ORIGINAL ARTICLE

GRAIN: a computer program to calculate ancestral and partial inbreeding coefficients using a gene dropping approach

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Summary

GRain is freely available software intended to enable and promote testing of hypotheses with respect to purging and heterogeneity of inbreeding depression. The program is based on a stochastic approach, the gene dropping method, and calculates various coefficients from large and complex pedigrees. GRain calculates, together with the 'classical' inbreeding coefficient, ancestral inbreeding coefficients proposed by Ballou, (1997) *J. Hered.*, **88**, 169 and Kalinowski *et al.*, (2000) *Conserv. Biol.*, **14**, 1375 as well as an ancestral history coefficient (A_{HC}), defined here for the first time. A_{HC} is defined as the number that tells how many times during pedigree segregation (gene dropping) a randomly taken allele has been in IBD status. Furthermore, GRain enables testing of heterogeneity and/or purging of inbreeding depression with respect to different founders/ancestors by calculating partial coefficients for all previously obtained coefficients.

Introduction

In genetically small populations, the mating of closely related individuals is unavoidable and results in inbred offspring. It is well documented that inbred offspring often are affected by inbreeding depression which is the reduction in performance per unit increase in inbreeding coefficient (Wright 1977; Hedrick & Kalinowski 2000; Leroy 2014). The magnitude of inbreeding depression often poses a considerable threat to the survival of inbred populations (Frankham *et al.* 2001). While conservation geneticists are looking for efficient strategies to overcome negative consequences of inbreeding (de Cara *et al.* 2013), one might question how realistic it is to hope that such strategies will be found at all. We believe that research focused on purging of inbreeding depression and heterogeneity of inbreeding depression is a promising position to start from.

There is empirical evidence indicating reduction or loss of negative consequences of inbreeding depression (Darwin 1876; Visscher *et al.* 2001). The current theoretical explanation, phrased 'purging the genetic load', is that negative effects of inbreeding depression can be purged if inbreeding is combined with selection (Crow 1970). Testing hypotheses focused on purging of inbreeding depression in animal populations were often based on the ancestral inbreeding concept. Ballou (1997) proposed an ancestral inbreeding coefficient as a measure of ancestral inbreeding and used it as a tool for evaluating the purging of deleterious alleles through inbreeding in 25 captive populations. He defined the ancestral inbreeding coefficient as 'the cumulative proportion of an individual's genome that has been previously exposed to inbreeding in its ancestors'. The idea behind this approach is as follows: if deleterious alleles contributing to inbreeding depression are effectively removed in ancestors by selection when in a homozygous state, then inbreeding depression would decline over time as ancestral inbreeding increases. A few years later, a slightly modified approach, breaking

inbreeding coefficients down into 'new' and 'old' inbreeding coefficients, was proposed by Kalinowski *et al.* (2000). Recently, statistical modelling (Boakes & Wang 2005) and the calculation method of the ancestral inbreeding coefficient (Suwanlee *et al.* 2007) were improved. Ballou's ancestral inbreeding concept was often cited, but less frequently tested with the exception of a few studies performed in laboratory (Lacy & Ballou 1998; Swindell & Bouzat 2006) and domestic (Curik *et al.* 2000; Köck *et al.* 2009; McParland *et al.* 2009; Hinrich *et al.* 2014) populations.

Heterogeneity of inbreeding depression has been observed, not only among species and among populations within species (e.g. Frankel & Soulé 1981; Thornhill 1993), but also within populations (e.g. Hohenboken *et al.* 1991; Pray & Goodnight 1995). Over the recent decades, research focused on heterogeneity of inbreeding depression within populations has been approached through the partial inbreeding concept. Therefore, each individual inbreeding coefficient has been further broken down with respect to the founders contributing to the individual's inbreeding level. (Here, founders are defined as unrelated individuals with unknown parents.). Using this concept for the first time, in an experimental study with the beach mouse *Peromyscus polionotus*, Lacy *et al.* (1996) demonstrated that the genetic load of deleterious alleles was unequally partitioned among founder pairs as well as that different founders contributed to the load affecting different fitness components. The concept of partial inbreeding has also been applied in the field of quantitative genetics of livestock populations. Rodrigáñez *et al.* (1998) found evidence of variation among founder lineages in inbreeding depression in Large White pigs. They also found that the alleles contributing to inbreeding depression descended from specific founder lineages. Further, a study with Jersey cattle indicated that a homogeneous effect of inbreeding on production may be an incorrect statistical specification in genetic evaluation models attempting to account for inbreeding depression (Gulisija *et al.* 2006). In addition, based on partial inbreeding coefficient concept, Gulisija & Crow (2007) have provided an alternative method how to estimate detrimental load in a well-recorded pedigree population.

