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Honda, Takeshi

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Ph. D. Thesis

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2005 January

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（黒毛和種集団の遺伝的多様性の維持に関する研究）

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GENERAL INTRODUCTION

A large proportion of the reproductive cows raised for beef production (about 0.64 million heads) in Japan is shared by the domestic breeds, i.e. Japanese Black, Japanese Brown, Japanese Poll, and Japanese Shorthorn. Among the four domestic beef breeds, the Japanese Black is the most common, with about 0.61 million reproductive females distributing over the country (MAFF 2002). The breed is characterized by the prominent intramuscular fat deposition (marbling), and has recently drawn attention of domestic (Zembayashi et al. 1995; Ozawa et al. 2000; Mukai et al. 2004) and foreign (Xie et al. 1996a, b; Kazala et al. 1999; Yang et al. 1998) researchers as important genetic resources.

The first organized breeding policy for the native cattle was the recommendation of crossbreeding with several foreign breeds (i.e. Simmental, Ayshire, and Brown Swiss; Namikawa 1992) in 1900. The foreign and crossbred cows were, however, immediately culled, because the crossing resulted in not only desirable gains such as increased body size and milk yield, but also undesirable changes such as increased feed intake and less aptitude for draft. Since then, the native cattle have been maintained by pure breeding over a century. In 1944, the Japanese Black was approved as a pure breed by the government on the basis of the uniformity in exterior appearance (Nishida 1973). Although the breed had been raised as labor cattle (e.g. cultivating rice fields and transporting farm products) in this period, the breed could rapidly turn to beef cattle according to the increased demands for beef products and the mechanization of farming and transportation from late 1950s. Accordingly, a two-stage testing scheme including performance test for growth ability and feed utilization efficiency and progeny test for carcass traits was initiated in 1968, and through the selection scheme, almost all the

calves are now produced by year-round artificial insemination by selected sires (Wagyu Registry Association 1992; Mukai 1994).

A notable feature in the breed history of Japanese Black is that most of breeding projects had been carried out within prefectures, though there was limited migration among prefectures, usually through males. For example, in the crossbreeding prior to the breed establishment, the breeds used and the degree of crossing varied among prefectures, and the testing scheme has also been carried out within a station of each prefecture. Nomura (1988) showed that the breed in 1960 had presented a remarkable genetic subdivision. In the subdivided breed structure, subpopulations in the five prefectures in western region of Japan (i.e., Hyogo, Tottori, Shimane, Okayama, and Hiroshima prefectures in Chugoku district) have played important roles as suppliers of breeding stocks to the whole breed. Among the breeders of the Japanese Black, it has been well known that the five subpopulations had their unique characteristics, such as the high meat quality in Hyogo and the high growth rate in Tottori and Shimane.

Since the liberalization of beef import restriction in 1991, following to the agricultural trade negotiations in the Uruguay Round, production environments of the Japanese Black have been drastically changed. To compete with the economical imported beef produced by mass production method, domestic production of high-quality beef has been more emphasized in Japan. In 1991, the genetic evaluation with best linear unbiased prediction (BLUP) under animal model using field carcass records was initiated by Wagyu Registry Association (Mukai 1994), and selection and mating of animals based on the predicted breeding values (PBV) have become common nowadays. Owing to this rapid prevalence of the genetic evaluation system, carcass traits have been remarkably improved. On the other hand, however, it should be noted that selection on PBV inevitably accelerates the rate of inbreeding in the population.

Parallel to the enhancement of genetic progress, the preservation of genetic diversity in the population is an important task in animal breeding, to allow the population to face future economic and environmental changes and to assure a possible long-term response to selection for traits of interest (Barker 2001). Furthermore, the reduction of genetic diversity, i.e., the reduction of the effective population size, associates with various unfavorable phenomena such as inbreeding depression in fitness-related traits and an increased fluctuation in selection response (Falconer and Mackay 1996).

Monitoring of genetic diversity in pedigreed populations of domestic animals has been traditionally based on the analysis of inbreeding and the estimation of the effective population size. Because the effective population size is not only inversely related to the rate of decay of genetic diversity in the population, but also determines the response to artificial selection and its variance (Hill 1985, 1986), it is a key parameter for designing both the conservation and genetic improvement programs. Many estimates of inbreeding (e.g. Queiroz and Lôbo 1993; Braake et al. 1994; Miglior and Burnside 1995; Young and Seykora 1996) and the effective population size (e.g. Wright 1977; Pirchner 1983, 1985; Boichard et al. 1997; Sölkner et al. 1998; Gutiérrez et al. 2003) have been reported for various cattle populations.

In addition to the effective population size, the recent theoretical development in conservation genetics has provided many analytical tools for monitoring genetic diversity. Although the new tools have been originally proposed for captive breeding of endangered wild species, the application to domestic animal populations is expected to give further insights beyond the effective population size (Rodrigáñez et al. 1998; Toro et al. 2000; Goyache et al. 2003). Lacy (1989) defined the effective number of founders to account for the loss of genetic diversity from unequal founder representations. He also introduced the concept of founder genome equivalents as

theoretically expected number of founders that would be required to generate the genetic diversity in the actual population if the founders were equally represented and had lost no alleles (Lacy 1989). Recently, Caballero and Toro (2000) showed the interrelations among these parameters and proposed a new parameter, the effective number of non-founders, which explains the amount of genetic diversity reduced by random genetic drift accumulated in non-founders' generations.

MaCluer et al. (1986) proposed the method of gene dropping simulation, in which different alleles are assigned to founders and the genotypes of all the descendants are generated along the actual pedigree. By replicating this process, distributions of frequencies of founders' alleles and probabilities of allele extinction in a population under question can bee estimated. Although the mean of the distribution of allele frequency for a founder should coincide with the genetic contribution computed from pedigree analysis, the probability of allele extinction can be obtained only through the gene dropping simulation.

In the Japanese Black cattle, Nomura (1988) estimated the effective size of breed in 1960 and 1980 using pedigree analysis. Although the effective size reduced from 1724 to 125 during the investigated period (Nomura 1988), the estimate in 1980 was within the range of a standard level for domestic animal population (i.e. $N_e \approx 100$; see Wright 1977; Pirchner 1983). He also showed that although the genetic subdivided structure within the population of each prefecture had disappeared by the prevalence of artificial insemination technique with frozen semen of sires, the whole breed could be still classified into several genetic groups according to the direct relationships between the subpopulations in Chugoku district (Nomura 1988; Nomura and Sasaki 1988). Since his pedigree analyses, there have been no reports on population structure and genetic diversity of the breed for the last two decades, in spite of the drastic change occurred in

selection and mating systems.

The objective of the first three chapters in this thesis is to update the information on the genetic structure and genetic diversity of the breed. In chapter I, the traditional pedigree analysis with the estimations of the inbreeding coefficient and the effective population size was carried out to survey the changes of population structure, with a special emphasis on the period after the introduction of the genetic evaluation with BLUP methodology. In chapter II, detail analysis with the new analytical tools of pedigree, such as the estimations of the effective numbers of ancestors and the gene dropping simulation, was conducted for the period from 1960 to 2000. In chapter III, the genetic relationships among subpopulations (i.e. populations of prefectures) were analyzed by means of multivariate analysis procedures. From these analyses, it was shown that although the genetic diversity was drastically reduced during the past two decades, the descendants form several founders in the five breeding units (i.e., Hyogo, Tottori, Shimane, Okayama, and Hiroshima prefectures) in Chugoku district could be effectively used to recover the genetic diversity of the entire breed.

Based on the results from the pedigree analysis, an optimum structure of the breed was theoretically assessed in the following three chapters (chapters IV, V and VI). In these chapters, a hierarchical breed structure was assumed, in which favorable genes are accumulated in the upper level of the hierarchy (breeding population) by artificial selection, and the achieved genetic progress is transferred to the lower level of hierarchy (commercial population) mainly through the migration of males. In Japanese Black cattle, the breeding population consists of the five breeding units in Chugoku district, since these units have played traditional roles as suppliers of breeding stocks to the entire breed. In chapter IV, a mating system to reduce the inbreeding in the commercial population was first examined. It was revealed that the rotational mating

with sires supplied from several closed lines in the breeding population is quite an effective system to suppress the inbreeding of commercial females. In chapter V, an optimum management plan to maintain the genetic diversity and reduce the inbreeding rate in the breeding population was theoretically assessed by assuming a situation where lines are constructed from the existing subpopulations. Based on population genetic theories, the optimum migration rate among lines and the required size of lines were determined. In the final chapter (chapter VI), the optimum structure of the entire breed was defined by combining the theories formulated in the previous two chapters. Since the hierarchical population structure has been found in many livestock breeds, the concept of the optimum breed structure defined in this thesis could be a general guideline to the conservation of genetic diversity and the suppression of inbreeding in livestock breeds.

CHAPTER I
Inbreeding and effective population size of the breed

Introduction

Since the liberalization of beef import restrictions in 1991, domestic production of high-quality beef has been receiving much emphasis in Japan, and the genetic evaluation project of meat quality with BLUP methodology was initiated in 1991 (Mukai 1994). Now, the breeding program is less stratified covering the populations of all the main prefectures. However, due to the intensive use of a few sires with high PBV of marbling, the increase of inbreeding is an increasing problem.

High rate of inbreeding causes some undesirable phenomena in populations of domestic animals, such as inbreeding depressions, reduced long-term genetic responses, and random fluctuation of selection responses (Falconer and Mackay 1996). As reviewed by Pirchner (1985), there are many evidences for the deleterious effects of inbreeding depression on traits related to the efficiency of beef production, such as fertility, maternal ability, viability, and feed efficiency. Genetic diversity is also rapidly reduced in the population with a high rate of inbreeding. The maintenance of genetic diversity is essentially important for the adaptation to unexpected changes of economic and environmental conditions, such as change of consumers' demands and prevalence of novel diseases in the near future.

In this chapter, the effects of recent change in breeding strategy on the genetic structure of the breed were assessed in terms of the amount of inbreeding and effective population size.

Materials and Methods

Pedigree records of the Japanese Black are kept by Wagyu Registry Association. For each animal, unique registered number of itself and its parents, names, and the date and place of birth are recorded in the database. They can be completely traced back to the ancestral population of the foundation period (1944 or before). Thus, the exact amount of inbreeding relative to the foundation period may be estimated. Furthermore, this breed was completely closed to foreign germplasm after its establishment. This is suitable for obtaining an accurate estimate of the rate of inbreeding (Woolliams and Mäntysaari 1995). Estimation of genetic parameters was performed for all the registered bulls and cows born from 1985 to 2000. Numbers of the bulls and cows born in each year are given in Table 1-1. The total number of individuals included in the pedigree file was 2,184,060 animals.

For the population with overlapping generations, generation intervals are estimated as the average ages of parents at which animals under study are produced (Falconer and Mackay 1996). The generation intervals of four gametic pathways, i.e., sire to son (L_{mm}), sire to daughter (L_{mf}), dam to son (L_{fm}), and dam to daughter (L_{ff}), were obtained from records of birth dates of animals in each reference year and their sires and dams. The average generation interval (L) was computed from

$$L = \frac{L_{mm} + L_{mf} + L_{fm} + L_{ff}}{4}.$$

To assess the amount of inbreeding and the population structure, Wright's F-statistics (Wright 1951, 1969), which consists of F_{IT}, F_{ST}, and F_{IS}, were estimated for each year. F_{IT} is the average inbreeding coefficient of registered animals and F_{ST} is the inbreeding coefficient expected under random mating. Following Wright (1951, 1977), F_{ST} was computed from a hypothetical population produced by matching sires and dams of the

Table 1-1. The numbers of bulls and cows born from 1985 to 2000.

Year	Bulls	Cows
1985	204	58,199
1986	187	61,744
1987	210	64,163
1988	250	74,606
1989	284	78,406
1990	301	76,624
1991	290	73,961
1992	248	65,399
1993	248	57,950
1994	307	56,272
1995	355	57,512
1996	346	53,923
1997	329	57,676
1998	293	56,557
1999	285	58,714
2000	296	63,838

registered animals in each year at random. In the preliminary analysis with the animals born in 1990 and 1995, 20 hypothetical populations were generated for each year, and F_{ST} was computed for each hypothetical population to obtain an empirical sampling error. Since the sampling errors were quite small (CV = 0.40% for both years), F_{ST} was computed for a single hypothetical population generated for each year of the examined period. The third statistic, F_{IS}, is given by the formula of Wright (1951, 1969):

$$F_{IS} = \frac{F_{IT} - F_{ST}}{1 - F_{ST}}.$$

This coefficient expresses the departure from randomness in the actual mating. When F_{IS} > 0, the actual inbreeding (F_{IT}) exceeds the level expected under random mating

(F_{ST}), implying that mating among more closely related parents than the average is predominant, or the population is partitioned into subpopulations and mating is more or less restricted within each subpopulation. In contrast, in the population with $F_{IS} < 0$, avoidance of inbreeding or mating between subpopulations is carried out predominantly.

Effective size of a population (Wright 1931, 1938) is defined as the size of an idealized population which would give rise to the variance of change in gene frequency or the rate of inbreeding observed in the actual population under consideration, each of which is so called variance and inbreeding effective size. In a regular breeding system, although each of them can be different for a given generation, they eventually converge to the same value so long as the population under consideration is not permanently subdivided into independent sublines and does not largely fluctuate in its size (Caballero and Toro 2000). The effective size can be estimated if pedigree data is available while the former necessitates genotypic frequency data. According to Wright (1977) and Caballero and Hill (1992), the effective size of the breed was estimated from the increasing rate of F_{ST} per generation. The annual increasing rate ($\Delta F_{ST,y}$) of F_{ST} was first computed by

$$\Delta F_{ST,y} = \frac{F_{ST,t} - F_{ST,t-1}}{1 - F_{ST,t-1}},$$

where $F_{ST,t-1}$ and $F_{ST,t}$ are the coefficients of F_{ST} in two successive years. Then, with the generation interval (L) in year t, the effective population size (N_e) was estimated as:

$$N_e = \frac{1}{2\Delta F_{ST,y} L}$$

(Hill 1972; Nomura 1996).

Results and Discussion

Table 1-2. Generation intervals of four genetic pathways and the average generation interval (yr).

Year	Pathway[a]				Average
	S-S (L_{mm})	S-D (L_{mf})	D-S (L_{fm})	D-D (L_{ff})	
1985	10.35	9.61	7.14	6.20	8.32
1986	11.11	10.22	7.20	6.36	8.72
1987	10.89	10.04	7.31	6.47	8.68
1988	11.69	9.92	7.70	6.57	8.97
1989	11.55	10.07	7.77	6.62	9.00
1990	11.57	10.20	7.35	6.62	8.94
1991	12.04	10.49	7.72	6.55	9.20
1992	12.47	11.11	7.48	6.55	9.40
1993	13.09	11.48	7.67	6.60	9.71
1994	13.54	11.62	7.55	6.60	9.83
1995	13.89	11.73	8.05	6.65	10.08
1996	13.64	11.78	7.92	6.75	10.02
1997	13.37	11.50	8.34	6.83	10.01
1998	13.17	11.54	7.93	6.87	9.88
1999	12.87	11.87	7.93	6.84	9.88
2000	12.73	11.55	8.11	6.83	9.80

[a]S-S: sire to son, S-D: sire to daughter, D-S: dam to son, D-D: dam to daughter.

Table 1-2 shows the estimates of generation interval in each year. The average generation interval remained essentially constant during the period 1985 to 1990. But in the following five years, the generation interval tended to increase, mainly due to increased generation intervals of the two paths from sires (L_{mm} and L_{mf}). Especially, the generation interval from sire to son was about 2 - 3.5 yr longer than that in 1985 to 1990. Since the liberalization of beef import restrictions in 1991, domestic production of high-quality beef has been received much emphasis in Japan. Accordingly, genetic

evaluation with BLUP under animal models was initiated in this year. Because the genetic evaluation project is based on records collected from carcass markets, there is a lag of about 2 yr between the completion of progeny testing in station (onset of service of sires) and the time when their PBVs become available. The increased generation intervals in the two pathways from sires after 1991 are largely attributed to the selective use of proven sires.

The change of *F*-statistics from 1985 to 2000 is presented in Figure 1-1. The drastic change of population structure in this period is characterized by the behavior of F_{ST}. The value of F_{ST}, which has a direct relation to the effective population size, showed a sharp increase from 2.4% in 1985 to 5.5% in 2000. On the other hand, F_{IT} increased more gradually from 4.7% to 6.0% during the same period. Thus, F_{IS} decreased almost linearly and reached 0.5% in 1996, indicating that the genetic subdivision among prefectures has essentially disappeared.

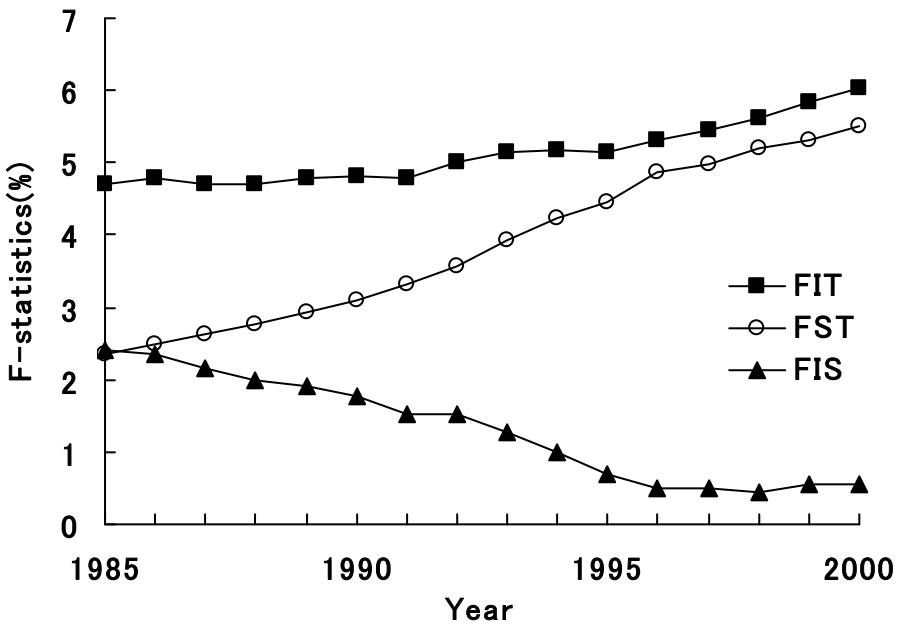


Figure 1-1. Changes of *F*-statistics in the breed from 1985 to 2000.

As shown in Table 1-3, the linear regression coefficients of *F*-statistics on years were significantly different between the periods before and after the initiation of the BLUP evaluation.

Table1-3. Regression coefficients of *F*-statistics on years.

Item	1985-1990	1991-1996	D[a]
F_{IT}	0.019	0.087	*
F_{ST}	0.152	0.304	**
F_{IS}	-0.133	-0.223	**

Significance of the difference of regression coefficients between the two periods. *$P < 0.05$, **$P < 0.01$.

Table 1-4 gives the estimates of effective population size. In the period before the initiation of the BLUP methodology (1985 to 1990), the breed maintained the effective size of approximately 30. However, after 1991 the effective size was sharply reduced. A surprising result is the small effective size of the recent population. Although the effective size fluctuated, the harmonic mean between 1991 and 2000 was only 20.3. Due to a temporal reduction of the annual increasing rate of F_{ST}, increments of the effective size were observed in 1997 and 1999. This would be presumably due to the onset of service of some sires less related to those widely used in the previous year, suggesting the possibility of recovering of the effective size.

