

ORIGINAL ARTICLE

Estimation of heritability for Tying-up syndrome in the Thoroughbred racehorse by Gibbs sampling

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Summary

Tying-up is a condition that primarily affects the muscles of horses. In this study, the heritability of the Tying-up syndrome in the Thoroughbred racehorse was estimated by Bayesian analysis with Gibbs sampling based on the threshold model for binary traits. The data used were the clinical data in racehorses diagnosed by veterinarians of the Racehorse Clinics of Japan Racing Association from 2000 to 2003. The health status of the Tying-up was treated as a binary trait. In the genetic analysis, the effect of changing the amount of the pedigree or inbreeding information on the estimation of heritability was investigated, too. The heritability estimates with non-zero probability in the posterior densities were approximately 0.16–0.18 in minimum, suggesting that the heritability of the Tying-up is not zero at least. The posterior density distributions of the heritability estimates were generally more pointed and sharp with using inbreeding coefficients than without using it, suggesting that more stable estimations were obtained when inbreeding coefficients were used. Among the different amounts of pedigree and inbreeding information, the heritabilities obtained with three or four generations of pedigree using inbreeding coefficients seems to be preferable, i.e. heritability of 0.42 or 0.43 for Tying-up.

Introduction

Tying-up may all be the name used to describe variations in the clinical picture of the same syndrome as 'azoturia', 'exertional rhabdomyolysis', 'paralytic myoglobinuria', 'Monday morning disease' and 'exertional myopathy'. It is an intermittently occurring condition that primarily affects the muscles of horses, resulting in clinical signs ranging from slight stiffness to immobility (Harris 1989, 1991). Tying-up can develop rapidly and most often occur when a horse in hard work is given a rest day without having its working diet reduced. As Tying-up develops, the muscles of the horse over the loins and quarters harden resulting in cramps and muscular stiffness. The horse's stride becomes shorter, it staggers behind

and then goes lame and may collapse if the work is continued. In severe cases the myoglobin released from the damaged muscles turns the urine dark red. Exertional rhabdomyolysis in horses has been recognized for more than a century as a syndrome of muscle pain and cramping associated with exercise (McKenzie *et al.* 2003).

As clinicians commonly experienced familial occurrences of Tying-up, it has been suggested to involve with a genetic predisposition. By using a pedigree-phenotype association analysis, Valberg *et al.* (1996) suggested an autosomal recessive pattern of inheritance for polysaccharide storage myopathy and associated exertional rhabdomyolysis in Quarter Horses. MacLeay *et al.* (1999) provided some evidences for the inheritance of recurrent exertional

rhabdomyolysis (RER) in the Thoroughbred racehorses as an autosomal dominant mode with expression variations by the pedigree analysis of four sire families.

Tying-up is inferred to be a threshold trait, because the disease is diagnosed with two phenotypic categories, affected or unaffected. The clue for understanding the inheritance of such categorical character lies in the idea that the character has an underlying continuity (so called as liability) with thresholds (Falconer 1989). In the case of the binary character, only one threshold is assumed to distinguish two categories of the health status (Ibi *et al.* 2003). Ibi *et al.* (2003) reported that the heritability of Laryngeal Hemiplegia in the Thoroughbred horses was estimated by treating the disease as the threshold trait and by using the computer program for genetic analysis with Gibbs Sampling for Threshold Models (GSTM).

In this study, the Tying-up diagnose was considered to be the threshold trait, and the health status of the Tying-up was treated as the binary trait. The aim of this study was to clarify the heredity of Tying-up in the Thoroughbred racehorse by estimating the heritability with GSTM. The effect of changing the amount of the pedigree or inbreeding information on the estimation of heritability is also discussed.