Many of the ideas and approaches linked to partial inbreeding coefficients have been pioneered by the work of John James on spread of genes and contributions of individual ancestors. James & McBride (1958) traced the percentages of genes contributed by founders in a closed poultry flock from pedigree information. James (1962a) showed that the variance of percentages of genes from male ancestors was much larger in a selected compared to a control flock. James (1962b) derived equations for the sampling variances of the proportions of genes of sires and dams from the matings of their progeny. An efficient way of calculating genetic contributions from pedigrees was presented by James (1972), and a FORTRAN program (called CØNTRIB) implementing this strategy was published by Miller & Rathie (1972) in a companion paper. More recently, Nicola Man, John James and Frank Nicholas explored partial inbreeding in Australian Holstein Friesian cattle (Man *et al.* 2001, 2002) and found that inbreeding depression on somatic cell count as well as milk, fat and protein production varies with respect to founders. The authors also employed the partial inbreeding concept to the descriptive epidemiology and management of recessive disorders (Man *et al.* 2007).

For the calculation of partial inbreeding coefficients, there are currently only a few programs publicly available, among them PARTINBR (Lacy & Pollak 2014), ENDOG (Gutiérrez & Goyache 2005) and PYPEDAL (Cole 2007). PYPEDAL is, to our knowledge, the only public software that provides the capacity of calculating Ballou's ancestral inbreeding coefficients. In this study, we describe freely available software that uses the gene dropping method to calculate a number of ancestral and partial inbreeding coefficients. An early version of the GRAIN software is implemented in PEDIG2007 (Boichard 2007) and has been already used in several papers (Köck *et al.* 2009; McParland *et al.* 2009; Nagy *et al.* 2010; Hinrich *et al.* 2014).

Methods

Formal definition of coefficients

The following coefficients can be calculated with GRAIN:

(i) Classical inbreeding coefficient (f) defined as the probability that the two alleles at any locus in an individual are identical by descent (IBD).

(ii) The ancestral inbreeding coefficient (f_{a_BAL}) as originally defined according to Ballou (1997). Alternatively, f_{a_BAL} can be defined as the probability that any allele in an individual has been autozygous (IBD) in previous generations at least once. In his definition, Ballou gives the following iterative formula:

$$f_{a\_BAL} = [f_{a(s)} + (1 - f_{a(s)})f_s + f_{a(d)} + (1 - f_{a(d)})f_d]/2,$$

where f_{a_BAL} is the ancestral inbreeding coefficient for an individual and f is the inbreeding coefficient with subscripts s and d representing these values for the sire and dam, respectively. In his formula, f_{a_BAL} and f are

assumed to be independent. If this is not the case, f_{a_BAL} would be overestimated. Here, we propose a stochastic approach for calculating f_{a_BAL}, as Suwanlee *et al.* (2007) showed that the gene dropping method performed better because f_{a_BAL} and f are dependent.

(iii) The ancestral inbreeding coefficient (f_{a_KAL}) as defined according to Kalinowski *et al.* (2000). The classical inbreeding coefficient f is split into two parts, alleles which had undergone inbreeding in the past ('old' i.e. f_{a_KAL}) and alleles IBD for the first time ('new' inbreeding coefficient, $f_{\_new}$). Thus, f_{a_KAL} represents the part of the genome where alleles are currently in IBD status and have also been IBD in an ancestor of the animal at least once. Therefore, Kalinowski *et al.* (2000) define ancestral inbreeding in a less broad sense than Ballou (1997). Alternatively, f_{a_KAL} can be defined as the probability that any allele in an individual is currently autozygous and has been autozygous (IBD) in previous generations at least once.

(iv) The ancestral history coefficient (A_{HC}). A_{HC} is defined and presented here for the first time. The concept behind A_{HC} is that alleles which have experienced inbreeding more often in the past are less likely to be deleterious than alleles which have undergone IBD less often. The proposed coefficient takes into account how often an allele in an individual's genome was exposed to IBD in the past and is defined as the number that tells how many times during pedigree segregation (gene dropping) a randomly taken allele has been in IBD status. This differs from Ballou's and Kalinowski *et al.*'s approach, where per definition an allele contributes to ancestral inbreeding if it has undergone IBD at least once. A_{HC} is an appropriate measure of ancestral inbreeding if selection against deleterious recessive alleles is less than fully efficient, so the probability of purging increases with the number of times alleles are homozygous during dropping (segregation) through the pedigree.