Wright (1977) and Pirchner (1983) summarized the estimates of effective size of various livestock breeds. Although the estimates for cattle breeds vary from 32 (Shorthorn in foundation period) to 330 (U.S. Holstein in 1881to1931), it is striking that most of the estimates are around 100 and, as pointed out by Pirchner (1983), they are essentially independent of the actual (census) size of the breed population. In the recent work of Woolliams and Mäntysaari (1995), the rate of inbreeding per generation

Table 1-4. Estimated effective population size per generation (N_e).

Year	N_e	Year	N_e
1985	29.0	1993	13.9
1986	43.0	1994	15.8
1987	39.2	1995	19.7
1988	37.3	1996	12.2
1989	31.2	1997	41.2
1990	32.8	1998	19.9
1991	23.3	1999	47.6
1992	23.0	2000	23.6

in Finnish Ayshire population was estimated to be 0.0147, leading to the estimated effective size of 34. Boichard et al. (1997) reported the effective sizes of 47 and 106 for two local dairy breeds in France. The effective size of current population of the Japanese Black is below the lower range of the published estimates.

Several researchers have addressed the question of a minimum effective size required for livestock populations. From a consideration of the net genetic response in economic merit in dairy cattle breeding, Goddard and Smith (1990) suggested 40 as a minimum effective size. Another approach towards defining minimum effective size was considered by Meuwissen and Woolliams (1994), which balanced inbreeding depression and gain in fitness through natural selection. This resulted in recommendations of the order of 30 to 250. Using the similar approach, a minimum viable effective size for conservation of endangered species has been estimated to be 50 (Franklin 1980; Lande and Barrowclough 1987). When the variation of selection response due to random genetic drift is used as a criterion, a much larger effective size (the order of several hundreds) is required for reducing the variation to an acceptable level (Nicholas 1989). The current effective size of Japanese Black is much smaller than these critical values.

In conclusion, the sharp reduction of the effective population size after the initiation of genetic evaluation project with BLUP methodology (1991) strongly suggests that the decay of genetic diversity in the breed drastically proceeded during this period. To clarify the causes of the reduction of effective size and develop an optimum management plan of the genetic diversity, further analysis is carried out in the following two chapters.

CHAPTER II

Survey on genetic diversity of the breed in terms of genetic contributions of founders

Introduction

In the field of conservation genetics for captive endangered wild species, several new analytical tools have been developed to monitor the genetic diversity in pedigreed animal populations. Lacy (1989, 1995) introduced the concepts of the effective number of founders and the founder genome equivalents. Caballero and Toro (2000) developed the idea of Lacy (1989, 1995), and proposed the effective number of non-founders. These effective numbers can be used to detect the causes of the change in genetic diversity in the population.

To analyze the genetic contributions of founder animals, MaCluer et al. (1986) proposed another analytical method, so-called the gene dropping simulation. Although the genetic contribution of a founder is the expected frequency of alleles originated from the founder to the current population, the actual allele frequency may be more or less varied around the expectation due to the stochastic process in the Mendelian segregation and the bottleneck effect. The gene dropping simulation accounts for this stochastic aspect of inheritance. The amount of variation of allele frequency would have an important meaning in designing management plans of genetic diversity (MacCluer et al. 1986; Ballou and Lacy 1995). For example, if two founders with the same genetic contribution show a large difference in their variations of allele frequencies, a priority for the conservation management should be put on the founder with the larger variation because the risk of allele extinction becomes larger.

A lot of pedigree analysis for genetic managements of captive wild animals (e.g.

Princée 1988; Haig et al. 1990; Gandini et al. 1994; Miller 1995; Folch and Jordana 1998; Belousova and Kudrjavtsev 2000) and domestic animals (e.g. Boichard et al. 1997; Gandini 1997; Rodrigáñez et al. 1998; Sölkner et al. 1998; Roughsedge et al. 1999; Toro et al. 2000; Nivet et al. 2001; Baumung and Sölkner 2002; Huby et al. 2003) have been conducted with these new analytical tools.

The purpose of this chapter was to estimate the amount of genetic diversity and analyze the genetic contributions of founder animals by applying these new analytical tools.

Materials and Methods

Pedigree data used in this chapter was the same as in chapter I. According to Boichard et al. (1997), the ancestors with unknown parents are defined as founders, which compose the base population of pedigree analysis, and all of their descendants as non-founders. Monitoring of genetic diversity was carried out for populations born in every five years from 1960 to 2000. These populations are referred to as reference populations. The number of registered bulls and cows for reproduction in each reference population are given in Table2-1, together with the number of their founders.

Table 2-1. Numbers of registered bulls and cows born in each year, and the actual number of founders (N_f) and the number of discrete generation equivalents (g_e).

Year	Bulls	Cows	N_f	g_e
1960	447	11,981	16,739	5.1
1965	256	13,681	17,462	6.0
1970	224	22,847	23,804	6.8
1975	232	36,681	29,314	7.6
1980	250	72,908	36,743	8.2
1985	204	58,199	29,640	8.7
1990	301	76,624	29,632	9.2
1995	355	57,512	24,575	9.7
2000	296	63,838	23,217	10.2

Concept of genetic diversity in terms of ancestral genetic contributions:

For simplicity, consider a single undivided population with constant size of N individuals and discrete generations under random mating. In generation 0, the population consists of unrelated founders, so that all the alleles in a given locus in that generation are assumed to be unique (not identical by descent), and all the gene pools of descendant generations are composed of alleles derived from them.

One of the most common measurements of genetic diversity is the expected heterozygosity (*GD*; Nei 1973), which is computed in the pedigree analysis as

$GD = 1 - \Pr(\text{two randomly chosen alleles from the gene pool are identical by descent})$.

Since there are *N* individuals, all the founder alleles distribute in founder generation with the frequency of $1/2N$, and the amount of genetic diversity is $GD = 1 - 1/2N$. However, because of unequal mating success among parents and random genetic drift, frequencies of founder alleles are varied during the allele transmission process in non-founder generations, and are unequalized in each generation. This inequality among frequencies of founder alleles is responsible for the reduction of genetic diversity, and is reflected in average coancestry of the population.

Letting *M* be a total number of individuals in a pedigree and N_0 be that of founders, average coancestry of the population in the latest generation *t* is

$$\bar{f}_t = \frac{1}{2N^2}\sum_{i=1}^{N_0} c_i^2 + \frac{1}{4N^2}\sum_{i=N_0+1}^{M} c_i^2\left(1 - \frac{F_{Si} + F_{Di}}{2}\right), \tag{2-1}$$

where c_i is the sum of direct relationships between individual *i* and the individuals in generation *t*, so that it reflects the amount of genetic contribution of individual *i* to generation *t*, and F_{Si} and F_{Di} are the inbreeding coefficients of sire and dam of individual *i*, respectively. Since the population size is assumed to be constant over generations, the mean contribution of individual in a given generation *k* to the descendants in generation *t* is one with variance

$$V_{k,t} = \sum_{i=1}^{N} \frac{c_{i(k,t)}^2}{N} - 1. \tag{2-2}$$

Substituting (2-2) into (2-1),

$$\bar{f}_t = \frac{1}{2N}(1 + V_{0,t}) + \frac{1}{4N}\sum_{k=1}^{t}(1 + V_{k,t})\left(1 - \bar{F}_{k-1}\right) \tag{2-3}$$

is obtained, where $\overline{F}_k$ is the average inbreeding coefficient in generation k. This equation indicates that the average coancestry of the population, i.e., the amount of reduced genetic diversity during the allele transmission process, is accounted for with the variance of genetic contributions of founders (the first term) and genetic drift accumulated in non-founder generations (the second term).

Pedigree depth and effective number of ancestors:

The depth of pedigree in each reference population was examined by computing the number of discrete generation equivalents (g_e), which is the expected number of generations from the base population to the reference populations if generation proceeded discretely. This parameter is obtained by

$$g_e = \frac{1}{N} \sum_{j=1}^{N} \sum_{i=1}^{n_j} \frac{1}{2^{g_{ij}}},$$

where n_j is the total number of ancestors of animal j in the reference population, g_{ij} is the number of generations between animal j and its ancestor i, and N is the number of animals in the reference population (Wooliams and Mäntysaari 1995).

To assess the amount of genetic diversity and clarify the causes of the reduction, three types of effective numbers of animals were estimated, i.e. effective number of founders, founder genome equivalents, and effective number of non-founders. The effective number of founders (N_{ef}) is estimated by

$$N_{ef} = \frac{1}{\sum_{i=1}^{N_f} \left(\frac{c_i}{N} \right)^2},$$

where N_f is the number of founders, N is the number of animals in the reference population, and c_i is the sum of direct relationships between founder i and animals in the reference population (Lacy 1989; Rochambeau et al. 1989). In this study, c_i /N is

defined as genetic contributions of founder *i*, so that it can be considered as the expected frequencies of alleles derived from founder *i* in the reference population. Since N_{ef} explains only the loss of genetic diversity due to unequal contributions of founders to the reference population, and does not account for the effect of genetic drift accumulated in the non-founders, it overestimates genetic variability of the reference population (see eq. (2-3)).

All the causes of the reduction of genetic variability are fully accounted for by founder genome equivalents (N_{ge}). This parameter is estimated by

$$N_{ge} = \frac{1}{2\sum_{i=1}^{N}\sum_{j=1}^{N} f_{ij} \Big/ N^2},$$

where f_{ij} is coancestry between individuals *i* and *j*. The denominator is the average coancestry of the reference population under consideration (Lacy 1995; Caballero and Toro 2000).

The third type of effective number of animals, the effective number of non-founders (N_{enf}), accounts only for the effects of genetic drift in non-founder generations. This effective number is obtained from the relation

$$\frac{1}{N_{ge}} = \frac{1}{N_{ef}} + \frac{1}{N_{enf}} \tag{2-4}$$

(Caballero and Toro 2000).

When genetic diversity is expressed with the expected heterozygosity (Nei 1973), the amount of genetic diversity (*GD*) in the reference population relative to the base population is estimated by

$$GD = 1 - \frac{1}{2N_{ge}},$$

(Lacy 1989, 1995). Analogously, the genetic diversity estimated by

$$GD^* = 1 - \frac{1}{2N_{ef}}$$

accounts only for the decay due to unequal contributions of founders (Caballero and Toro 2000). From equation (2-4), the difference of these two indices of genetic diversity is

$$GD^* - GD = \frac{1}{2N_{enf}}.$$

This difference represents the amount of the genetic diversity reduced by the genetic drift accumulated over non-founders' generations (Caballero and Toro 2000).

Gene dropping simulation:

Although theories for computing the distribution of allele frequency have been developed in the analysis of human pedigrees (Thomas and Thompson 1984; Thomas 1986), the application to animal populations has been limited to small populations (Thompson 1986), because of the computational problems arising from the complex pedigree structure and a large number of ancestors included in the pedigree. As an alternative, gene dropping simulation proposed by MacCluer et al. (1986) is available for this purpose (Thomas 1990). To estimate the distributions of founder allele frequencies in all the current reproductive cows, the simulation was applied to those alive in August 2001 (612,959 heads).

The procedure of the gene dropping simulation is illustrated in Figure 2-1, in which the process of one trial of the simulation is presented with a simple pedigree. In the Figure, individuals *a*, *b*, *c*, *d*, and *e* are founders, and individuals *x*, *y*, and *z* consist of a reference population. At first, two unique hypothetical alleles are assigned to each founder (G_1 to G_{10} in Figure 2-1 (A)). Then the alleles are dropped along the pedigree

following the Mendelian segregation rules, and all the genotypes of the reference population are determined, as exemplified in Figure 2-1 (B). To obtain reliable distributions of allele frequencies in the reference population, we replicated this process 20,000 times. Theoretically, the expected value of the distribution of allele frequencies for a founder agrees with the genetic contribution of the founder (Lacy 1989; Rochambeau et al. 1989; Caballero and Toro 2000). From the estimated distribution, the probability of extinction of alleles originated from a given founder (Pr(lost)) was calculated as the proportion of replicates in which both alleles from the founder had not existed in the reference population. We should note that although genetic contributions of founders (c_i /N) are exactly determined by their direct relationships to the reference population, this probability depends not only on the pedigree structure but also on the stochastic process in the Mendelian segregation.

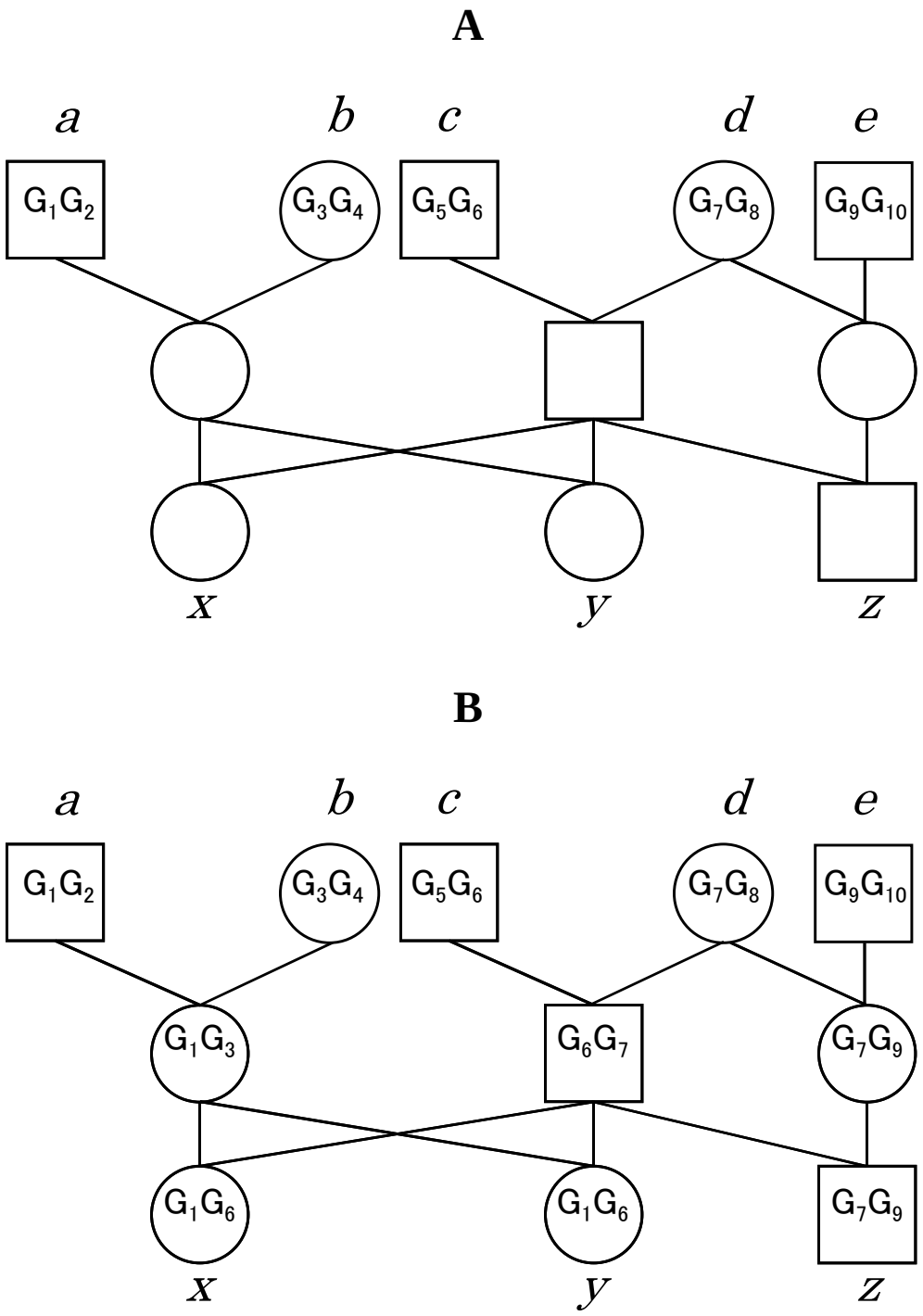


Figure 2-1. Illustration of gene dropping simulation. (A) Assignment of unique hypothetical alleles (G_1 to G_{10}) to founders (*a*, *b*, *c*, *d*, and *e*). (B) Result of one trial of simulation. Four founder alleles, G_1, G_6, G_7, G_9, have retained in the reference population (*x*, *y*, and *z*) in this trial.

Results and Discussion

The depth of pedigree in each reference population is shown in the last column of Table 2-1. From 1960 to 2000, discrete generation equivalents (g_e) increased from 5.1 to 10.2. Dividing the period by the increment of g_e gives an estimate of average generation interval in the last four decades as 7.8 years. The increase of g_e along years was essentially linear, implying that the change of generation interval was not large during the analyzed period.

The estimates of the three effective numbers of ancestors are given in Table 2-2. The most comprehensive effective number, founder genome equivalents (N_{ge}), decreased from 86.6 to 7.3 during the period analyzed. According to the definition by Lacy (1989), the amount of genetic diversity in the recent population could be generated by only 7 or 8 non-related founders. The sharp decline of the effective number of founders (N_{ef}) in the earlier period (1960-80) suggests that selection enhancing unequal contributions of founders, such as selection among strains, had been practiced, and genes in minor strains would be mostly lost during this period. Nevertheless, throughout the investigated period, N_{ef} was much larger than the effective number of non-founders (N_{enf}), and the latter effective number showed close value to N_{ge}, suggesting that for the cause of reduction of genetic diversity, random genetic drift accumulated in the non-founder generations is more serious than the unequal contributions of founders.

Assuming a closed population with a constant selection scheme, Wray and Thompson (1990) predicted that although variation among contributions of founders is inflated by selection, the variance approaches an asymptotic value within several generations. From this prediction, it is expected that the effective number of founders (N_{ef}) attains a steady value within several generations (Caballero and Toro 2000). Although the

Table 2-2. Founder genome equivalents (N_{ge}), effective number of founders (N_{ef}), and effective number of non-founders (N_{enf}) in each reference population.

Year	N_{ef}	N_{ge}	N_{enf}
1960	418.4	86.6	109.2
1965	272.3	55.4	69.5
1970	281.2	44.2	52.4
1975	220.3	34.6	41.1
1980	96.2	21.7	28.0
1985	74.1	14.9	18.7
1990	69.3	12.6	15.5
1995	52.4	9.4	11.4
2000	50.2	7.3	8.5

breeding objectives in the breed have been altered during the past four decades, the rapid decrease of N_{ef} in the earlier period and the much smaller decrease of N_{ef} after 1980 are roughly in agreement with the theoretical expectation.

In the recent work of Boichard et al. (1997), N_{ef} for three cattle breeds in France (i.e., Abondance, Normande, and Limousine) were estimated to be 69, 132, and 790, respectively. The corresponding estimates of N_{ge} were 17, 22, and 206, respectively. Sölkner et al. (1998) reported that N_{ef} and N_{ge} for four main cattle breeds in Austria (i.e., Simmental, Braunvieh, Pinzgauer and Grauvieh) varied from 66.2 to 220.8 and from 20.8 to 94.3, respectively. In addition, those values for three endangered breeds in the same country (i.e., Original Pinzgau, Tux-Zillertal, and Carinthian Blond) ranged from 21.0 to 65.4 and from 9.7 to 22.6, respectively (Baumung and Sölkner 2002). Estimate of N_{ef} for UK Holstein-Friesian population by Roughsedge et al. (1999) was 93. Comparison with these published estimates characterizes the extremely limited genetic diversity in the current population of the Japanese Black cattle.

The changes of two indices of genetic diversity, GD and GD^*, are shown in Figure

2-2. Index *GD* enhanced its decreasing rate after 1975 and 1990. Accordingly, difference between two indices has rapidly increased, implying that accelerated accumulation of the genetic drift has diminished *GD*.