Materials and methods

All the racehorses which are registered by Japan Racing Association (JRA) need to be accommodated and trained within the confines of either the Miho or Ritto Training Centre of JRA. Both the training centres have the latest racehorse clinic facilities (the Racehorse Clinics of JRA) staffed by JRA veterinarians, who are resident at the training centres. The racehorse clinics offer facilities and expertise for diagnosis and treatment of the disorders of the racehorses. In this study, we used the clinical data on racehorses diagnosed for Tying-up syndrome by veterinarians of the racehorse clinics.

For genetic analysis, 6538 individual racehorses registered twice by semiannual medical examinations (spring and autumn) of 2001 in the Miho and Ritto Training Centres were used. Among these horses, totally 501 individuals (7.7%) were diagnosed as Tying-up from 2000 to 2003 at the Ritto or Miho Training Centres. The record of the diagnosis was treated as a binary trait and classified under only two categories, i.e. 'normal' (classified as 0) and 'Tying-up' (classified as 1). Table 1 shows the num-

Table 1 The frequency of Tying-up and normal horses by sexes

Sex	Normal	Tying-up	Prevalence ratio (%)
Filly	2175	268	11.0
Colt	3666	221	5.7
Gelding	196	12	5.8
Total	6037	501	7.7

ber of individuals in this study by the status of diagnosis and sex. A total of 501 Tying-up horses consisted of 268 fillies (53.5%), 221 colts (44.1%) and 12 geldings (2.4%).

In order to estimate the heritability for the Tying-up syndrome, the pedigree of the 6538 horses was traced back to the ancestors with two, three and four generations. The inbreeding coefficient of each individual was estimated by Sato (2000). With the combination of the three pedigree structures and the two settings of inbreeding coefficients (i.e. using or not using), totally six datasets were used and the results were compared each other.

The six datasets were analysed by Bayesian analysis with Gibbs sampling based on the threshold model for binary traits, using the GSTM program (Ibi *et al.* 2003). A preliminary analysis with the SAS LOGISTIC procedure (SAS Institute Inc. 2000) revealed that the sex effect (filly, colt and gelding) was highly significant ($p < 0.0001$). Therefore, the sex effect was included in the mathematical model with GSTM. The equation for the threshold model was:

$$L_{ij} = \text{SEX}_i + u_j + e_{ij} \quad \text{and} \\ Y_{ij} = 0 \quad (L_{ij} \leq t) \quad \text{and} \quad Y_{ij} = 1 \quad (t < L_{ij}),$$

where L_{ij} is the liability of each animal, SEX_i is the effect of the i th sex, u_j is the breeding value of the j th individual [i.e. it follows normal distribution $N(\mathbf{0}, \mathbf{A}\sigma_u^2)$], e_{ij} is the residual effect [$N(\mathbf{0}, \mathbf{I}\sigma_e^2)$], Y_{ij} is the binary phenotype for the Tying-up (0 or 1), t is threshold, $\mathbf{A}$ is numerator relationship matrix (Henderson 1973), σ_u^2 is additive genetic variance, σ_e^2 is environmental variance. Then, heritability (h^2) is defined as

$$\frac{\sigma_u^2}{(\sigma_u^2 + \sigma_e^2)}.$$

The sex effect was treated as a random effect with $N(\mathbf{0}, \mathbf{I}\sigma_{\text{sex}}^2)$ following Hoeschelle & Tier (1995) and Moreno *et al.* (1997), where σ_{sex}^2 is the variance of sex.

In the Gibbs sampling scheme with GSTM, the total number of samplings was 10 000 000. In order to get the final random samples used for the construc-

tion of the posterior density, the optimal burn-in and the spacing value for the Gibbs chain were determined based on the method of Raftery & Lewis (1996). Then the mode value of the constructed posterior density was chosen as the estimate. In addition to the mode, the mean and median were also calculated.