(v) Partial inbreeding coefficients (pf). Generally, the partial inbreeding coefficient is the probability that an individual is autozygous (IBD) for an allele descended from a specified founder. The sum across all founders of the partial inbreeding coefficients for a descendant is equal to the corresponding 'total' inbreeding coefficient for that individual (Lacy *et al.* 1996; Lacy 1997).

(vi) Partial ancestral inbreeding coefficient (pf_{a_BAL}). Here, we extended Ballou's definition and defined pf_{a_BAL} as the probability that any allele in an individual descended from a specified founder and has been autozygous (IBD) in previous generations at least once.

(vii) Partial ancestral inbreeding coefficient (pf_{a_KAL}) derived from f_{a_KAL}. By extending the definition of f_{a_KAL}, we defined pf_{a_KAL} as the probability that any allele in an individual descended from a specified founder is currently autozygous and has been autozygous (IBD) in previous generations, at least once.

(viii) Partial ancestral history coefficient (pA_{HC}). Following previous definitions, pA_{HC} quantifies how many times in previous generations any allele in an individual descended from a specified founder has been autozygous (IBD).

Computational strategy

The program is named GRAIN (acronym) because the calculations of all coefficients mentioned are based on the gene dropping approach, like 'Genetic Rain' when a large number of drops are performed. This approach is based on the idea of gene flow through a pedigree, first introduced by Edwards (1968) and further developed by MacCluer *et al.* (1986). The procedure of gene dropping in GRAIN involves assigning two unique alleles to each founder and generating the genotypes of all descendants along the actual pedigree following Mendelian segregation rules. For each half-founder, that is an animal with just one parent known, a dummy founder is created and the unknown second parent is assigned an artificially created new identification number and also provided with two unique alleles. The gene dropping process is repeated many times. The proportion of IBD loci out of all loci in an individual genome is considered as its inbreeding coefficient (f). Therefore, GRAIN calculates the 'classical' inbreeding coefficient as the total number of IBD replications divided by the total number of replications of dropping.

The main reason for using a stochastic approach instead of a deterministic one lies in the calculation of f_{a_BAL}, where gene dropping provides correct values (Suwanlee *et al.* 2007). To adapt the approach to the calculation of ancestral inbreeding coefficients, one needs to keep track of IBD events in the pedigree of an individual. This is performed by flagging alleles once they are in IBD state for the first time and keeping this information over the replications of the dropping process. This allows simulating a genome for each animal where the number of replications refers to the number of biallelic unlinked loci. The proportion of already flagged alleles out of all alleles in an individual genome is considered as its f_{a_BAL}. GRAIN calculates f_{a_BAL} as the total number of flagged alleles divided by the total number of alleles in the genome

(two times the number of replications of the gene dropping process).

The proportion of alleles actually IBD and already flagged due to past IBD events out of all alleles in an individual's genome is considered its f_{a_KAL}. GRAIN calculates f_{a_KAL} as the total number of flagged alleles which are currently IBD, divided by two times the total number of replications of the gene dropping process. For the A_{HC}, the number of IBD events for each allele in an animal's ancestors is summed over all replications of the gene dropping process. This sum is divided by the number of all alleles in an individual's genome (two times the number of replications). Per definition, values for f_{a_BAL} and f_{a_KAL} must lie between 0 and 1 and can be interpreted as probabilities. This does not hold for A_{HC}, which can exceed 1.

Partial inbreeding coefficients can be determined using a modification of the tabular method for calculating inbreeding coefficients from the additive relationship or kinship matrices, an approach used in the PARTINBR program (Lacy 2003), ENDOG v4.8 (Gutiérrez & Goyache 2005) and PYPEDAL (Cole 2007). As GRAIN is based on the stochastic gene dropping approach, it calculates partial inbreeding coefficients just by taking the allele's origin from each founder into account. Therefore, for each animal, there are as many partial inbreeding coefficients as founders found in the pedigree we define. Partial coefficients pf, pf_{a_BAL}, pf_{a_KAL} and pA_{HC} are calculated for f, f_{a_BAL}, f_{a_KAL} and A_{HC}, respectively.