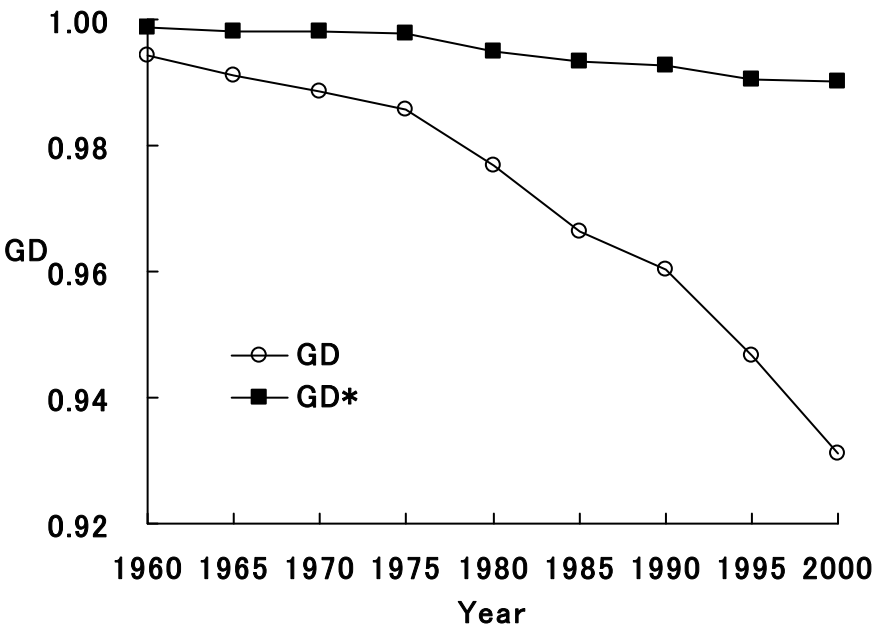


Figure 2-2. Changes of genetic diversity in each reference population.

Table 2-3 shows the number of sires in each reference population, average, variance and coefficient of variation of their progeny number, and percentage of progeny sired by five bulls most intensively used in each year. The number of sires was approximately 1,000 during the period from 1965 to 1990, but showed a gradual decline after 1990. Although this reduction might be partially responsible for the genetic drift, more pronounced change can be seen in the coefficient of variation of progeny number among sires. Although it was essentially constant over the period from 1960 to 1975, it was inflated to almost twice in 1990, and showed further rapid increase from 1990 to 2000. This increase of the coefficient of variation was due to the intensive use of a few popular sires, which is also reflected in percentage of progeny sired by five bulls

most intensively used in each year. After 1975, the percentage of the progeny began to increase remarkably from 7.3% and reached 17.3% in 1985. Furthermore, it rapidly increased again from 16.8% in 1990, and progenies of only five bulls made up more than 50% of all the registered cows born in 2000. These results strongly indicate that the accelerated accumulation of genetic drift is caused by the bottleneck effect due to the concentrated use of a few famous sires. In addition, it must be also a major reason for the disappearance of genetic subdivision observed in chapter I.

Table 2-3. The numbers of sires in reference populations, basic statistics of their progeny number, and percentages of progenies sired by five bulls most intensively used in each year (P(5bulls) (%)).

		No. of progeny / sire			
Year	No. of sires	Average	Variance	CV (%)	P(5bulls) (%)
1960	1209	10.3	234	148.6	4.1
1965	1052	13.3	386	148.3	5.0
1970	997	23.1	1441	164.0	6.5
1975	1083	34.1	4382	194.2	7.3
1980	1062	68.9	30975	255.5	10.3
1985	1004	58.2	37196	331.6	17.3
1990	1063	72.4	69899	365.3	16.8
1995	832	69.6	121146	500.4	31.9
2000	732	87.6	483807	793.9	52.4

In considering the preservation or the recovery of genetic diversity of the breed, the genetic contributions of subpopulations (populations of prefectures) in the foundation period will provide useful information. In Figure 2-3, the genetic contributions of founders to the whole breed, summed up by their birth prefecture, are presented. As expected from the breed history, the genetic contributions of the five traditional breeding units (i.e., Hyogo, Tottori, Okayama, Shimane and Hiroshima prefectures, see

general introduction) have predominated since the early time. Until late 1970s, genetic contribution of Tottori prefecture was the largest because high growth ability had been preferred in beef production in Japan. However, due to the current emphasis moved on the meat quality, the genetic contribution of Hyogo prefecture increased after 1980 and the contribution to the breed in 2000 was close to 50%. The results in Figure 2-3 indicate that genes originated from the subpopulations less represented in the current breed (i.e., Okayama, Shimane and Hiroshima prefectures) are of special importance for the recovery of genetic diversity.

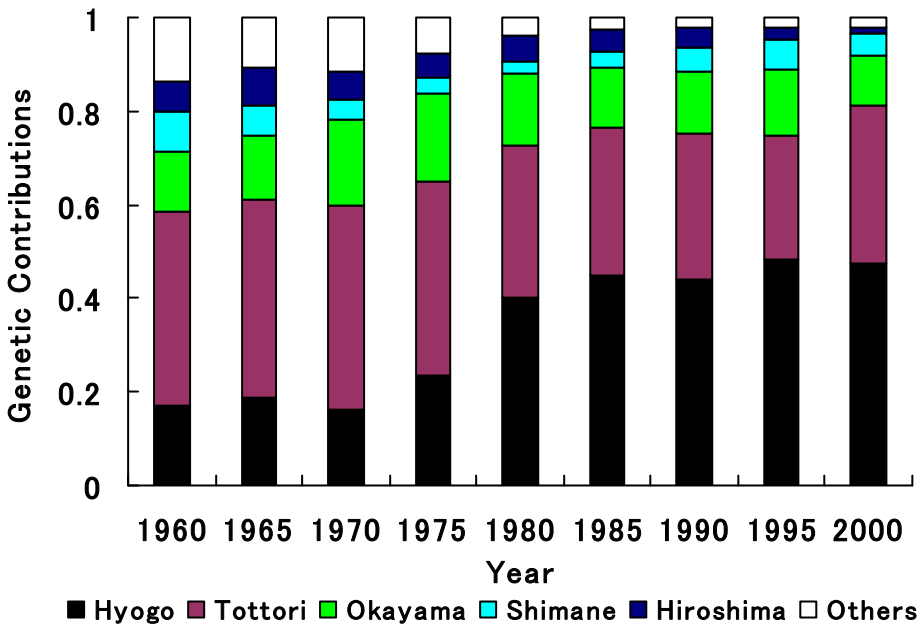


Figure 2-3. Classification of genetic contributions by the birth place of founders.

Information additional to the genetic contributions will be obtained from gene dropping simulation. The results of simulation are summarized in Table 2-4, in which the probabilities of allele extinction (Pr(lost)) are given for the five highest contributors (founders) in each of the five traditional subpopulations. Under the assumption that all the founders (39,966 heads) equally contribute to the current live animals, genetic

Table 2-4. Genetic contributions (*gc*) and probabilities of allele extinction (Pr(lost)) of five founders with the highest genetic contributions in each of five traditional breeding prefectures.

Prefecture	Founders' name	Sex [a]	*gc*	Pr(lost)
Hyogo	Fukue	F	0.1134	0.000
	Moku	F	0.0211	0.575
	Hana	F	0.0192	0.028
	Ume	F	0.0143	0.018
	Kumanami	M	0.0134	0.530
Tottori	Nishikawa	F	0.0144	0.184
	5th Shige	F	0.0121	0.288
	Akahori	F	0.0080	0.097
	Kiku 3	F	0.0072	0.464
	Kotani 2	F	0.0063	0.002
Shimane	4th Ryuun	M	0.0086	0.000
	1st Kamezu	F	0.0035	0.011
	Nitowakazuki	F	0.0035	0.000
	2nd Hidaka	F	0.0025	0.136
	Moriwaki	F	0.0021	0.023
Okayama	13th Hanayama	M	0.0093	0.000
	Senka 4	F	0.0048	0.556
	Nitta	F	0.0042	0.000
	Nakamura	F	0.0038	0.002
	Hanahide	M	0.0026	0.016
Hiroshima	4th Kakefuda	M	0.0046	0.000
	3rd Yasuda	F	0.0020	0.109
	20th Okuda	F	0.0013	0.143
	10th Hayashi	F	0.0011	0.078
	2nd Hiwa	M	0.0010	0.279

[a] M: male, F: female

contributions of each founder would be equivalent to 2.5×10^{-5}. The female founder 'Fukue' in Hyogo showed an exceptionally high genetic contribution, since her famous son 'Tajiri' had played an important role in the production of breeding stocks with high genetic merits of marbling. Although 'Tajiri' is not a founder, he produced 183 sires and his genetic contribution to the current breed is 0.168.

In considering the management of genetic diversity, Pr(lost) gives a useful information. For example, two founders, 'Moku' and 'Hana' in Hyogo contribute to the current breed with a similar proportion, but Pr(lost) of 'Moku' is much higher than 'Hana'. A detail survey of pedigree revealed that 'Moku' showed a low genetic contribution in the early period of the breed history. Thus, the alleles had passed a strong bottleneck in the early period, leading to the allele extinction with a high probability. In contrast, the low probability of allele extinction of the founder 'Hana' implies that she has lineage through which her alleles can be surely transmitted to the current breed. Although the genetic contributions of the founders in Shimane, Okayama and Hiroshima prefectures were relatively low, several founders showed low probabilities of allele extinction. This result suggests that a part of the current population is connected with these founders without severe bottleneck of lineages.

By the pure breeding policy of the Japanese Black cattle, an increase of genetic variation by an introduction of foreign germ plasm will not be expected. Consequently, for the maintenance of genetic diversity within the current population, it is the only way to keep genetic variability that had presented in founder population. However, breeding strategy with a large emphasis on carcass traits, especially on marbling, inevitably caused the extensive loss of genetic diversity. The results of the present chapter strongly suggested that the use of limited sires should be avoided urgently.

Although the current genetic evaluation project has been limited to carcass traits, the

project is now planned to cover other economically important traits, such as reproductive performance, maternal ability, and feed utilization efficiency (Oyama et al. 1996; Mukai et al. 2000; Shojo et al. 2004). This extended project is expected to relax the concentration of the use of AI semen from a few sires. Nevertheless, establishment of active conservation strategy should be strongly required. Gene dropping simulation would be useful for the strategy. Although we have used the simulation to examine allele survival in the breed, this technique could be applied to each live animal or local group of animals, to detect genetically important animals or a local group surely having target founder alleles for preservation. Breeding scheme based on the information could effectively contribute to the recovery of genetic diversity in the Japanese Black population.

CHAPTER III
Survey on genetic subdivision in the breed by multivariate analysis

Introduction

Population structure has a significant impact on genetic improvement of the breed. Wright (1922) noted that if the trait under selection exhibit epistasis, improvement would be more rapid in a population subdivided into small subpopulations, with regular selection and crossing among lines, than in a population with the same size maintained as a single one. A number of empirical (Katz and Young 1975; Banks 1986) and simulation (Enfield and Anklesaria 1986; Rutledge 1970) studies have confirmed his expectation.

The population of Japanese Black cattle has been also maintained with the structure subdivided into several local populations of prefectures (referred to as subpopulations hereafter). However, the subdivision of the breed has been disappeared by the nation-wide use of a few popular sires (chapter II). In designing a conservation strategy of genetic diversity, the genetic structure of the whole breed should be clarified on the basis of the current genetic relationships among subpopulations.

Scientists in psychology and biology have been often imposed to deal with a large number of variables having effects on each other. However, it is extremely difficult for human to grasp relationships of more than three variables simultaneously. To solve the problem, multivariate analysis has been wildly adopted in their fields since the early 20th century. This method is also useful to clarify genetic relationships among a large number of subpopulations. According to the development in technique for detecting molecular polymorphisms, increasing studies on genetic relationships among

subpopulations using allelic frequency data have been reported in domestic animal species, such as dogs (Kim et al. 2001), goats (Li 2002), poultries (Eding et al. 2002), pigs (Laval et al. 2000; Óvilo et al. 2000; Fabuel et al. 2004), and cattle (Cañón et al. 2001). In this chapter, two methods of multivariate analysis, i.e., principal component analysis and cluster analysis, were applied to genetic relationships of subpopulations in the breed.

Materials and Methods

In August 2001, about 0.61 million cows were raised for reproduction. From them, the cows younger than or equal to 10 years old were extracted, and genetic relationships among 25 subpopulations with more than 2,000 reproductive cows were examined. The limitation on the age of the cows (≤ 10) was chosen according to the average generation interval of the current breed (chapter I). The numbers of the cows in the 25 subpopulations are shown in Table 3-1. Pedigree data used in this chapter was the same as in the previous two chapters.

Table 3-1. Abbreviations of subpopulations and the number (*n*) of reproductive cows analyzed in each subpopulation.

Subpopulation	Abbre.	*n*	Subpopulation	Abbre.	*n*
Hokkaido	HK	27,225	Tottori	TT	2,570
Aomori	AM	7,013	Shimane	SM	8,128
Iwate	IW	28,680	Okayama	OK	3,441
Miyagi	MG	19,891	Hiroshima	HR	4,167
Akita	AK	5,279	Yamaguchi	YC	3,355
Yamagata	YT	3,366	Saga	SG	5,768
Fukushima	FK	16,837	Nagasaki	NS	19,533
Ibaragi	IB	3,417	Kumamoto	KM	9,139
Tochigi	TC	7,213	Ooita	OT	13,453
Gunma	GM	3,534	Miyazaki	MZ	66,180
Nagano	NN	2,317	Kagoshima	KG	76,917
Gifu	GF	5,454	Okinawa	ON	26,400
Hyogo	HY	11,135	Total	-	392,346

The genetic relationships among the 25 subpopulations were expressed by two different measurements. The first measurement of the genetic relationship is the average additive relationship coefficient. The value between two different subpopulations *X* and *Y* can be calculated as

$$\bar{a}_{XY} = \frac{\sum_{i=1}^{N_X}\sum_{j=1}^{N_Y} a_{XY(i,j)}}{N_X N_Y},$$

where $a_{XY(i,j)}$ is the additive relationship coefficient between individuals *i* and *j* each from the subpopulations *X* and *Y*, and N_X and N_Y are the numbers of cows in the subpopulations *X* and *Y*, respectively. The average additive relationship coefficient represents the expected covariance between breeding values of two cows randomly chosen each from the two subpopulations. Analogously, the value within a subpopulation is estimated by

$$\bar{a}_X = \frac{\sum_{i=1}^{N_X}\sum_{j=1}^{N_X} a_{X(i,j)}}{{N_X}^2},$$

where $a_{X(i,j)}$ is the additive relationship between individuals *i* and *j* in the subpopulation *X*. When $i = j$, $a_{X(i,i)} = 1 + F_i$ (where F_i is the inbreeding coefficient of individual *i*). With the average additive relationships, 25×25 matrix (**A** matrix) was constructed, which has $\bar{a}_X$ and $\bar{a}_{XY}$ on the diagonals and off-diagonals, respectively.

Principal component analysis (PCA) is one of the multivariate analyses, which summarizes multi-dimensional information into principal variation, and allows one to grasp the characteristics of the data structure in lower dimension. Consider the vector of *p* variables $\mathbf{x}' = [x_1, x_2, \ldots, x_p]$. The *k* th principal component (PC) is expressed in the form of $z_k = \mathbf{l}_k'\mathbf{x}$, where $\mathbf{l}_k'$ is the vector of weighting coefficients. Letting **S** be a variance and covariance matrix of the *p* variables, derivation of PCs is equivalent to solving eigenvalue problem

$$(\mathbf{S} - \mathbf{\Lambda I})\mathbf{Q} = \mathbf{0}$$

under the condition of determinating equation

$$|\mathbf{S} - \mathbf{\Lambda I}| = \mathbf{0},$$

where $\mathbf{\Lambda}$ is a diagonal matrix of eigenvalues in which the *k* th largest value (λ_k) is the (*k*, *k*) element, **Q** is a matrix including eigenvectors associated with λ_k in the *k* th column ($\mathbf{q}_k$), and **I** is an identity matrix. Then, the vector of coefficients for the *k* th PC ($\mathbf{l}'_k$) corresponds to the eigenvector with the *k* th largest eigenvalue λ_k (i.e. $\mathbf{l}'_k = \mathbf{q}_k$).

The method is often used for the grouping of operational taxonomic units (OTUs; Okuno et al. 1975). Regarding the 25 subpopulations as OTUs, the **A** matrix is a variance and covariance matrix among OTUs. In this study, PCA was applied to the **A** matrix, and factor loadings of subpopulations, i.e., correlations between subpopulations and principal components, were plotted on a plane to visualize the genetic configuration of the subpopulations.

The second measurement of the genetic relationship used in the present study is Nei's standard genetic distances (Nei 1972). This distance was originally proposed as a measurement of the accumulated number of the differences of the codon between two alleles randomly chosen each from two populations. Letting j_{XY} and j_X be the probabilities that two alleles randomly chosen each from subpopulations *X* and *Y*, and both from subpopulation *X*, are identical at a certain locus, respectively, the standard genetic distance (d_{XY}) between subpopulations *X* and *Y* is calculated as

$$d_{XY} = -\log_e \frac{J_{XY}}{\sqrt{J_X J_Y}}, \qquad (3\text{-}1)$$

where J_{XY} and J_X are the averages of j_{XY} and j_X for all the analyzed loci, respectively. According to Caballero and Toro (2000), expectations of J_{XY} and J_X under the assumption of an infinite number of loci can be related to the average additive relationships as

$$J_{XY} = \sum_{i=1}^{N_X}\sum_{j=1}^{N_Y} f_{XY(i,j)} \Big/ N_X N_Y = \bar{a}_{XY}/2 \qquad (3\text{-}2)$$

$$J_X = \sum_{i=1}^{N_X}\sum_{j=1}^{N_X} f_{X(i,j)} \Big/ N_X{}^2 = \bar{a}_X/2\,, \qquad (3\text{-}3)$$

where $f_{XY(i,j)}$ is the coancestry between individuals *i* and *j* each from subpopulations *X* and *Y*, and $f_{X(i,j)}$ is the coancestry between individuals *i* and *j* in subpopulation *X*. Substituting (3-2) and (3-3) into (3-1), the standard genetic distances were calculated as

$$d_{XY} = -\log_e \frac{\bar{a}_{XY}}{\sqrt{\bar{a}_X \bar{a}_Y}}\,.$$

Then, 25×25 distance matrix (**D** matrix) was constructed, which has 0 on the diagonals and d_{XY} on the off-diagonals. Applying cluster analysis based on the unweighted pair group method with arithmetic mean (UPGMA; Sneath and Sokal 1973) to the **D** matrix, the dendrogram of 25 subpopulations was drawn to understand the process of grouping.

Results and Discussion

The average additive relationship coefficients between two subpopulations ($\bar{a}_{XY}$) and within subpopulations ($\bar{a}_X$) are presented in Table 3-2. The coefficients in the last two rows (θ and F) are the half of the average additive relationships and the average inbreeding coefficients within each subpopulation, respectively. Because almost completely closed breeding has been performed in Hyogo prefecture since the early times (Mukai et al. 1989), the average additive relationship coefficient and the inbreeding coefficient of the subpopulation are much higher than those of the others. The coefficient θ gives an estimate of F_{ST} (the inbreeding coefficient expected under random mating) of Wright's *F*-statistics (Wright 1951, 1969). In all the subpopulations, the coefficient θ exceeds the actual inbreeding coefficient *F*, suggesting that genetic subdivisions within subpopulations have essentially disappeared.

The mean of the average additive relationship coefficients within subpopulations was significantly ($p<0.01$) larger than the mean of the average additive relationship coefficients between all the possible pairs (300 pairs) of subpopulations (0.160 vs. 0.103). From the estimates of *F*-statistics in 1985-1996 (chapter I), rapid increase of the genetic homogeneity within the breed has been shown. However, the above result implies that the genetic subdivision still remains within the current breed.

