examinations and increased knowledge would have led to improved patellae health even despite the lacking PL score regulation. Yet, only in the Chihuahua and the Japanese Spitz a positive genetic trend could be seen (Figure 7 and 10). The Pomeranian's genetic trend seems to go up which means that the patellae health is degenerating (Figure 8). The Pomeranian is a tiny dog with a profuse coat which might make it the most challenging breed to score for PL. In the Finnish Spitz the genetic trend seems rather neutral (Figure 9). As in the Finnish Spitz's breeding practices it is allowed to mate a dog once without a patellae check, this result can be considered decent.

This study's heritability estimates are in line with the previous results of other similar studies (Tables 1 and 20). PL is a complex disorder in which the environmental factors have a great impact. One possible source of bias in PL scores is the evaluating veterinarian. One could speculate that the differences in heritability estimates between left and right patellae (Table 20) could be explained by sidedness of veterinarians (i.e. either right- or left handed) since the genetic correlation estimates (Table 21) suggest that the same genes are involved. In Finland, all veterinarians graduate from the same university faculty. In order to be officially approved to evaluate patellae, the veterinary students need to attend an optional orthopaedics course. At this course, the students practice the scaling of a few live animals' patellae according to the Putnam's protocol. After these exercises all students are approved as official examiners. This practice might require re-evaluation. What is more, the importance of accurate phenotyping should be emphasized to students since a PL score of an animal plays a big role not just for the dog (owner) in question but for the whole breed. Another approach could be to abandon the Putnam's scale and turn to X-ray images. Mortari et al., (2009) and Marttinen (2016) got promising results for grading PL from various angulations. The downside of X-ray pictures for some dog owners though is the price and the need for anaesthesia. Also, a new protocol should be carefully studied, validated, and established first.

For some breeds the obtained heritabilities were low. This could be due to genetic background of the trait (genetic variation is small in the breed) or it could be due to phenotyping problems. In addition, the applied statistical model may not encounter all systematic factors that may have an effect on PL scores. Phenotyping could be improved by decreasing the number of the scoring veterinarians and collecting more data from more animals. In addition, as described on chapter 4.2, the LPL and MPL scores were combined into one PL score. Afterwards it seems that MPL and LPL should have kept apart since it is possible that they are genetically two different traits. As soon as these problems are tackled, the selection should be based on breeding indexes. This is what also Wangdee et al. (2014) and Lavrijsen et al. (2013) recommended based on their work and results. Currently the selection is based on phenotypes. It is well known that this practice is not the best for traits with a low heritability (Oldenbroek and van der Waaij 2014, p. 162-163). Also, PL is a developmental disorder that might show symptoms only after a dog is already used for breeding.

In earlier studies, there has been a discussion on whether or not sex has an effect on the risk of getting PL. In this study, the effect of sex was significant for all breeds (Tables

14-17) in the phenotypic data. The estimated effect of the sex to EBVs are given in the Table 23. The BLUE values in each breed indicates that females have slightly worse patellae health than males that is in line with the earlier studies (Priester 1972, Bound et al. 2009). Some breeders consciously avoid taking their female dogs to patellae check during the dogs' estrus (personal communications with dog breeders). They have an idea that a dog in heat tends to get worse patella scores. To the author's knowledge, this matter has not been studied in dogs. However, the biochemical connection between female hormones and tendon laxity has indeed been studied and shown both in humans and in rats (Lee et al. 2013, Dehghan et al. 2015 and Leblanc et al. 2017). This suggests that there could be a connection between worse PL scores and estrus in dogs as well. Yet, further studies are needed to make practical conclusions on the matter.

There is no doubt that PL is a serious welfare problem for several dog breeds and as a consequence a burden for the dog owners. PL is a problem that could be solved with sophisticated breeding plans. Before action some challenges remain: how to make the diagnostics more accurate, reliable and objective? How to accomplish this without the expenses rising beyond the tolerance limit of dog owners? The more observation data there is, the higher the rating reliability gets. Therefore, not only stud dogs ought to be screened but also the ordinary family dogs. This should indeed be emphasized to all puppy owners. There is also a concern that clinically affected, worst cases do not end up in the breeding database. An unofficial survey for the Pomeranian owners in spring 2019 suggested that surgical PL operations seem more common than the breeding database indicates (unpublished data). Perhaps breed clubs could more actively try to spread the knowledge about genetic health issues and the value of comprehensive breeding databases.

7 CONCLUSIONS

In this study, the heritability of PL was studied in four dog breeds: the Chihuahua, the Pomeranian, the Finnish Spitz and the Japanese Spitz. To the author's knowledge there has not been similar studies made in the Finnish Spitz and the Japanese Spitz before. The results revealed new information on the additive genetic component and the genetic trend of PL. Based on the results, a new approach to breeding against PL and a development of a better phenotyping protocol is recommended. The author concluded that decades of PL examinations have not solely led to improved patellae health in the studied breeds and since PL is a complex trait, the breeding selection should not be based on phenotypes. The author would find it beneficial if the diagnostics of PL could be improved in the future and if the breeding indexes would be introduced to the breeding practices to make the selection more accurate and efficient.

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Figure 4: Finnish Spitz ©Päivi Ruotanen

Figure 5: Japanese Spitz ©Pekka Uimari

Figure 6: Distribution of the mean HD scores.

Figure 7: The genetic trend of PL in the Chihuahua.

Figure 8: The genetic trend of PL in the Pomeranian.

Figure 9: The genetic trend of PL in the Finnish Spitz.

Figure 10: The genetic trend of PL in the Japanese Spitz.