More about implementation and usage of the coefficients presented can be seen in papers that were based on the previous version of GRAIN (Köck *et al.* 2009; McParland *et al.* 2009; Nagy *et al.* 2010; Hinrich *et al.* 2014).

Running the program

Setting up a run

GRAIN is a Fortran90 program, for which an R extension is available. It executes the compiled executable file in a user friendly way and creates additional graphical output. The R function with all function arguments is the following:

GRain(execName="GRain.exe", pedFile="...", option="...", ancestorFile="...", nAncestors="...", nDrops="...", yearCol=0, UNIXlike=F),

where

execName: The name of the compiled executable file. The default value is set to GRain.exe, with possibility to adapt.

pedFile: The name of the initial pedigree file. It should be an ASCII text file, one animal per line with three columns for each line: animal, sire and dam code. Any extra columns are ignored when calculating inbreeding coefficients. This file should contain the birth years of individuals, if the user requires the visualization of annual inbreeding trends (see *yearCol*). Animal's identity should be coded. Identity codes of all pedigree members should be ordered sequentially from 1 to n without interruption, while the identity code of parents should be lower than in their offspring; otherwise, erroneous results will be obtained. For founder individuals, sires and dams are denoted with zero. Users are recommended to prepare the original pedigree data by the program PED_UTIL included in the PEDIG software package (Boichard 2002). An example of the pedFile, for the pedigree illustrated in Figure 1 is presented in Figure S1A.

option: The user might request the calculation of total inbreeding coefficients ('totalOnly'), or the total and partial inbreeding coefficients according to Ballou ('faBallouAndPartial'), Kalinowski ('faKalinowskiAndPartial'), as well as the classical inbreeding ('fAndPartial') and ancestral history ('AHCAndPartial') coefficients. The option 'all' runs all total and partial inbreeding computations.

ancestorFile: The name of the file containing the list of ancestors for calculation of partial inbreeding coefficients. This file should contain only one column
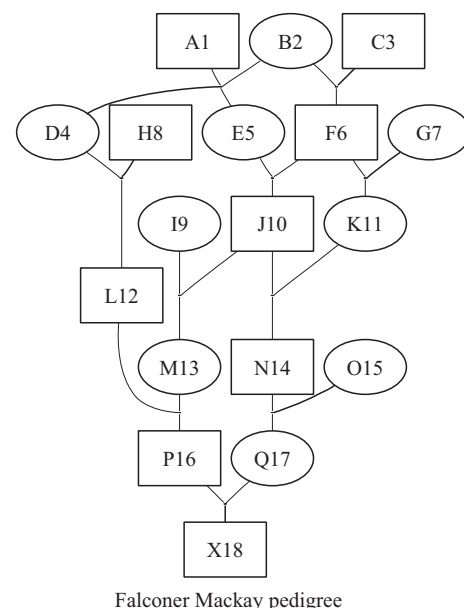
Falconer Mackay pedigree

Figure 1 Sample pedigree taken from Falconer & Mackay (1996).

with the sequentially ordered animal codes. See Figure S1C for calculation of partial inbreeding coefficients for the first four founders.

nAncestors: The number of ancestors to be used for calculating partial inbreeding coefficients. If there is no *ancestorFile* specified, the first *nAncestors* animals are taken to calculate partial inbreeding coefficients from the *pedFile*.

nDrops: The number of repetitions for gene drops in the simulation. A substantial number of gene drops are required, as explained in the accuracy and speed section.

yearCol: The column number for the birth year in *pedFile* should be provided here if inbreeding trends are requested.

UNIXlike: The R extension works in Windows machines by default. The *UNIXlike=T* function argument allows to run the extension on non-Windows computers.

Out of these function arguments, *pedFile, option* and *nDrops* are mandatory. The function argument *nAncestors* is mandatory when partial inbreeding coefficients are requested, otherwise ignored. If the function arguments *execName, yearCol* and *UNIXlike* are ignored, their default values will be used, as presented above.

The prerequisite to run the R extension is to import it via the source() function and specify the directory containing the data, the GRain.r interface script and the compiled GRain executable file using the setwd() function. After that, a typical run to compute all total and partial inbreeding coefficients is the following:

source("GRain4R.r")

setwd("Absolute_or_relative_PATH_to_your_working_directory_here")

GRain(pedFile="Fig 1_Ped.txt",option="all",nAncestors="7",nDrops="1000000")

Output files

Up to five outputs text files and graphical illustrations will be produced.