Applying PCA to the **A** matrix shown in Table 3-2, the first five PCs were extracted. The cumulative contribution of the five PCs was 86.9%. This is contrast to the result (17.1%) obtained by Nomura and Sasaki (1988), who applied an analogous analysis to the population of the reproductive cows registered in 1980. The high cumulative contribution obtained from the current breed is apparently due to the sharp increase of the average relationships among the subpopulations.

Table 3-2. The average additive relationship coefficients within and among 25 subpopulations, and the average inbreeding coefficients of each subpopulation.

	HK[1)]	AM	IW	MG	AK	YT	FK	IB	TC	GM	NN	GF	HY	TT	SM	OK	HR	YC	SG	NS	KM	OT	MZ	KG	ON
HK	0.12																								
AM	0.11	0.12																							
IW	0.11	0.12	0.13																						
MG	0.11	0.12	0.12	0.17																					
AK	0.12	0.12	0.12	0.13	0.15																				
YT	0.12	0.13	0.13	0.15	0.14	0.16																			
FK	0.11	0.12	0.12	0.12	0.12	0.13	0.13																		
IB	0.11	0.11	0.11	0.12	0.12	0.13	0.11	0.12																	
TC	0.12	0.12	0.12	0.12	0.13	0.14	0.12	0.12	0.15																
GM	0.11	0.12	0.12	0.11	0.12	0.13	0.12	0.11	0.13	0.13															
NN	0.11	0.12	0.11	0.12	0.12	0.13	0.12	0.11	0.13	0.12	0.12														
GF	0.12	0.12	0.12	0.13	0.13	0.14	0.12	0.12	0.13	0.12	0.13	0.18													
HY	0.13	0.17	0.16	0.17	0.15	0.19	0.15	0.15	0.14	0.15	0.15	0.17	0.41												
TT	0.08	0.08	0.09	0.07	0.08	0.08	0.09	0.07	0.09	0.09	0.09	0.09	0.07	0.18											
SM	0.12	0.09	0.09	0.09	0.12	0.09	0.09	0.09	0.12	0.09	0.11	0.11	0.06	0.09	0.20										
OK	0.09	0.08	0.07	0.07	0.09	0.08	0.08	0.07	0.09	0.08	0.08	0.09	0.08	0.07	0.11	0.15									
HR	0.06	0.06	0.05	0.08	0.06	0.07	0.05	0.06	0.06	0.05	0.06	0.06	0.08	0.03	0.04	0.03	0.19								
YC	0.11	0.11	0.11	0.11	0.11	0.12	0.11	0.11	0.12	0.11	0.11	0.13	0.15	0.09	0.10	0.08	0.05	0.12							
SG	0.11	0.12	0.12	0.11	0.12	0.13	0.12	0.11	0.13	0.13	0.12	0.13	0.14	0.10	0.10	0.08	0.05	0.12	0.15						
NS	0.10	0.11	0.11	0.11	0.11	0.12	0.11	0.10	0.11	0.11	0.11	0.12	0.14	0.09	0.10	0.08	0.05	0.11	0.13	0.12					
KM	0.10	0.11	0.11	0.10	0.11	0.12	0.11	0.11	0.11	0.12	0.11	0.12	0.14	0.09	0.09	0.07	0.05	0.11	0.13	0.11	0.12				
OT	0.08	0.08	0.08	0.08	0.09	0.08	0.08	0.08	0.10	0.09	0.08	0.09	0.10	0.07	0.09	0.07	0.04	0.08	0.09	0.09	0.10	0.20			
MZ	0.09	0.10	0.10	0.09	0.09	0.10	0.10	0.10	0.10	0.11	0.11	0.11	0.13	0.08	0.08	0.06	0.05	0.10	0.11	0.10	0.12	0.08	0.16		
KG	0.08	0.09	0.09	0.09	0.08	0.09	0.10	0.08	0.08	0.11	0.10	0.10	0.12	0.09	0.05	0.05	0.04	0.10	0.12	0.11	0.12	0.07	0.10	0.19	
ON	0.11	0.11	0.10	0.10	0.12	0.11	0.11	0.10	0.12	0.11	0.11	0.11	0.13	0.08	0.11	0.08	0.05	0.10	0.11	0.10	0.10	0.08	0.09	0.09	0.12
Θ[2)]	0.06	0.06	0.06	0.09	0.08	0.08	0.06	0.06	0.07	0.06	0.06	0.09	0.21	0.09	0.10	0.08	0.09	0.06	0.07	0.06	0.06	0.10	0.08	0.10	0.06
F[3)]	0.05	0.05	0.05	0.07	0.05	0.06	0.05	0.05	0.04	0.04	0.05	0.06	0.17	0.07	0.07	0.06	0.07	0.05	0.04	0.04	0.05	0.04	0.05	0.05	0.04

1) For abbreviations of subpopulations, see Table 3-1. 2) Inbreeding coefficient expected under random mating within subpopulation. 3) Average inbreeding coefficient within subpopulation.

Figure 3-1 (A) shows the plots of factor loadings of the subpopulations on PC1 and PC2 plane. Hiroshima showed the smallest factor loading for PC1. This will be due to the fact that Hiroshima had relatively low relationships with all the other subpopulations (the mean of the average additive relationships between Hiroshima and the other subpopulations was 0.053). Thus, Hiroshima seems to have a unique genetic constitution. For PC2, Hyogo took the smallest factor loading, and the three subpopulations with the lowest relatoinships to Hyogo, i.e., Shimane, Tottori and Okayama (cf. Table 3-2), showed the largest factor loadings. Thus PC2 would be the factor mainly determined by the relationship to Hyogo.

By plotting the factor loadings of 25 subpopulations on the PC1 and PC2 plane (Figure 3-1 (A)), eight subpopulations (i.e., Hyogo, Tottori, Shimane, Okayama, and Hiroshima in Chugoku district, and Ooita, Miyazaki, and Kagoshima in Kyushu district) could be separated from the other 17 subpopulations. In contrast to the wide disperse of the eight subpopulations, the other 17 subpopulations formed a cluster. The locations of subpopulations on the PC1 and PC2 plane could be partially interpreted as the reflections of the breeding policies within them. In Table 3-3, the proportions of reproductive cows produced by own bred sires (P_S) and by own bred dams (P_D) are presented for each subpopulation. Both the proportions for the eight dispersed subpopulations were much higher than the 17 grouped subpopulations, implying that these eight subpopulations are more or less keeping their unique breeding policies.

Plots of factor loadings on PC1 and PC3 plane are illustrated in Figure 3-1 (B). Kagoshima showed the largest factor loading for PC3. Since the three subpopulations with the lowest relationships to Kagoshima, i.e., Shimane, Okayama and Hiroshima (cf. Table 3-2), took the smallest factor loadings, PC3 seems to be a factor mainly determined by the relationships to Kagoshima. According to the degree of relationship

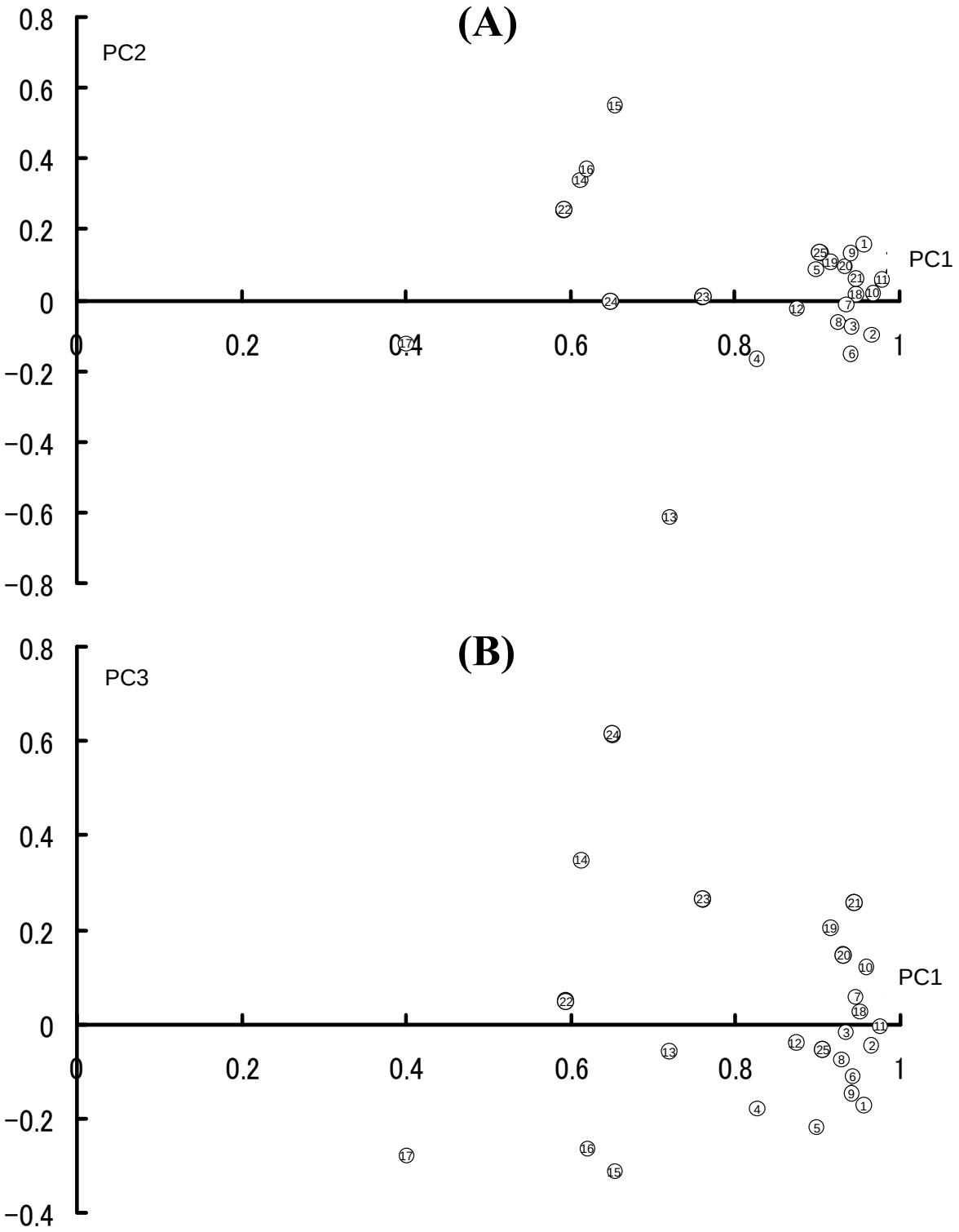


Figure 3-1. Plots of factor loadings of 25 subpopulations on (A) PC1 and PC2 plane and (B) PC1 and PC3 plane.

1:Hokkaido, 2:Aomori, 3:Iwate, 4:Miyagi, 5:Akita, 6:Yamagata 7:Fukushima, 8:Ibaragi, 9:Tochigi, 10:Gunma, 11:Nagano, 12:Gifu, 13:Hyogo, 14:Tottori, 15:Shimane, 16:Okayama, 17:Hiroshima, 18:Yamaguchi, 19:Saga, 20:Nagasaki, 21:Kumamoto, 22:Ooita, 23:Miyazaki, 24:Kagoshima, 25:Okinawa

Table 3-3. Proportions of cows produced by own bred sires (P_S) and own bred dams (P_D) in each subpopulation.

Subpopulation	P_S	P_D	Subpopulation	P_S	P_D
Hokkaido	0.01	0.46	Tottori	0.74	0.85
Aomori	0.03	0.62	Shimane	0.90	0.93
Iwate	0.09	0.68	Okayama	0.59	0.88
Miyagi	0.32	0.53	Hiroshima	0.78	0.92
Akita	0.05	0.74	Yamaguchi	0.03	0.51
Yamagata	0.01	0.37	Saga	0.01	0.38
Fukushima	0.16	0.54	Nagasaki	0.08	0.58
Ibaragi	0.22	0.42	Kumamoto	$\cong 0$	0.20
Tochigi	$\cong 0$	0.30	Ooita	0.84	0.94
Gunma	0.01	0.27	Miyazaki	0.79	0.86
Nagano	0.11	0.21	Kagoshima	0.68	0.95
Gifu	0.17	0.46	Okinawa	0.25	0.77
Hyogo	1.00	1.00			

to Kagoshima, Tottori could be separated from Shimane and Okayama.

Although plots of factor loadings on PC4 and PC5 are not presented in figures, Hiroshima and Ooita was largely separated from the other subpopulations on the PC4 and PC5 plane.

The result from the cluster analysis applied to the **D** matrix is presented in Figure 3-2. The result agreed well with the structure suggested from PCA. In the earlier stage of clustering, the 17 subpopulations that formed a group in Figure 3-1 (A) were connected into a single cluster. The eight subpopulations with higher proportions of own bred breeding stocks (Table 3-3) were sequentially included in the cluster at the later stages. Because of the unique genetic composition (Figure 3-1 (A)), Hiroshima joined to the cluster at the final stage of clustering.

Throughout the breed history, the five subpopulations in Chugoku district (i.e., Hyogo, Tottori, Shimane, Okayama, and Hiroshima) have played important roles as

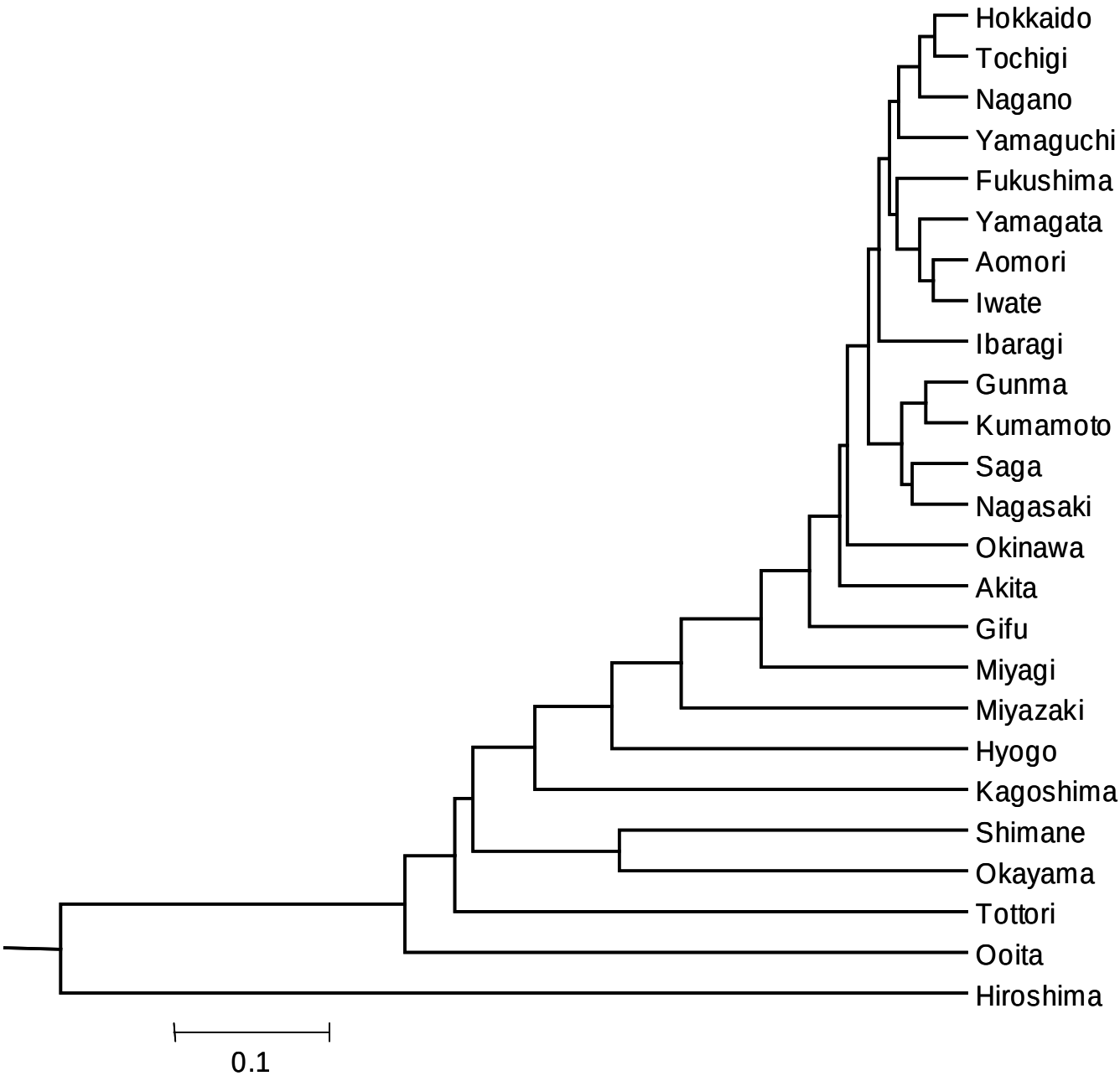


Figure 3-2. Dendrogram showing genetic relationships among 25 subpopulations based on Nei's standard genetic distance.

suppliers of breeding stocks to the entire breed (see general introduction). Nomura and Sasaki (1988) showed that most of the subpopulations of the breed in 1980 could be classified into five genetic groups each with unique relationships to the five subpopulations in Chugoku district. The grouping pattern shown in Figure 3-2 indicates that the breed structure had drastically changed during the past two decades. In the current breed, most of the subpopulations formed a single cluster. Among the five subpopulations in Chugoku district, Hyogo joined to the cluster in the earliest stage, indicating that the importance of Hyogo as a supplier of breeding stocks has been remarkably increased during the past two decades. Apparently, this is a reflection of the current emphasis on meat quality as the breeding objective of the breed.

Reduction of genetic diversity in the Japanese Black seems to have accompanied the disappearance of genetic subdivision (progress of genetic homogeneity). According to several researchers (Chesser et al. 1980; Lacy 1987, 1994), subdivided population structure with smaller breeding units undergoing different selections and connected by occasional migration is favorable for the maintenance of genetic diversity in the whole population. Caballero and Toro (2002) recently gave a basic theory to conserve genetic diversity in a subdivided population, and showed that genetic distances among subpopulations are one of the essential factors for genetic diversity of the whole population. Sölkner et al. (1998) also reported that large genetic diversity retained in the Austrian Simmental owes much to the population structure subdivided into partially isolated subpopulations. These studies suggest that reconstruction of genetically subdivided structure should be one of the effective solutions for the recovery of genetic diversity in the breed. For this purpose, creation and maintenance of several breeding herds supplying different types of genes to the whole population will be an urgent task. It would also largely contribute to the improvement for economically important traits

other than the carcass traits.

In the remaining three chapters, assuming a hierarchical population structure, in which favorable genes are accumulated in the breeding population (upper level of hierarchy) by artificial selection and the achieved genetic progress is transferred to the commercial females (lower level) mainly through the migration of males (Robertson and Asker 1951; Vu Tien Khang 1983), I will assess an optimum population structure of the breed by taking into account of the following four problems; 1) restriction of inbreeding coefficient of the commercial females, 2) restriction of inbreeding in the breeding population by the construction of several breeding herds (referred to as ‘sire lines’ hereafter), 3) maintenance of genetic diversity of the whole set of sire lines, and 4) maintenance of genetic diversity between the sire lines.

CHAPTER IV

Reduction of inbreeding in commercial females by rotational mating with several sire lines

Introduction

Control of the increase of inbreeding is a common policy in the maintenance of animal populations. To reduce inbreeding rate in conserved populations or control lines in selection experiments, many strategies, such as equalization of family sizes (Gowe et al. 1959; Wang 1997a), choice of parents to minimize average coancestry (Toro et al. 1999; Caballero and Toro 2000) and various systems of group mating (Rochambeau and Chevalet 1985; Nomura and Yonezawa 1996; Wang 1997a) have been proposed.