Results and discussion

In order to carry out the genetic analysis of the Tying-up syndrome in the Thoroughbred racehorse, we used the data solely obtained from the semiannual medical examinations in spring and autumn of 2001 in JRA, considering this data as a sample of whole racehorse population in JRA. In Table 1, the prevalence ratio of the Tying-up individual in the data was 7.7%, and the prevalence ratio in fillies (11.0%) was slightly greater than those in colts (5.7%) and geldings (5.8%). The sex difference was significant ($p < 0.0001$) and therefore the sex was included in the model as an effect. MacLeay *et al.* (1999) also reported that in their study the prevalence ratio in female was larger than that in male. The reason for the sex difference in the prevalence ratio is still not clear, and further studies are awaited for this question.

In Table 2, the estimates of heritability are shown as the mean, mode and median of the final samples for the six different datasets. In general, very high heritability estimates (0.41–0.47) were obtained, even though minor differences were still recognized among the six datasets.

The posterior density distribution for the heritability estimates with and without using inbreeding coefficients are shown in Figures 1 and 2 respectively. In these figures, the heritability estimates with

non-zero probability in the posterior densities were approximately 0.16–0.18 in minimum, suggesting that the heritability of Tying-up is not zero at least.

The posterior density distributions of the heritability estimates were generally more pointed and sharp with using inbreeding coefficients than without using inbreeding coefficient. In other words, the peaks of the density were more clearly recognized in Figure 2. This suggests that using inbreeding coefficients allowed us to obtain better convergence in Gibbs sampling scheme, probably because of the full computation of **A** matrix in the model without approximation. In addition, the precision of the estimates would be improved, too.

In genetic analysis with mixed models, the variance components in the base population is generally estimated because of the property of **A** matrix (Sorensen & Kennedy 1984). Therefore, modifying the amount of pedigree information may change the estimate of heritability importantly. However, in this study as increasing the pedigree information from two to four generations, the heritability estimates were reduced merely slightly. Especially with using inbreeding coefficients (Figure 2), the peaks of the three densities were almost identical. Therefore, this suggests that the ancestors (i.e. animals in base population) in each dataset might have similar values for the variance components.

On the contrary, modifying the amount of pedigree information may affect the convergence of the Gibbs sampling. In general, the larger is the pedigree size, the more difficult is the convergence of Gibbs sampling. As shown in Table 2, the total numbers of individuals in the pedigree were 16 857, 20 848 and 24 386 for the datasets with two, three and four generations respectively. These figures generally require huge number of samples in Gibbs sampling

Table 2 Estimates of heritability, numbers of horses, sample sizes and inbreeding coefficient (F) with pedigree information traced back to 2, 3 and 4 generations for six datasets composed of combinations of the pedigree structures and the setting of F in the Thoroughbred racehorse

Item	Generations traced back to					
	2	2 with F^1	3	3 with F^1	4	4 with $4F^1$
Number of horses diagnosed	6538	6538	6538	6538	6538	6538
Total number of individual horses	16 857	16 857	20 848	20 848	24 386	24 386
Heritability estimates						
Mean	0.46	0.44	0.44	0.44	0.45	0.45
Mode	0.47	0.45	0.43	0.42	0.41	0.43
Median	0.46	0.44	0.44	0.43	0.45	0.44
Average inbreeding coefficient	0	0.0002	0	0.0014	0	0.0038
Final sample size ²	702	1203	1015	979	791	802

¹Inbreeding coefficients of individuals are used in the genetic analysis.

²Sample size of the Gibbs sampling used for the construction of posterior density distributions.

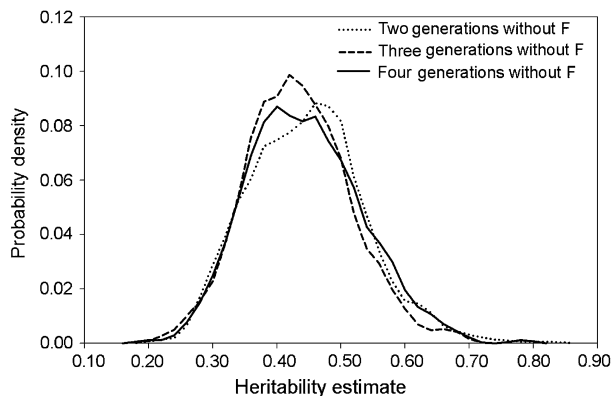


Figure 1 The posterior density distribution of the heritability estimates for the Tying-up by pedigree information traced back to 2, 3 and 4 generations without inbreeding coefficient (F) in the model in the Thoroughbred horse.