In general, the first three columns in all output files contain the codes for individual, sire and dam. Please note that the output files might contain more animals than the original pedigree input file due to dummy founders required for allele assignment as discussed in the subsection 'Calculation strategy'. An example of the output file (tot_Fig 1_ped.txt) is shown in Figure S1B.
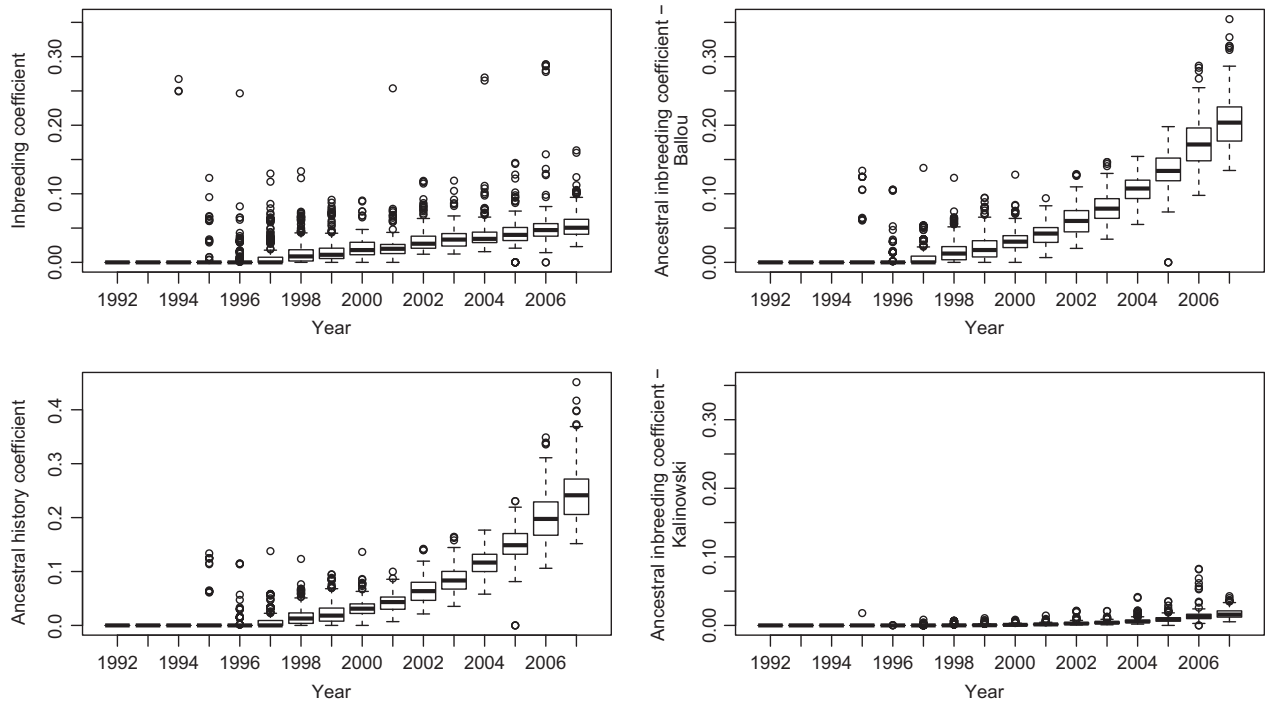
Figure 2 Demonstration of inbreeding and ancestral inbreeding trends (box plots) based on a Pannon White rabbit population data set (Nagy *et al.* 2010).

When requested via the parameter *option*, GRAIN provides other output files with the following:

(i) *f* and the corresponding partial inbreeding coefficients (file with prefix 'F_'),

(ii) f_{a_BAL} with corresponding partial coefficients (file with prefix 'Fa_BAL_'),

(iii) f_{a_KAL} with corresponding partial coefficients (file with prefix 'fa_KAL_'),

(iv) A_{HC} with their corresponding partial coefficients (file with prefix 'AHC_').

In these files, the total coefficients together with a number of corresponding partial coefficients are recorded. An example for an output file ('Fa_bal_Fig 1_ped.txt') presenting f_{a_BAL} is shown in Figure S1C. General information on the computation process is provided in the 'Summary' file.