The hierarchical population structure has been reported in many livestock breeds in commercial use (e.g. Robertson and Asker 1951; Barker 1957; Vu Tien Khang 1983). In such a structure, different systems are required for reducing the inbreeding rate in the breed according to the levels of the hierarchy. As shown by many authors (Robertson 1961; Wray and Thompson 1990; Santiago and Caballero 1995; Nomura 1999), selection is inevitably accompanied by an increase in inbreeding. Thus, the main concern as to inbreeding in the upper level of the hierarchy is to maximize the genetic progress under restricted increase of inbreeding, and a large number of selection and mating systems for this purpose has been developed (e.g. Toro and Perez-Enciso 1990; Caballero et al. 1996; Meuwissen 1997; Sonesson and Meuwissen 2000).

Farmers in the lower level of the hierarchy usually rear females to produce commercial stocks and their replacements. Since the traits related to commercial production and reproduction can express strong inbreeding depression (Pirchner 1985;

Falconer and Mackay 1996), the suppression of increased inbreeding in the commercial females will also be a practically important issue.

In this chapter, a situation where males are rotationally supplied by several sire lines in the upper level of the hierarchy was supposed. One of the most efficient systems for the suppression of inbreeding in the commercial females will be the rotational use of the sire lines, as in rotational crossing with several breeds. The use of this mating system to reduce the inbreeding in commercial females was first proposed by Nozawa (1960). Using the methodology of path analysis, he worked out the recurrence equation of the inbreeding coefficient under rotational mating with sire lines mated by full-sib mating, and showed that this type of mating is quite an effective system to reduce the long-term inbreeding accumulation in the commercial females (Nozawa 1960). In this chapter, more general recurrence equations, which allow the evaluation of the effects of the number and size of sire lines and the initial relationship among them were derived. Based on numerical computations with the equations, the practical efficiency of the rotational mating system was examined.

Theory

(1) Theory of group coancestry

In the derivation, we apply group coancestry theory (Cockerham 1967,1970), which is an extension of the coancestry of individuals (Malécot 1969) to groups of individuals. Under random mating, the group coancestry has the same operational rule as the ordinary coancestry. For example, consider the group *x* of individuals with parental groups of *p* and *q*, each of which is descended from grandparental groups of *a*, *b*, and *c*, *d*, respectively. Letting $\phi_{p\cdot q}$ be the group coancestry between two groups *p* and *q*, the expected inbreeding coefficient (F_x) of individuals in group *x* is expressed as:

$$F_x = \phi_{p\cdot q} = \frac{1}{4}\left[\phi_{a\cdot c} + \phi_{a\cdot d} + \phi_{b\cdot c} + \phi_{b\cdot d}\right]. \tag{4-1}$$

The group coancestry of group *x* with itself is defined as the average pairwise coancestry including reciprocals and self-coancestries (Caballero and Toro 2000). Thus,

$$\phi_{x\cdot x} = \frac{1+F_x}{2N} + \frac{N-1}{N}\bar{\phi}_x, \tag{4-2}$$

where *N* is the number of individuals in group *x*, and $\bar{\phi}_x$ is the average pairwise coancestry among individuals (excluding self-coancestries).

(2) Mating scheme and population structure

We suppose a single commercial population of females, maintained by mating with sires rotationally supplied from *n* sire lines, each with the same constant size of N_m males and N_f females over generations. The sire lines are assumed to be completely closed to each other after the initiation of rotational mating, but with various degrees of relationships in the initial generations. Within each sire line, random mating and

discrete generations are assumed. Thus, the inbreeding coefficient in each sire line at generation t (F_t*) is computed by the recurrence equation

$$F_t^* = F_{t-1}^* + \frac{1}{2N_e}(1 - 2F_{t-1}^* + F_{t-2}^*)\,, \tag{4-3}$$

where $N_e = 4N_m N_f / \left(N_m + N_f\right)$ is the effective size of the sire line (Wright 1931).

The line supplying sires to the commercial females in a given generation is referred to as the supplier at that generation. We give sequential numbers 1, 2, …, n to the suppliers in generations 0, 1, …, n-1, respectively. Letting S_{t-i} be the sequential number of the supplier in generation t-i, S_{t-i} could be determined by

$$S_{t-i} = \mathrm{MOD}\left(t - i, n\right) + 1\,,$$

where $\mathrm{MOD}\left(x, n\right)$ is the remainder of x divided by n. Note that, because of the nature of rotational mating, $S_{t-i} = S_{t-i-kn}$ for a given integer number k. The groups of males and females in the sire line S_{t-i} are denoted by $m(S_{t-i})$ and $f(S_{t-i})$, respectively. The group coancestries within and between male and female groups are assumed to be equal in a given generation t-1:

$$\bar{\phi}_{m(S_{t-1}),\,t-1} = \bar{\phi}_{f(S_{t-1}),\,t-1} = \phi_{m(S_{t-1})\cdot f(S_{t-1}),\,t-1} = F_t^*\,. \tag{4-4}$$

The population of commercial females is denoted by c. Discrete generations with the same interval as the sire lines and random mating with supplied sires are assumed in the commercial population.

(3) Recurrence equation for inbreeding coefficient of commercial females

Rotational mating with unrelated sire lines:

We first consider the case with unrelated sire lines. In this case, it is apparent that the inbreeding coefficient (F_t) of the commercial females within the first cycle of rotation is zero; $F_t = 0$ for $t \le n$. In Figure 4-1, the pedigree diagram for $t \ge n+1$

is illustrated. Applying the operational rule of coancestry (eq. (4-1)) to the diagram, we get an expression of the inbreeding of the commercial females in generation $t \geq n+1$ as

$$F_t = \phi_{m(S_{t-1})\cdot c,t-1}$$

$$= \frac{1}{2^{n+1}}\left[\phi_{m(S_{t-1})\cdot m(S_{t-1})} + \phi_{f(S_{t-1})\cdot m(S_{t-1})} + \phi_{m(S_{t-1})\cdot c} + \phi_{f(S_{t-1})\cdot c}\right]_{t-n-1}. \qquad (4\text{-}5)$$

From (4-2) and (4-4), the first two group coancestries in (4-5) are

$$\phi_{m(S_{t-1})\cdot m(S_{t-1}),t-n-1} = \frac{1+F^*_{t-n-1}}{2N_m} + \frac{N_m-1}{N_m}\bar{\phi}_{m(S_{t-1}),t-n-1} = \frac{1+F^*_{t-n-1}}{2N_m} + \frac{N_m-1}{N_m}F^*_{t-n}$$

and

$$\phi_{f(S_{t-1})\cdot m(S_{t-1}),t-n-1} = F^*_{t-n}.$$

Furthermore, by noting that the supplier in generation *t*-1 should be also the supplier in generation *t-n*-1 (*i.e.* $S_{t-1} = S_{t-n-1}$), the last two group coancestries in (4-5) could be written as

$$\phi_{m(S_{t-1})\cdot c,t-n-1} = \phi_{f(S_{t-1})\cdot c,t-n-1} = F_{t-n}.$$

Substituting these expressions into equation (4-5) leads to the recurrence equation for the inbreeding coefficient of the commercial females as

$$F_t = \frac{1}{2^{n+1}}\left[\frac{1+F^*_{t-n-1}}{2N_m} + \frac{2N_m-1}{N_m}F^*_{t-n} + 2F_{t-n}\right]. \qquad (4\text{-}6)$$

Note that when *n*=1, the assumed mating system reduces to the closed nucleus breeding system. It can be verified that the asymptotic rate of inbreeding ($\Delta F = (F_t - F_{t-1})/(1-F_{t-1})$) of equation (4-6) with *n*=1 depends only on the effective size of sire line, and is approximated by $\Delta F = 1/(2N_e)$, agreeing with the previous result for the closed nucleus breeding system (James 1977, 1978).

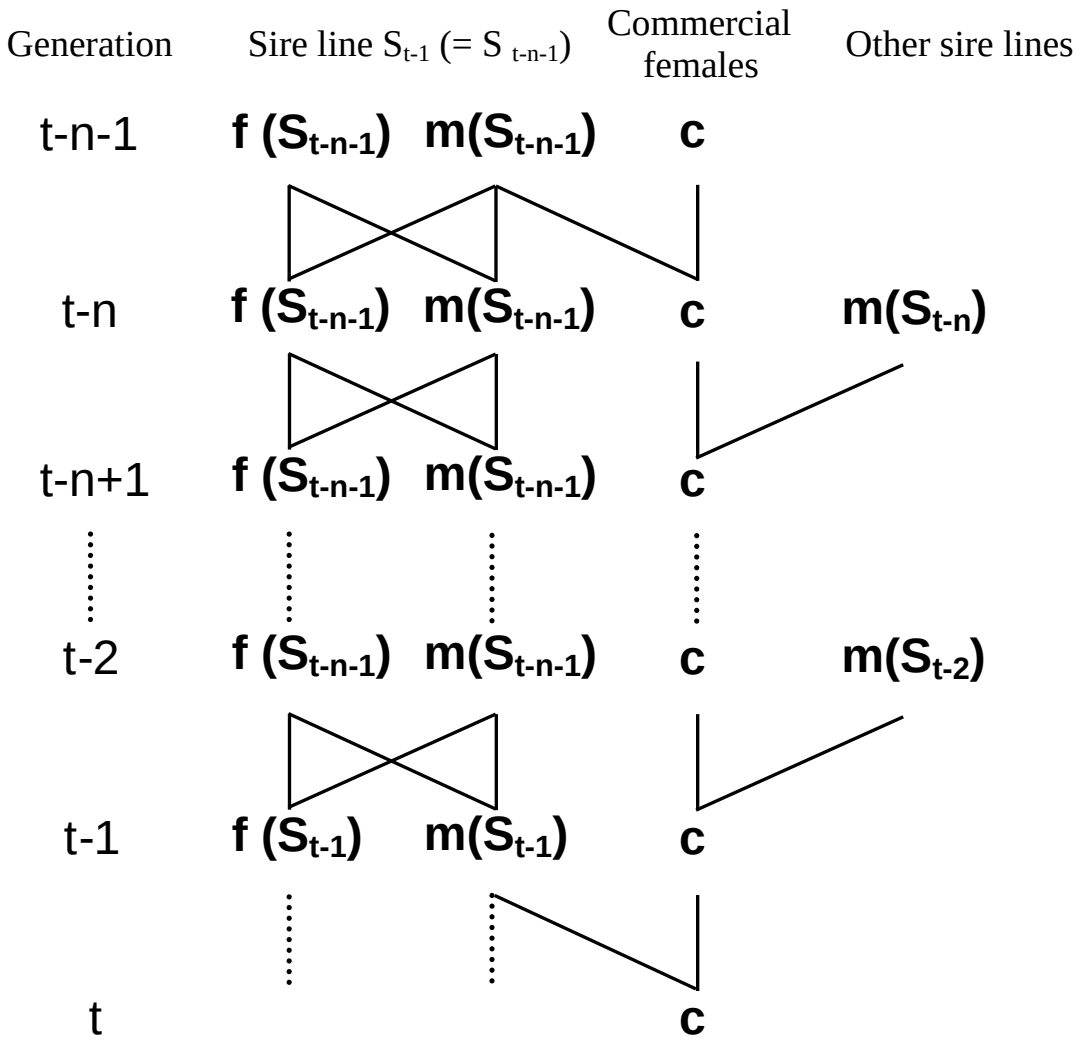


Figure 4-1. Rotational mating with *n* sire lines for $t \geq n+1$. The commercial females are rotationally mated with sires supplied from male group of *n* sire lines (m(·)). The sire line S_{t-1}, which supplies sires for the mating at generation *t*-1, appeared as S_{t-n-1} in the previous cycle of mating (at generation *t*-*n*-1). Between these two generations, *n*-1 different sire lines (from S_{t-n} to S_{t-2}) supply sires rotationally. Mating within each sire line, except for S_{t-1}, are omitted for simplification.

Rotational mating with related sire lines:

When related sire lines are used, the inbred commercial females appear within the first cycle of rotation. Prior to the derivation, we derive a fundamental property of group coancestry between two closed lines. Figure 4-2 shows two closed lines *x* and *y* with initial relationships $\phi_{m(x)\cdot m(y),0}$, $\phi_{m(x)\cdot f(y),0}$, $\phi_{f(x)\cdot m(y),0}$ and $\phi_{f(x)\cdot f(y),0}$. On the assumption of random mating within each line, the group coancestry between lines *x* and *y* at generation $t \geq 1$ is constant over generations, i.e.,

$$\phi_{x\cdot y,t} = \frac{1}{4}\left[\phi_{m(x)\cdot m(y)} + \phi_{m(x)\cdot f(y)} + \phi_{f(x)\cdot m(y)} + \phi_{f(x)\cdot f(y)}\right]_0 = R_{(x,y)}. \tag{4-7}$$

Consider first the inbreeding coefficient of commercial females within the first cycle of rotation. Applying the operational rule (eq. (4-1)) to the pedigree diagram of Figure 4-3 (1) leads to the inbreeding coefficient of commercial females in generation 2 as

$$F_2 = \phi_{m(2)\cdot c,1} = Q_{(2,1)}, \tag{4-8}$$

where $Q_{(2,1)}$ is generally defined as $Q_{(x,1)} = \frac{1}{4}\left[\phi_{m(x)\cdot m(1)} + \phi_{f(x)\cdot m(1)}\right]_0$. In Figure 4-3 (2), the pedigree diagram for $t = 3\,(n \geq 3)$ is illustrated. With the application of equations (4-1) and (4-7) to this diagram, the inbreeding coefficient in generation 3 is obtained as

$$\begin{aligned} F_3 &= \frac{1}{4}\left[\phi_{m(3)\cdot m(2)} + \phi_{f(3)\cdot m(2)}\right]_1 + \frac{1}{8}\left[\phi_{m(3)\cdot m(1)} + \phi_{f(3)\cdot m(1)}\right]_0 \\ &= \frac{1}{2}R_{(3,2)} + \frac{1}{2}Q_{(3,1)}. \end{aligned}$$

With the same argument, the inbreeding coefficient in generation $3 \leq t \leq n$ can be generally expressed as

$$F_t = \sum_{i=2}^{t-1} \frac{1}{2^{i-1}} R_{(t,t-i+1)} + \frac{1}{2^{t-2}} Q_{(t,1)}. \tag{4-9}$$

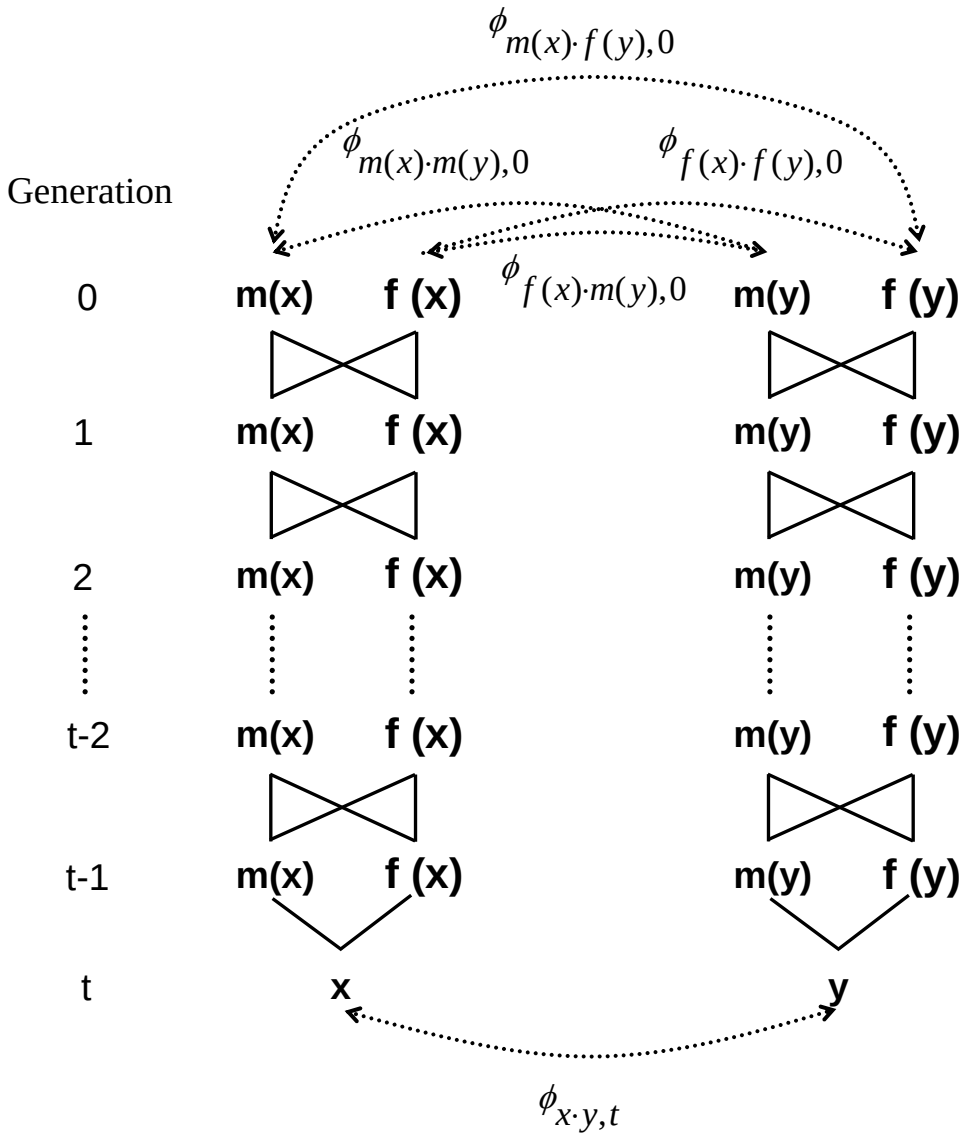
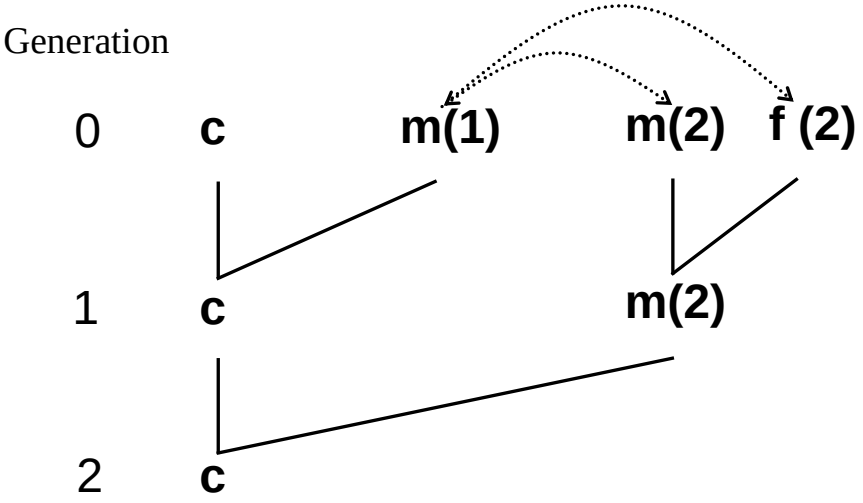


Figure 4-2. Illustration for relationships between two closed sire lines x and y at generation $t \geq 1$, with the initial relationships $\phi_{m(x)\cdot m(y),0}$, $\phi_{m(x)\cdot f(y),0}$, $\phi_{f(x)\cdot m(y),0}$ and $\phi_{f(x)\cdot f(y),0}$.