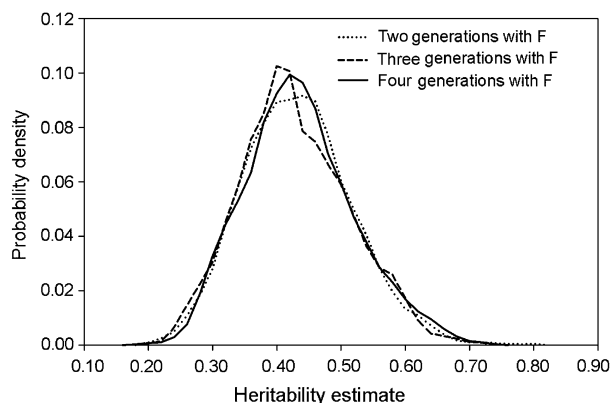


Figure 2 The posterior density distribution of the heritability estimates for the Tying-up by pedigree information traced back to 2, 3 and 4 generations with inbreeding coefficient (F) in the model in the Thoroughbred horse.

schemes in order to achieve good convergence of the samples. In our Gibbs sampling scheme, the single long Gibbs chain with the total number of 10 000 000 samples was obtained. This sample size was determined so that the number of final random samples was large enough for the construction of the posterior density distributions; i.e. author recommends approximately 500 final samples or more. Consequently, with this long chain the final sample sizes for each dataset were between 702 and 1203 (Table 2). These final sample sizes seem to be large enough for the construction of the density distributions, and therefore for the estimation of the heritability based on the density. When the total length of Gibbs chain is determined as this study, the larger is the pedigree size, the smaller is the final sample size.

Our result generally follows this rule except for the dataset with generation 2 without inbreeding coefficients, suggesting that the convergence in this dataset was most difficultly achieved in our six datasets.

Regarding the pedigree and inbreeding information as discussed above, it is suggested that the values of heritability obtained with three or four generations with using inbreeding coefficients are the most preferable estimate, i.e. 0.42 or 0.43 for Tying-up.

In the history of Thoroughbred horse breeding, most animals suffered from any genetic diseases have been eliminated in order to prevent propagation. However, the Tying-up syndrome, clarified as a genetic disorder in this study, would be one of many genetic disorders that have not been recognized as genetic disorders. Concerning the Tying-up related traits, Valberg *et al.* (1996) and MacLeay *et al.* (1999) also suggested the recessive mode of inheritance for polysaccharide storage myopathy and dominant mode for RER, respectively, by using pedigree-phenotype association analysis. These results were conflicting each other for the mode of inheritance but it may be due to the limited number of families used in their pedigree analyses. Once recognized as genetic disorders, we would start to discuss how to decrease the prevalence ratio of such genetic disorders by introducing some preventive techniques or early treatments based on the development of symptoms mechanisms. For example, McKenzie *et al.* (2003), who considered that RER might be induced by physical or mental stress, advocated management strategies to reduce stress and excitability, including turnout, exercising or feeding these horses first before other horses, and the judicious use of low-dose tranquilizers during training.

It is noteworthy to consider further investigation on the associations of such highly heritable (heritability of 0.42–0.43) incidences in the Tying-up syndrome with polymorphic markers, which are available from recent reported studies on the equine genetic linkage maps (Penedo *et al.* 2005). Then we can start to develop the evidence-based method of treatment for Tying-up in the Thoroughbred horses.

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