A box plots illustrating trends for all four calculated coefficients, if birth years are provided, are presented in Figure 2 for the Pannon White rabbit data set analysed in Nagy *et al.* (2010). Those are analytical graphs intended to enable first insight into inbreeding and ancestral inbreeding trends of the population under analysis.

Accuracy and speed

The performance and results obtained by GRAIN have been tested and checked for consistency using alternative software when available and/or manually using conventional formulas. GRAIN performance has also been tested on several pedigrees of three different sizes: (i) small pedigrees (pedigrees presented in Figures 1 and 3); (ii) medium pedigrees (synthetic Pannon rabbit population and Large White pig pedigree with 7271 and 65 784 animals, respectively), such pedigrees are characteristic for zoo and endangered domestic animal populations; and (iii) large pedigrees (Austrian Brown Swiss cattle pedigree with 2 124 145 animals). Pedigrees that are extremely large in size are characteristic of intensively used domestic animals (pig and cattle populations), often handled by national breeding organizations.

Because GRAIN is based on a stochastic process (gene dropping), its accuracy depends proportionally on the number of replications (gene drops) performed. To analyse the accuracy of GRAIN, the program was run 100 times for the complex pedigree in Figure 3 and the results are compared using 10^4, 10^5 and 10^6 replications. In addition, the program is run 10 times on this same pedigree using 10^9 replications for 'classical' and partial inbreeding coefficients, and the results are compared with

ATP45i
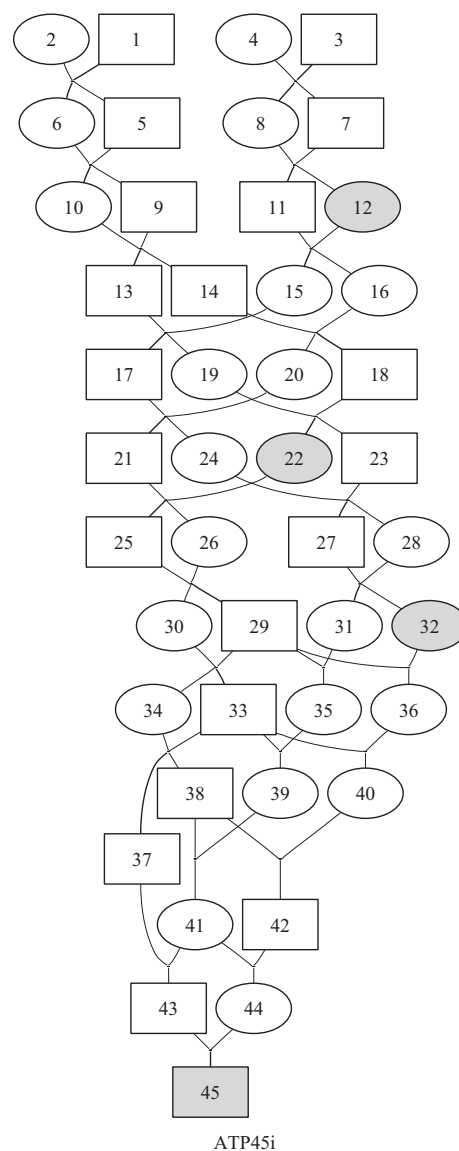
Figure 3 Complex sample pedigree artificially created for testing the accuracy of the GRain program.

values obtained using a deterministic approach, implemented in PARTINBR software. No difference could be observed between deterministically obtained results and those coming from runs with 10^9 replications, to a precision of four decimal places. Thus, it was assumed that runs with 10^9 replications provide adequate reference results, even for testing the accuracy of coefficients that can only be calculated stochastically. The comparison of values for f, f_{a_BAL}, f_{a_KAL} and A_{HC} obtained with different numbers of replications (10^4, 10^5, 10^6, and 10^9) is presented in Table 1 for four individuals (the animals shaded in Figure 3). Very similar