(1) t =2



(2) t =3

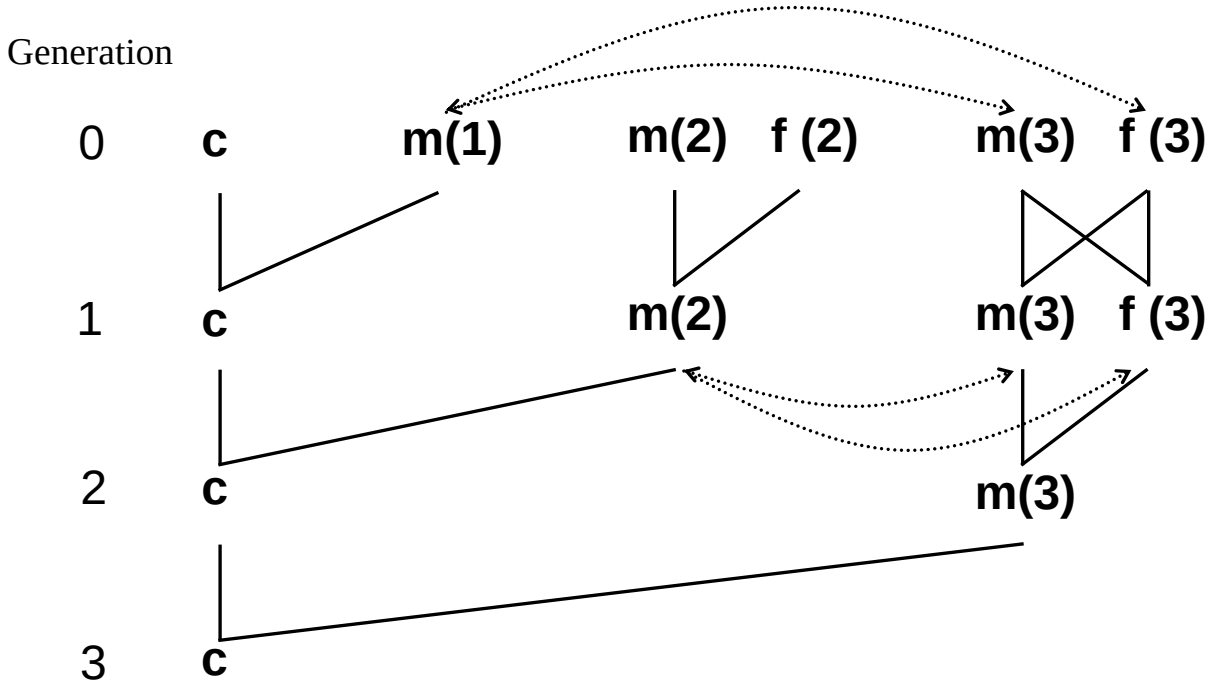


Figure 4-3. Pedigree diagrams to derive the inbreeding coefficient (F_t) of commercial females within the first cycle of rotational mating at (1) t =2 and (2) t =3 (for $n \geq 3$). The group coancestries relevant to F_t are shown by dashed curves with arrows of both directions.

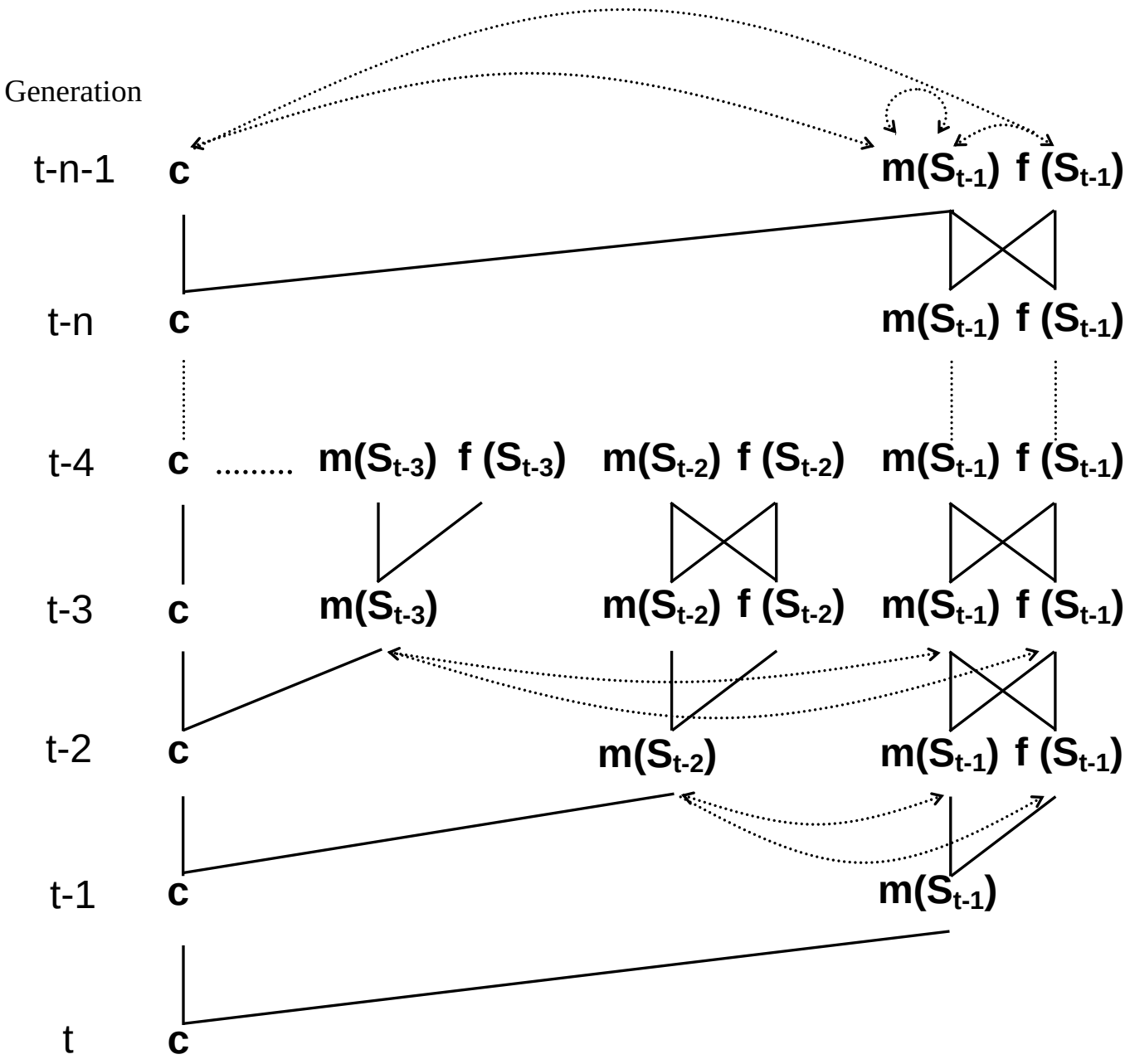


Figure 4-4. Pedigree diagram to derive the inbreeding coefficient (F_t) of commercial females after the first cycle of rotational mating ($t \geq n+1$). The group coancestries relevant to F_t are shown by dashed curves with arrows of both directions.

Next, consider the inbreeding coefficient of commercial females after one cycle of rotational mating, *i.e.* $t \geq n+1$. The pedigree diagram is illustrated in Figure 4-4. Applying the operational rule (equation (4-1)) to the pedigree diagram, we get an expression of F_t as

$$F_t = \frac{1}{2^{n+1}}\left[\phi_{m(S_{t-1})\cdot m(S_{t-1})} + \phi_{f(S_{t-1})\cdot m(S_{t-1})} + \phi_{m(S_{t-1})\cdot c} + \phi_{f(S_{t-1})\cdot c}\right]_{t-n-1}$$
$$+\sum_{i=2}^{n}\frac{1}{2^i}\left[\phi_{m(S_{t-1})\cdot m(S_{t-i})} + \phi_{f(S_{t-1})\cdot m(S_{t-i})}\right]_{t-i}.$$

With equations (4-6) and (4-7), the above expression reduces to

$$F_t = \frac{1}{2^{n+1}}\left[\frac{1+F^*_{t-n-1}}{2N_m} + \frac{2N_m-1}{N_m}F^*_{t-n} + 2F_{t-n}\right] + \sum_{i=2}^{n}\frac{1}{2^{i-1}}R_{(S_{t-1},S_{t-i})}\,. \qquad (4\text{-}10)$$

(4) Asymptotic inbreeding coefficient of commercial females

Rotational mating with unrelated sire lines:

When unrelated sire lines are used, the inbreeding coefficient of the commercial population eventually reaches an asymptotic value. The asymptotic value F_∞ can be obtained by the following consideration.

Since there is no gene flow among sire lines, each line will eventually be fixed, and then

$$F^*_\infty \equiv F^*_{t-n-1} = F^*_{t-n} = 1\,.$$

Substituting this into equation (4-6) gives

$$F_t = \frac{1}{2^n}(1+F_{t-n})\,.$$

Since F_t and F_{t-n} can be replaced by F_∞ in the asymptotic state, the asymptotic value is obtained as

$$F_{\infty} = \frac{1}{2^n - 1}. \tag{4-11}$$

Rotational mating with related sire lines:

With initial relationship among sire lines, the asymptotic expression for the inbreeding coefficient of the commercial females is complicated because the second term in (4-10) does not converge to a single asymptotic value when $n \geq 3$. For a sufficiently large *t*, we denote the suppliers before i generations as S_{-i}. With an analogous argument to the previous case, an asymptotic expression could be obtained as

$$F_{\infty} = \frac{1}{2^n - 1}\left[1 + \sum_{i=2}^{n} 2^{n-i+1} R_{(S_{-1}, S_{-i})}\right]. \tag{4-12}$$

Equation (4-12) converges to a single value for *n*=2, but shows a regular oscillation with a cycle of *n* generations for $n \geq 3$.

Numerical computations

Rotational mating with unrelated sire lines:

To assess the effects of the number (n) and the size (N_m and N_f) of sire lines on the accumulation of inbreeding in the commercial females (F_t), numerical computations with (4-3) and (4-6) were carried out for the combinations of $n = 2$, 3, 4 and 5 and $N_m = 2$, 5 and 10. Figures 4-5 (A), (B) and (C) show the results of $N_m = 2$, 5 and 10, respectively, under various n and a fixed N_f (=200). For a given size of sire line, an increase of n reduces F_t, but the effect becomes trivial when $n \geq 4$. Comparison among Figures 4-5 (A)-(C) reveals that although an increase of N_m has a pronounced effect on F_t for a relatively small n (say $n \leq 3$), the effect is diminished as n becomes larger. For example, the inbreeding coefficients of commercial females with $n = 2$ reached 22.5%, 12.4% and 7.1% in generations 20 for $N_m = 2$, 5 and 10, respectively, while the corresponding values with n=5 were 2.0%, 1.1% and 0.6%, respectively.

As seen from (4-3) and (4-6), the number of females in each sire line (N_f) affects the inbreeding coefficient of the commercial females only through the effective size of sire lines (N_e in equation (4-3)). Since the individual number of the less numerous sex, *i.e.* the number of males in the domestic animals case, is the major factor for determining the effective size, it is expected that an increase of N_f has little effect on the accumulation of inbreeding in the commercial females. For example, the inbreeding coefficient in the commercial females for the case of N_m=5 and N_f =1000 showed no essential differences from that for the case of N_m=5 and N_f =200.

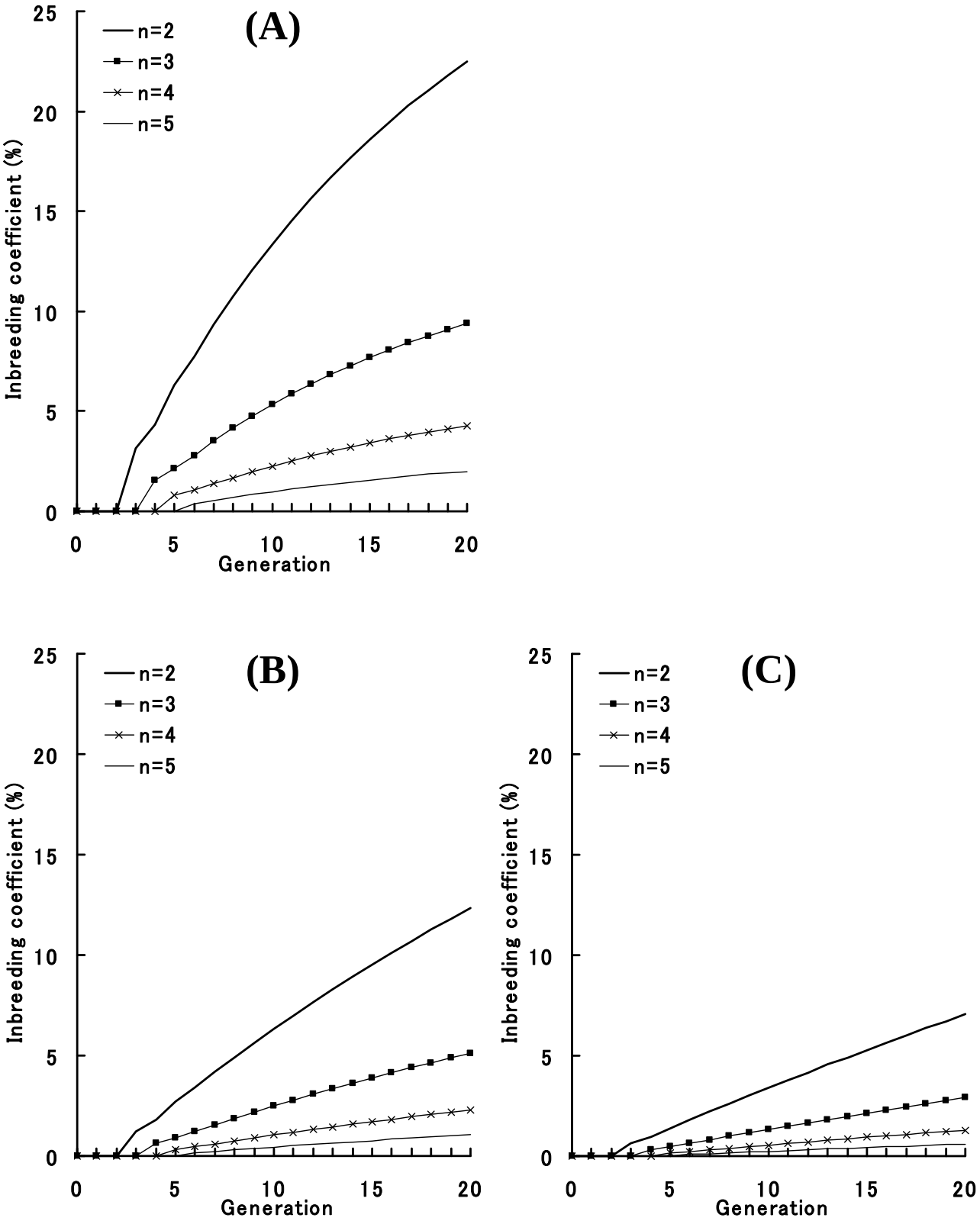


Figure 4-5. Inbreeding coefficient of the commercial females under rotational mating using *n* unrelated sire lines, with the sizes of (A) N_m=2 males and N_f =200 females, (B) N_m=5 and N_f =200, and (C) N_m=10 and N_f =200.

Rotational mating with related sire lines:

The average coancestries among five breeding herds (*i.e.* Hyogo (HY), Tottori (T), Shimane (S), Okayama (O) and Hiroshima (HR) prefectures) of the Japanese Black were used to illustrate the effect of initial relationships among sire lines. The average coancestries among the five herds estimated in the chapter III are reproduced in Table 4-1. We suppose a simple situation where a sire line with N_m=2 and N_f =200 is constructed from each of the five herds, and the rotational mating system is applied to a hypothetical population of commercial females. For simplicity of the computation, the five sire lines are assumed to be initially inbred with the same degree of $F_0^* = 0.06$, which is the average inbreeding coefficient in the current breed (see chapter I). The orders of the use of sire lines in the commercial population were assumed to be HY-T, HY-T-S, HY-T-S-O and HY-T-S-O-HR for *n*=2, 3, 4, and 5, respectively. The inbreeding coefficient of the commercial population computed from equation (4-10) is shown in Figure 4-6.

Although the inbreeding coefficient in the commercial population is higher than the corresponding value of the case with unrelated sire lines (c.f. Fig. 4-5 (A)), the rotational mating with four or five sire lines can essentially suppress the increase of inbreeding in the commercial females. As seen from the additional term due to the initial relationships in equation (4-10), the oscillation pattern observed in the cases with $n \geq 3$ depends on the order of the use of sire lines, and a sharp increase of inbreeding manifests when highly related sire lines are used in two consecutive generations.

Table 4-1. The average coancestries of the Japanese Black cattle in five subpopulations of traditional breeding prefectures.

Subpopulation*		HY		T		S		O		HR	
	Sex**	M	F	M	F	M	F	M	F	M	F
HY	M	-	-								
	F	-	-								
T	M	0.020	0.021	-	-						
	F	0.035	0.037	-	-						
S	M	0.039	0.038	0.040	0.052	-	-				
	F	0.031	0.031	0.035	0.045	-	-				
O	M	0.018	0.018	0.021	0.028	0.054	0.053	-	-		
	F	0.037	0.038	0.025	0.034	0.059	0.056	-	-		
HR	M	0.040	0.038	0.016	0.021	0.033	0.027	0.016	0.021	-	-
	F	0.040	0.038	0.015	0.017	0.024	0.019	0.011	0.016	-	-

* Subpopulations of Hyogo (HY), Tottori (T), Shimane (S), Okayama (O), and Hiroshima (HR) prefectures.

** M: male, F: female

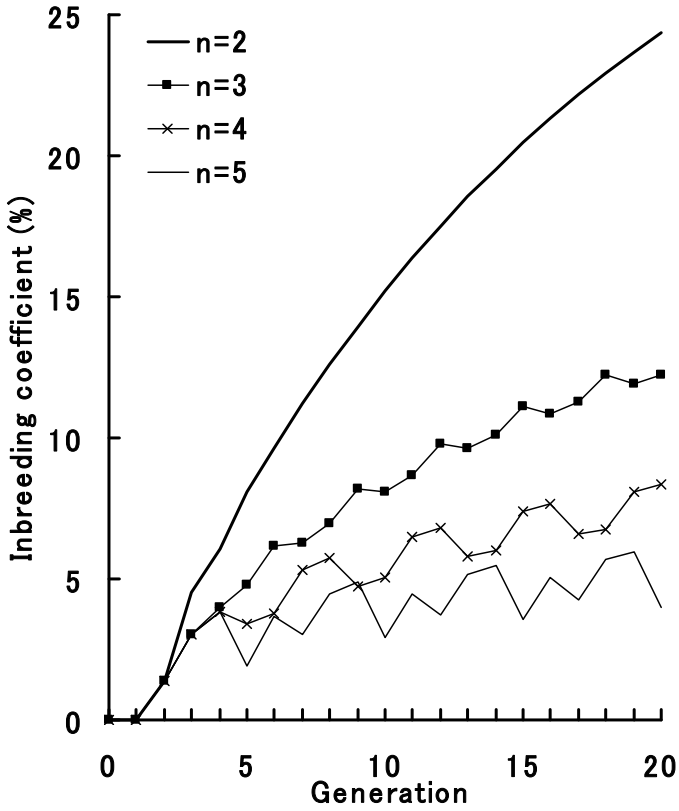


Figure 4-6. Inbreeding coefficient of the commercial females using *n* related sire lines shown in Table 4-2, each with the size of N_m=2 males and N_f =200 females.

Asymptotic inbreeding coefficient in commercial females:

Asymptotic inbreeding coefficients of commercial females are presented in Table 4-2, for the cases with unrelated and related sire lines. With unrelated sire lines, the asymptotic inbreeding coefficient of the commercial females can be suppressed to less than 10% when $n \geq 4$. Even if the sire lines are initially related, the initial relationships generate no serious increases of the asymptotic inbreeding.