Table 1 Accuracy of calculated coefficients tested on the four shaded pedigree members in Figure 3 where we analysed distribution of differences from deterministic values. Medians and upper 95th percentile ($T_{95\%}$) for the absolute differences (Δ) between values obtained by 10^4, 10^5 or 10^6 gene drops and the particular reference values (10^9 gene drops) are calculated over 100 runs. Reference values are shown in italics

| | Individuals | | | | | | | |
| | 12 | | 22 | | 32 | | 45 | |
Source	Median	$T_{95\%}$	Median	$T_{95\%}$	Median	$T_{95\%}$	Median	$T_{95\%}$
f (10^9)	*0.2500*		*0.2500*		*0.4609*		*0.6887*	
Δf (10^4)	0.0024	0.0071	0.0029	0.0089	0.0034	0.0089	0.0036	0.0087
Δf (10^5)	0.0010	0.0029	0.0011	0.0026	0.0013	0.0037	0.0010	0.0032
Δf (10^6)	0.0003	0.0008	0.0004	0.0011	0.0003	0.0009	0.0003	0.0009
fa_{BAL} (10^9)	*0.0000*		*0.5000*		*0.6670*		*0.9394*	
Δfa_{BAL} (10^4)	–	–	0.0026	0.0078	0.0022	0.0062	0.0011	0.0033
Δfa_{BAL} (10^5)	–	–	0.0007	0.0025	0.0008	0.0027	0.0004	0.0011
Δfa_{BAL} (10^6)	–	–	0.0002	0.0008	0.0003	0.0007	0.0001	0.0004
fa_{KAL} (10^9)	*0.0000*		*0.1640*		*0.3611*		*0.6718*	
Δfa_{KAL} (10^4)	–	–	0.0025	0.0079	0.0028	0.0083	0.0031	0.0084
Δfa_{KAL} (10^5)	–	–	0.0008	0.0023	0.0011	0.0033	0.0010	0.0031
Δfa_{KAL} (10^6)	–	–	0.0003	0.0008	0.0003	0.0008	0.0003	0.0009
A_{HC} (10^9)	*0.0000*		*0.6250*		*1.1718*		*3.8312*	
ΔA_{HC} (10^4)	–	–	0.0034	0.0110	0.0068	0.0174	0.0152	0.0396
ΔA_{HC} (10^5)	–	–	0.0013	0.0037	0.0020	0.0063	0.0051	0.0122
ΔA_{HC} (10^6)	–	–	0.0003	0.0012	0.0006	0.0017	0.0014	0.0041

Inbreeding coefficients = f, Ballou's ancestral inbreeding coefficients = f_{a_BAL}, Kalinowski *et al.*'s ancestral inbreeding coefficients = f_{a_KAL} and ancestral history coefficients = A_{HC}.

results, not presented here, were obtained for partial coefficients. Depending on the computing capacity, the user can, by choosing the number of replications, 'control' the accuracy of the results. The time required for calculations of all coefficients on non-professional equipment was also tested, that is on a laptop computer (Compaq nx6310) with dual-core processors (x86 Family 6 Model 15 Stepping 6 Genuine Intel 1662 MHz) and 512 MB of total physical memory. The performance obtained is as follows: (i) for both pedigrees presented in Figures 1 and 3, calculations with 10^6 replications lasted <2 s and <10 s, respectively; (ii) for the Pannon White rabbit pedigree with 7271 animals, the calculation with 10^6 replications lasted 33 min and results were highly correlated with inbreeding coefficients obtained by the deterministic approach ($r = 0.9998$); (iii) for the Large White pig pedigree with 65 784 animals, the calculation with 10^6 replications lasted 637 min (10 h and 37 min); (iv) for the Austrian Brown Swiss cattle pedigree with 2 124 145 animals, the calculation with 10^5 replications lasted 1440 min (24 h). Computing time is linear in the number of replicates. We recommend users to run the program at low numbers of replications (1000 or 10 000) first and then, depending on computing time (stored in the summary.txt file)

and accuracy desired, repeat the analysis with a higher number of replications (from 10^5 to 10^9).

Calculation of partial coefficients for large numbers of founders/ancestors does increase computing time. Please note that computing time will be reduced drastically by providing more powerful computational environments, also because gene drops are independent and easily parallelized.

Availability and usage

GRAIN was written in Fortran90 and can be run standalone. The source code, compiled GRAIN program for Windows, source code for the R interface and example data files are available at http://www.nas.boku. ac.at/nuwi/software/. An R version, GRAIN4R, was produced above while the code is available at the same link. Please note that at the current state, the user needs to provide a complied version of the Fortran software in the R working directory.

An earlier version of GRAIN is also included in the PEDIG package (Boichard 2007).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 An example of the input file "Fig 1_ped.txt" (see Figure S1A), output file "tot_Fig 1_ped.txt" (see Figure S1B) and output file "Fa_bal_-Fig 1_ped.txt" (see Figure S1C) obtained for a small-size pedigree from Falconer & Mackay (1996).