Table 4-2. Asymptotic inbreeding coefficients (%) of commercial females with *n* unrelated sire lines and related sire lines. With related sire lines, inbreeding shows a regular oscillation according to coancestries among consecutive suppliers, HY, T, S, O, and HR, shown in Tab. 4-1.

		Related sire lines				
n	Unrelated sire lines	HY	T	S	O	HR
1	100	-	-	-	-	-
2	33.3	35.2	35.2	-	-	-
3	14.3	17.1	17.1	17.8	-	-
4	6.7	9.4	9.5	10.6	10.7	-
5	3.2	6.6	5.7	7.1	7.3	5.2

Discussion

There is much evidence that the inbreeding coefficients of females have substantial effects on commercially important traits, such as milk yield and egg production (Pirchner 1985). The traits relevant to meat production, such as carcass weight and daily gain, are also affected by the inbreeding of dams (Pirchner 1985). In recent years, various mating and selection strategies have been proposed to reduce the rates of inbreeding in selection programmes while keeping genetic gains at a given level (e.g. Toro and Perez-Enciso 1990; Caballero et al. 1996; Meuwissen 1997; Sonesson and Meuwissen 2000; Oyama and Mukai 2001). However, most of these methods target small nucleus populations, in which selection and mating can be intensively managed, and may not be implemented in the commercial female populations, where the choice of sires to be mated with the females depends on the strategies of individual farmers. The mating system considered in the present chapter will have practical merit in its application to the commercial female population, because it does not require systematic controls of mating over the population, such as the systematic mating allocation required in the minimum coancestry mating (Caballero et al. 1996). The proposed system can be arranged by individual farmers according to their strategies. For example, if five sire lines (say A, B, C, D and E) are available, some farmers could rotationally use the four lines A, B, C and D, while others could adopt the mating with the four lines B, C, D and E.

Although I have revealed that genetic diversity of the Japanese Black has been reduced by the intensive use of sires from limited strains (chapters I and II), five regional subpopulations still maintain their unique genetic compositions (see chapter III). In this chapter, it was theoretically shown that reconstruction of several breeding herds with existing genetic materials and the rotational use of them will largely

contribute to the reduction of inbreeding in the commercial females. In addition, it will also contribute to the maintenance of genetic diversity in the breed through the balanced genetic contributions of the herds. The proposed mating system could be applied also to the other livestock breeds with hierarchical structure.

Numerical computations have shown that the critical factor for determining the inbreeding in commercial females is the number of sire lines, and the size of each sire line has a minor effect. In practice, if four or five sire lines are available, rotational mating could efficiently function to reduce the short- and long-term inbreeding of the commercial females, irrespective of the effective size of each sire line. When the sire lines are initially related, an oscillation of the inbreeding coefficient may occur in commercial females. A sharp increase in inbreeding in one generation may cause a serious inbreeding depression, as experimentally shown by Beilharz (1982). To minimize the oscillation of inbreeding, the use of two highly related lines in consecutive generations should be avoided.

Ultimately, the inbreeding coefficient of commercial females reaches an intermediate value (c.f. (4-11) and (4-12)). This is a special case of the theoretical result of Robertson (1964). He showed that a population split into permanent sublines will not genetically fix, because allele fixation occurs independently in each subline. In the present study, the assumption of closed sire lines will lead to fixation of the lines with different alleles, and thus the commercial females retain heterozygosity in the ultimate state. In the study of crossbreeding, Dickerson (1973) showed that the fraction of heterosis expected under rotational crossing with n breeds is $\left(2^n-2\right)/\left(2^n-1\right)$. This agrees with the expected ultimate heterozygosity in the commercial females predicted by equation (4-11), that is $1-F_\infty=1-1/\left(2^n-1\right)$.

In the derivation of the theory, we have made several simplifications. Among them,

the most critical one is the neglect of selection in sire lines. Selection will inflate the inbreeding coefficient in a sire line (Robertson 1961; Wray and Thompson 1990; Santiago and Caballero 1995; Nomura 1999). Due to the inflated inbreeding, the inbreeding in the commercial females will be also enhanced (c.f. (4-6) and (4-10)). This effect could be approximately taken into account by computing the effective size of sire lines with the formula incorporating the effect of selection. Theories for predicting the effective population size have been developed for various types of selection (e.g. Wray et al. (1994) for index selection, and Bijma et al. (2001) for BLUP selection).

In a strict sense, the application of derived equations is limited to neutral loci. If intense selection for the same breeding objective is carried out in all sire lines, it will tend to increase the genetic resemblance between sire lines, even if they are maintained separately. Thus the same favorable alleles will be fixed in most sire lines, and the heterozygosity for the loci in the commercial females may be less than that predicted.

In practice, sire lines may have different sizes. The problem of unequal sizes of sire lines could be solved straightforwardly by applying the recurrence equation (4-3) to each sire line. The inbreeding coefficient of the commercial females is then obtained by replacing F^*_{t-n} and F^*_{t-n-1} in (4-6) and (4-10) by the corresponding inbreeding coefficient of the supplier in generation *t*-1.

To apply rotational mating to species with overlapping generations, the sire lines should be rotated based on the turnover of generations in commercial females. Although the recurrence equations (4-6) and (4-10) can not be strictly applied, the inbreeding coefficient could be approximated with these equations, so long as there are no large differences among generation intervals of sire lines and commercial females. Since the asymptotic equations (4-11) and (4-12) hold exactly even in the case of

overlapping generations, the upper limit of inbreeding can be estimated with these equations.

Finally, although we have assumed that sire lines are closed to each other after the initiation of rotational mating, some amount of migration should be actually allowed among the sire lines to avoid the deleterious inbreeding effect within each of them. A recent study by Wang (1997b) predicts that managed migration among subpopulations could reduce the inbreeding rate in each subpopulation to an acceptable level. However, through migration of animals, new relationships would be generated among sire lines, and will inflate the inbreeding of the commercial females. More detailed theoretical study on this subject is carried out in the final chapter.

CHAPTER V

Genetic management of partially isolated lines in the breed

Introduction

In the previous chapter, only the suppression of inbreeding coefficient in the lower level of the hierarchy was considered. However, for sustainable use of sire lines, a well-designed management plan should be established in the breeding population.

In designing genetic conservation programs of subdivided animal populations, a crucial question is how much gene flow should be allowed between the subpopulations. In designing a conservation program for endangered species with fragmented habitat, much effort has been devoted to finding a migration rate to integrate the subpopulations into an essentially single breeding population (e.g. Franklin 1980; Frankel and Soulé 1981; Roux 1995).

In domestic animal populations with subdivided structure, a considerable amount of genetic diversity exists between subpopulations (e.g. Sölkner et al. 1998). Genetic diversity between subpopulations could be a greater potential for the future breeding materials than the same amount of the diversity, but dispersed over the entire population, because it saves time to assemble genes conforming to various commercial demands. Thus, an appropriate amount of migration in a population with subdivided structure should be determined so as to maintain genetic diversity both within and between subpopulations. Apparently, this problem involves a trade-off. If there are large amount of gene flow among subpopulations, rate of inbreeding within each of them will be effectively suppressed, but genetic diversity among them will be rapidly decayed, and vice versa.

As shown in chapter II, the five subpopulations in Chugoku district (i.e., Hyogo,

Tottori, Shimane, Okayama and Hiroshima prefectures) have played important roles as suppliers of breeding stocks. These subpopulations still maintain their unique genetic compositions (see Chapter III). Thus, they could be candidates for the base population, from which sire lines are constructed.

In this chapter, I supposed a situation where lines are constructed from these five subpopulations, and a management plan to conserve the genetic diversity within and between the lines is applied to the set of lines. Based on population genetic theories, the optimal migration rate among lines and the required size of lines are assessed.

Materials and Methods

Model and assumptions:

Consider a situation where n lines are constructed each from n subpopulations. Each line has a constant size of N_m males and N_f females over generations. Mating within line is assumed to be at random, and the numbers of male and female offspring (which become parents in the next generation) per parent follow independent Poisson distributions within the sex of parent, giving the effective size of line as $N_{e,s} = 4N_m N_f \big/ \left(N_m + N_f\right)$.

As a migration pattern among the lines, we first assume the island model (Wright 1943), in which each line receives a proportion of d_m males and d_f females drawn at random from the other lines before mating of each generation. Effects of departure from this simplified model will be discussed later.

Probabilities of gene identity by descent:

To describe the progress of the gene identity by descent under the island model, three coefficients, i.e. the inbreeding coefficient within each line ($F_{(t)}$), and coancestries between two individuals within and among lines before migration ($\theta_{(t)}$ and $\alpha_{(t)}$, respectively) should be considered (Wang 1997b). Note that when the numbers of male and female offspring follow independent Poisson distributions, there is no need to distinguish the coancestry between individuals according to their sexes (see Wang (1997b) for more detail). Let q_{mf} and q'_{mf} be the probabilities that two individuals of different sexes, taken at random from the same line and the different lines after migration, come from the same line before migration, respectively. Similarly, q_{vv}, and q'_{vv} are defined as the probabilities that two individuals of the same sex v (=m for male or f for female), taken at random and without replacement from the same line and the

different lines after migration, come from the same line before migration, respectively. In the ordinary finite island model (e.g. see Wang 1997b), emigrants from a line are allowed to come back to their birth line as migrants. However, since it should be considerably a rare phenomenon, this possibility is removed from the present model, giving expressions for the four probabilities q_{mf}, q'_{mf}, q_{vv}, and q'_{vv} as

$$q_{mf} = (1-d_m)(1-d_f) + \frac{d_m d_f}{n-1}$$

$$q'_{mf} = \frac{(d_m + d_f)(n-1) - n d_m d_f}{(n-1)^2}$$

$$q_{vv} = (1-d_v)\left(1 - \frac{N_v d_v}{N_v - 1}\right) + \frac{d_v (N_v d_v - n + 1)}{(N_v - 1)(n-1)}$$

$$q'_{vv} = \frac{d_v (2n - n d_v - 2)}{(n-1)^2}.$$

With these probabilities, the inbreeding and coancestry coefficients are obtained from the recurrence equation of Wang (1997b) as follows,

$$\begin{bmatrix} F_{(t)} \\ \theta_{(t)} \\ \alpha_{(t)} \end{bmatrix} = \begin{bmatrix} 0 & q_{mf} & 1-q_{mf} \\ \frac{1}{2N_{e,s}} & 1 - \frac{Q}{4} - \frac{q_{mm}}{4N_m} - \frac{q_{ff}}{4N_f} & \frac{Q}{4} - \frac{1-q_{mm}}{4N_m} - \frac{1-q_{ff}}{4N_f} \\ 0 & 1 - \frac{Q'}{4} & \frac{Q'}{4} \end{bmatrix} \begin{bmatrix} F_{(t-1)} \\ \theta_{(t-1)} \\ \alpha_{(t-1)} \end{bmatrix} + \begin{bmatrix} 0 \\ \frac{1}{2N_{e,s}} \\ 0 \end{bmatrix}, \quad (5\text{-}1)$$

where

$$Q = 4 - q_{mm} - 2q_{mf} - q_{ff},$$

and

$$Q' = 4 - q'_{mm} - 2q'_{mf} - q'_{ff}.$$

Genetic diversity of the sire lines:

Recent study of Caballero and Toro (2002) showed that total genetic diversity of subdivided population can be partitioned into the components of within and between subpopulations. Suppose a subdivided population of n subpopulations, in which subpopulation i is consisted of N_i individuals ($\sum_{i=1}^{n} N_i = N_T$). Let the average self-coancestry and the average inbreeding coefficient of subpopulation i be s_i and $F_i = 2s_i-1$, and let the average distance between individuals of subpopulations i and j and Nei's minimum distance (Nei 1987) between subpopulations i and j be D_{ij} and $\mathbf{D}_{ij}$ ($\mathbf{D}_{ii}$=0), respectively. With the average coancestry over the whole population ($\bar{f}$), total genetic diversity (expected heterozygosity; Nei 1973) of the population (GD) is $GD = 1 - \bar{f}$, which is decomposed as

$$GD = (1 - \tilde{s}) + \tilde{D} + \overline{\mathbf{D}}, \tag{5-2}$$

where

$$\tilde{s} = \frac{\sum_{i=1}^{n} s_i N_i}{N_T}, \tag{5-3}$$

$$\tilde{D} = \frac{\sum_{i=1}^{n} D_{ii} N_i}{N_T}, \tag{5-4}$$

$$\overline{\mathbf{D}} = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n} \mathbf{D}_{ij} N_i N_j}{N_T^2} \tag{5-5}$$

(Caballero and Toro 2002). The first and the second term on the right hand side are genetic diversity within individuals and between individuals, respectively, and the sum of these two elements gives genetic diversity within subpopulations. The third term is genetic diversity between subpopulations (GD_{BS}), i.e., $GD_{BS} = \overline{\mathbf{D}}$.

Noting the symmetric property of the island model and using three gene identities

obtained from equation (5-1),

$N_i = N_m + N_f$, $s_i = \frac{1+F_{(t)}}{2}$, $D_{ii} = \frac{N-1}{N}\left(\frac{1+F_{(t)}}{2} - \theta_{(t)}\right)$ for any i, and

$\mathbf{D}_{ij} = \frac{1+F_{(t)} + 2(N-1)\theta_{(t)} - 2N\alpha_{(t)}}{2N}$ for any combinations of i and j ($i \neq j$),

respectively. Finally, from equation (5-5), genetic diversity between sire lines at generation *t* can be obtained as

$$GD_{BS(t)} = \frac{(n-1)\{1+F_{(t)} + 2(N-1)\theta_{(t)} - 2N\alpha_{(t)}\}}{2nN},$$

and from equation (5-2), (5-3) and (5-4), total genetic diversity of the whole set of lines at generation *t* can be obtained as

$$GD_{(t)} = 1 - \frac{1+F_{(t)} + 2(N-1)\theta_{(t)} + 2(n-1)N\alpha_{(t)}}{2nN}.$$

Distribution of genes over the set of lines:

For the assessment of the maintainability of the initial genetic diversity between the lines, the distribution of genes over the set of lines originated from each line in generation 0 was computed with the migration matrix defined by Bodmer and Cavalli-Sforza (1968).

Let m_{ij} be the proportion of individuals migrating from line j to line i in each generation. If the migration rates are estimated for separate sexes, $m_{ij} = (d_{m,ij} + d_{f,ij})/2$, where $d_{m,ij}$ and $d_{f,ij}$ are the male and female migration rates, respectively. We define the migration matrix as $\mathbf{M} = [m_{ij}]$. Letting $\mathbf{Q}_t = [q_{ij(t)}]$ be the matrix of frequency of allele *j* in line *i* at generation *t*,

$$\mathbf{Q}_t = \mathbf{M}\mathbf{Q}_{t-1},$$

therefore,

$$\mathbf{Q}_t = \mathbf{M}^t \mathbf{Q}_0 .$$

The element in the matrix $\mathbf{M}^t$ in generation *t*, $m_{ij(t)}$, gives the proportion of genes in line *i* at generation *t* that have descended from line *j* in generation 0 (Hedrick, 1983). The main variables relevant to the structure and genetic parameters of sire lines are listed in Table 5-1.

Numerical analysis:

We suppose that five sire lines ($n = 5$) are constructed each from the five regional subpopulations (Hyogo, Tottori, Shimane, Okayama and Hiroshima) in the breed. Table 5-2 shows the annual census numbers of breeding animals, generation intervals (*L*), annual effective population sizes ($N_{e(y)}$), and annual effective numbers of males ($N_{m(y)} = N_{e(y)}/4$) in the five subpopulations. The effective population size was estimated with the formula given by Nomura (2002), i.e.,

$$N_e = \frac{4N_m}{1+C^2},$$

in which the census number of breeding males (N_m) and the coefficient of variation of progeny numbers among the males (C^2) are accounted for.

In the numerical analysis, maximum number of males in each line was chosen as 10. Since the generation interval averaged over the five subpopulations is around 10 (Table 5-2), $N_m = 10$ implies that one male (in the effective number) is selected per year in each line. The estimated annual effective numbers of males in the five subpopulations (Table 5-2) suggest that a realizable maximum would not be largely differ from $N_{m(y)}$=1 in each line. The number of females (N_f) was fixed to 200, because preliminary analysis showed that the number of females has a minor effect on results.

Various rates of male migration (d_m=0~0.5) were examined by fixing the female migration rate to zero ($d_f = 0$).

Table 5-1. List of main variables used in computations.

Variables	Definition
Structure of sire lines.	
n	The number of sire lines.
N_v	The number of animals of sex v (=m for male or f for female) in each line.
$N_{e,s}$	Effective size of each line, i.e., $N_{e,s} = 4N_m N_f / (N_m + N_f)$.
d_v	Migration rate of animals of sex v.
Probabilities of gene identity by descent.	
$F_{(t)}$	Inbreeding coefficient within each line before migration in generation t.
$\theta_{(t)}$	Coancestries between two individuals within line before migration in generation t.
$\alpha_{(t)}$	Coancestries between two individuals among lines before migration in generation t.
Measurements of genetic diversity.	
$GD_{(t)}$	Total genetic diversity of the set of lines in generation t.
$GD_{BS(t)}$	Genetic diversity among the lines in generation t.
Migration matrix.	
$d_{v,ij}$	Migration rate through animals of sex v from line j to line i in each generation.
m_{ij}	Proportion of animals migrating from line j to line i in each generation, i.e., $m_{ij} = (d_{m,ij} + d_{f,ij})/2$.
M	Migration matrix composed of m_{ij} in the element (i , j).
$m_{ij(t)}$	Proportion of genes in line i at generation t that have descended from line j in generation 0. This is obtained as the element (i , j) of $\mathbf{M}^t$.

Table5-2. Annual census numbers of breeding males and females, the generation interval (L), the annual effective size ($N_{e(y)}$), and the annual effective number of males ($N_{m(y)}$) in the five subpopulations of the Japanese Black.

	Annual number				
Subpopulation	Male	Female	L	$N_{e(y)}$	$N_{m(y)}$
Hyogo	9.2	1260.3	10.2	2.06	0.52
Tottori	8.7	418.8	9.8	3.92	0.98
Shimane	18.3	1106.0	10.2	2.85	0.71
Okayama	20.0	506.7	8.9	6.34	1.59
Hiroshima	10.7	558.2	8.2	3.91	0.98
Average	13.4	770.0	9.5	3.82	0.96

All the values are the averages over 1996-2001.

Results and discussion

Figures 5-1 (A), (B) and (C) exemplify the changes of the inbreeding coefficient within line ($F_{(t)}$), total genetic diversity of the set of lines ($GD_{(t)}$), and genetic diversity between lines ($GD_{BS(t)}$) over 20 generations, respectively, for the cases of $N_m = 5$ and various male migration rate (d_m). Although an increase of d_m reduces the increasing rate of $F_{(t)}$, the effect is rapidly diminished as d_m becomes larger (Fig. 5-1 (A)). As seen from Figure 5-1 (B), the total genetic diversity retained in the set of lines, $GD_{(t)}$, is less affected by d_m. In contrast, even with a small amount of male migration (say $d_m \leq 0.2$), increase of d_m effectively suppresses the accumulation of genetic diversity between lines (Fig. 5-1 (C)). Insensitivity of $GD_{(t)}$ to d_m observed in Figure 5-1 (B) is the reflection of the fact that the two opposing effects of migration on the genetic diversity (i.e., the enhanced maintainability of genetic diversity within line, and the suppress of the accumulation of genetic diversity among lines) are largely canceled for any amount of male migration.

Figure 5-2 shows the effects of N_m and d_m on the increasing rate of inbreeding coefficient within line (ΔF) averaged over the initial 10 generations. Based on knowledge from animal breeding, conservation geneticists have recommended that the rate of inbreeding in animal populations should remain below 1% per generation (e.g. see Franklin 1980; Soulé 1980). From Figure 5-2, it is found that a relation $N_m d_m \cong 1$ gives an approximated critical condition to satisfy the criterion $\Delta F \leq 1\%$. This implies that irrespective of the number of males within line (N_m), each line should receive more than one male migrant per generation.

Another criterion for the evaluation of conservation plans is the genetic diversity retained in a conserved population. As a criterion, Soulé et al. (1986) proposed that the goal of conservation management should be to preserve at least 90% of the genetic

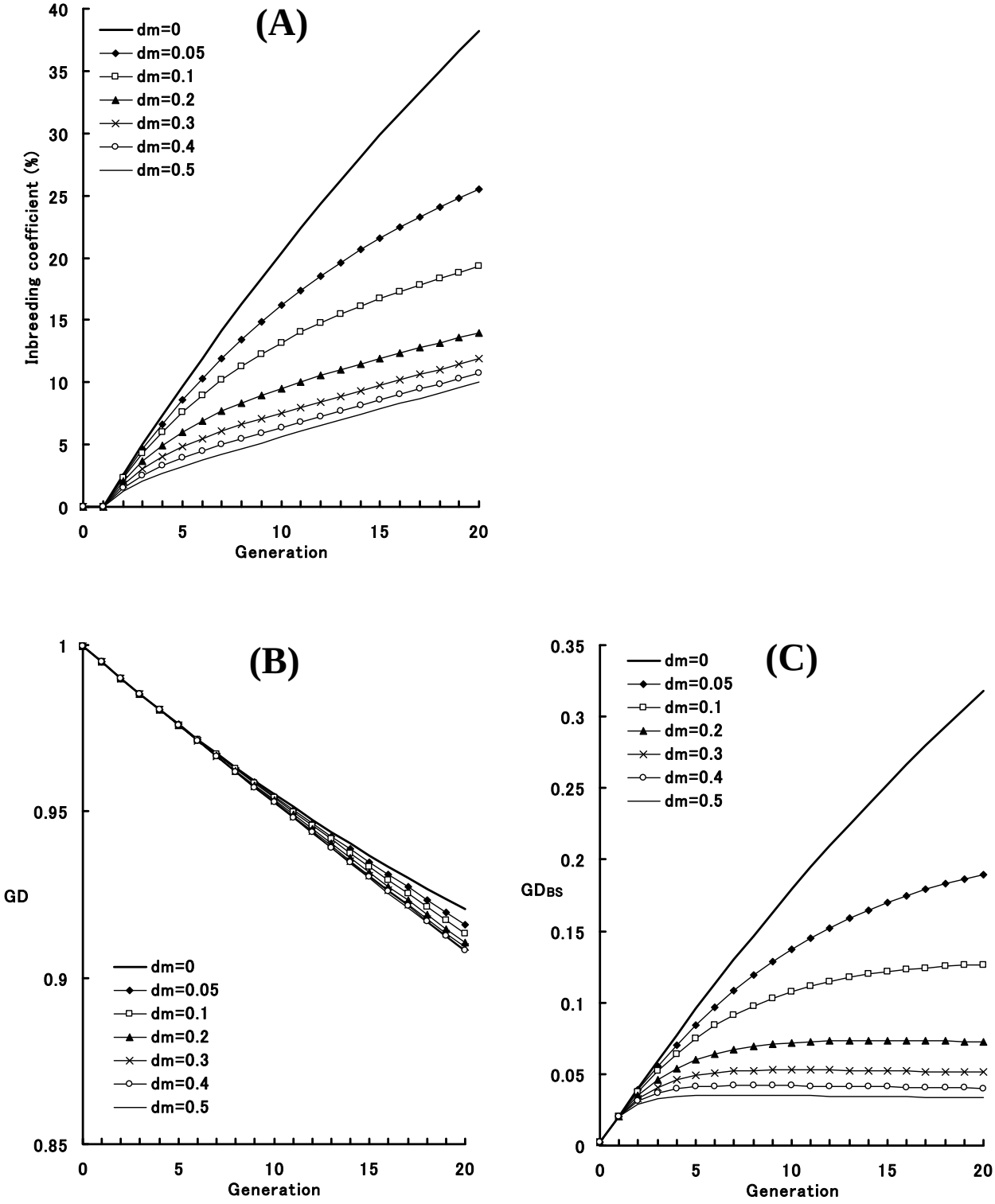


Figure 5-1. Inbreeding coefficient within line, *F*, (A), total genetic diversity of the set of lines, *GD*, (B), and genetic diversity between lines, GD_{BS}, (C), in the set of five lines (n=5) each with N_m=5 males and N_f =200 females.

d_m = male migration rate

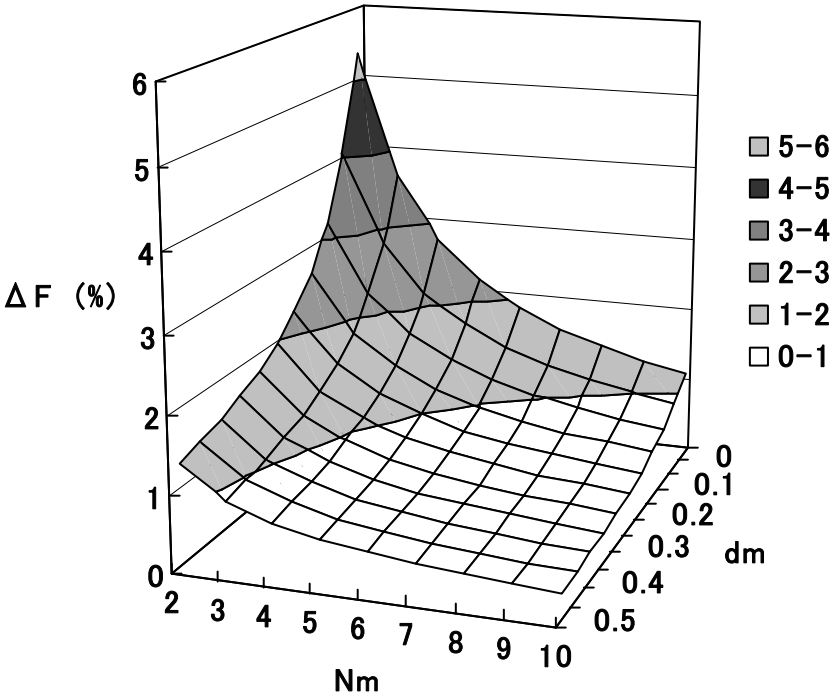


Figure 5-2. Effects of the number (N_m) and the migration rate (d_m) of males on the rate of inbreeding (ΔF) within line averaged over the initial 10 generations.

diversity that existed in the initial population over 200 years. This criterion has been applied to the conservation of endangered wild animal species with various modifications (Frankham et al. 2002). For example, in the conservation of the golden lion tamarin, *Leontopithecus rosalia*, the objective is defined as the maintenance of 98% of genetic diversity for 100 years (Frankham et al. 2002). Similar criteria have been quoted in the context of conservation of rare livestock breeds (Mace 1990; Gill and Harland 1992).

In Figure 5-3, the effects of N_m and d_m on the total genetic diversity after 10 generations ($GD_{(10)}$) are illustrated. In the population of Japanese Black cattle, 10 generations are approximately equal to 100 years (c.f. Table 5-2). As already shown in Figure 5-1 (B), $GD_{(10)}$ is less affected by d_m for a given N_m. If the objective is defined as the conservation of at least 97% of the total genetic diversity over 10

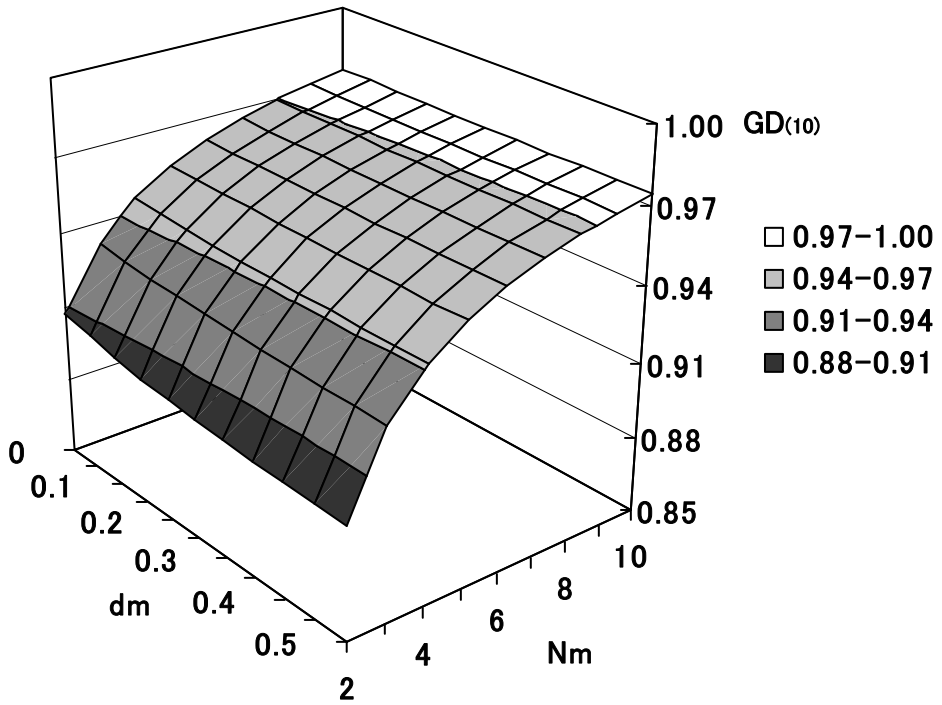


Figure 5-3. Effects of the number (N_m) and the migration rate of males (d_m) on the total genetic diversity after 10 generations ($GD_{(10)}$).

generations ($GD_{(10)} \geq 0.97$), $N_m \geq 8$ will be required.

The genetic diversity generated between lines (Fig. 5-1 (C)) could be future breeding materials. However, maintenance of the initial genetic differentiation among lines will be more important for our purpose. The maintainability could be approximately evaluated by the proportion of genes in a line in generation *t* that have descended from the line in generation 0. The proportion is obtained by a diagonal element $m_{ii(t)}$ of the matrix $\mathbf{M}^t$ (note that in the island model, $m_{ii(t)}$ is equal for all the diagonal elements). When the five lines ($n = 5$) are completely mixed, $m_{ii(t)} = 1/n = 0.2$. Figure 5-4 illustrates the proportion in generation 10. If we choose $m_{ii(10)} \geq 0.5$ as a criterion, which indicates that 50% of original genes retain in each line, male migration rate should be restricted to $d_m \leq 0.15$. The criterion $m_{ii(10)} \geq 0.5$ is apparently arbitrary, but in a line satisfying this criterion, genes originated from the line in

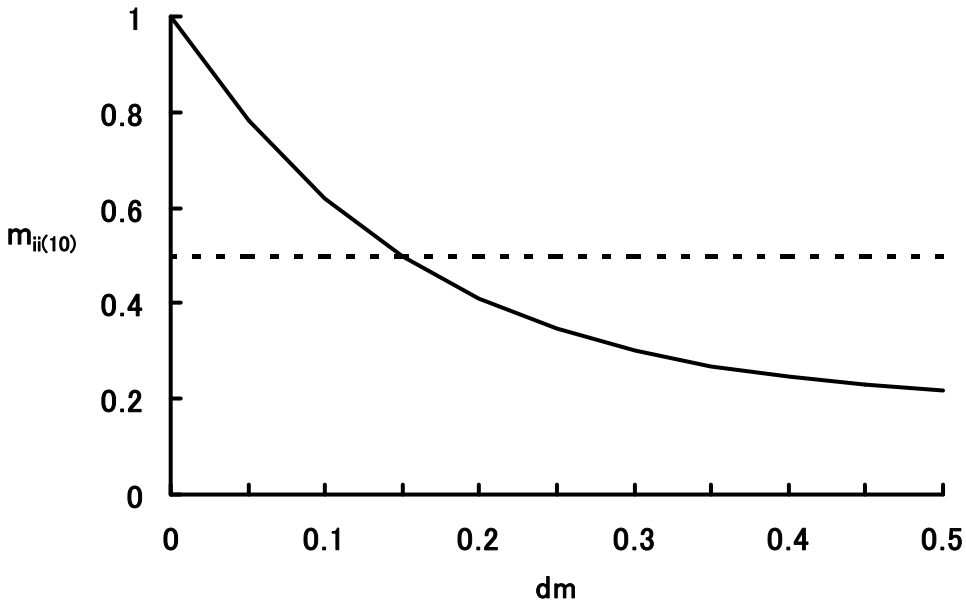


Figure 5-4. Proportion ($m_{ii(10)}$) of genes in a line in generation 10 that have descended from the line in the initial generation. The dashed line indicates the proportion of $m_{ii(10)} = 0.5$.

generation 0, if necessary, could be effectively assembled by selection within the line.

Optimum values of N_m and d_m simultaneously satisfying the three criteria ($\Delta F \leq 1\%$, $GD_{(10)} \geq 0.97$ and $m_{ii(10)} \geq 0.5$) are indicated by the shaded zone in Figure 5-5. From this figure, $N_m \cong 10$ and $d_m \cong 0.1$ will be recommended as an optimum design for the assumed situation. This implies that in each line, approximately one breeding male should be selected per year, and one breeding male should be received as a migrant per generation.

Most of recommendations for the genetic management of fragmented subpopulations are originated from Wright's work on the infinite island model. Based on the results obtained by his analysis, OMPG rule (introducing one migrant per generation from the other subpopulations) is widely accepted in the field of conservation biology in terms of the avoidance of the inbreeding depression and the population differentiation (Mills & Allendorf 1996; Wang 2004). Although this rule has been widely accepted among

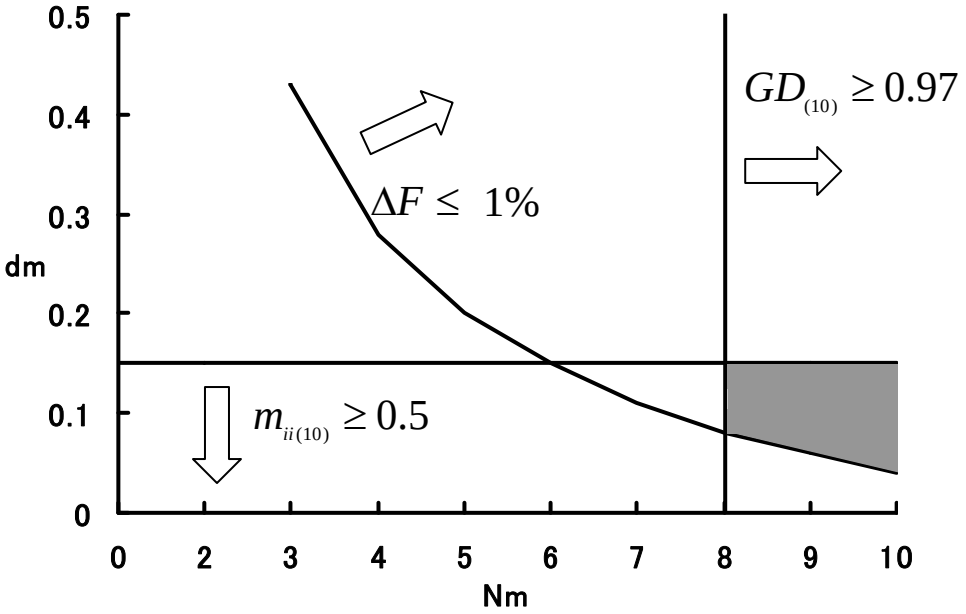


Figure 5-5. Graphical solution for the optimum number of males (N_m) and male migration rate (d_m), which satisfy the three criteria ($\Delta F \leq 1\%$, $GD_{(10)} \geq 0.97$, and $m_{ii(10)} \geq 0.5$) simultaneously.

conservation biologists, Wang (2004) emphasized that in its application the deviation of the real population from the simplified Wright's island model should be carefully examined. One of the most important deviations in domestic animal populations would be the migration pattern. Although the migration pattern in the island model is symmetric, a herd predominated in economically important traits would tend to supply larger amount of migrants than inferior herds. Table 5-3 gives the estimated male migration rates among the five subpopulations in the Japanese Black. Although the male migration rate averaged over all the possible pathways (20 pathways) is 0.05, quite an asymmetrical migration pattern is observed. For example, Hyogo is completely closed, but supplies males to all the other subpopulations. In contrast, Okayama and Hiroshima receive migrants without supplying any emigrants.

To examine the effect of the asymmetrical pattern of migration, I computed the distribution of genes in a line in generation 0 over the set of the lines in generation *t*, by

Table 5-3. Male migration rates among the five subpopulations in the Japanese Black.

	Source				
Recipient	HY	T	S	O	HR
Hyogo (HY)	1.0	0	0	0	0
Tottori (T)	0.11	0.81	0.08	0	0
Shimane (S)	0.07	0.03	0.90	0	0
Okayama (O)	0.18	0.07	0.16	0.59	0
Hiroshima (HR)	0.14	0.07	0.09	0	0.70

All the rates are the averages over 1996-2001.

Figures on the diagonal are the proportions of genes from own bred sires.

substituting the male migration rates given in Table 5-3 into the migration matrix $\mathbf{M}$. Table 5-4 shows the results for t=3 and 10. After three generations of migration (t=3), the two lines constructed each from Okayama and Hiroshima reduce their original genes to 51 and 61%, respectively. These proportions further decrease to 11 and 20% after ten generations (t=10), respectively. Note that genes originated from the two subpopulations exist only in the lines constructed from themselves. These results emphasize that for a successful management of the set of partially isolated lines, migration among lines should be managed so as to avoid an extremely asymmetrical pattern of migration.

The success of the proposed plan will also critically depend on how base animals in the initial line are sampled from the subpopulation. Using the gene dropping simulation (MacCluer et al. 1986), I have shown that several important founders in the five subpopulations have lineages through which their alleles are surely transmitted to the current subpopulations, suggesting that the simulation could be a powerful tool to detect genetically important group of animals surely having target alleles for preservation (chapter II). Construction of the lines should be based on such

Table 5-4. Proportions of genes in lines in generation *t* (=3 and 10), that have descended from lines in generation 0. The computation is based on the migration rates in Table 5-2.

		Originated from:				
t	Subpopulation	Hyogo	Tottori	Shimane	Okayama	Hiroshima
3	Hyogo	1.0	0	0	0	0
	Tottori	0.17	0.73	0.10	0	0
	Shimane	0.12	0.03	0.86	0	0
	Okayama	0.23	0.09	0.19	0.51	0
	Hiroshima	0.19	0.09	0.10	0	0.61
10	Hyogo	1.0	0	0	0	0
	Tottori	0.44	0.36	0.20	0	0
	Shimane	0.34	0.05	0.61	0	0
	Okayama	0.53	0.11	0.29	0.11	0
	Hiroshima	0.48	0.13	0.19	0	0.20

information together with detail examination on genetic merits of the animals.

CHAPTER VI

Prediction of inbreeding in commercial females maintained by rotational mating with partially isolated sire lines

Introduction

In chapter IV, it was shown that the inbreeding coefficient of commercial females in the lower level of hierarchy could be effectively suppressed to a negligible level by the rotational mating with more than four isolated sire lines. In practice, however, some amount of migration should be allowed for the avoidance of rapid increase of inbreeding within each line, as illustrated in chapter V. Since the migration generates genetic relationships among the lines, inbreeding of commercial females kept by rotational mating will be more or less inflated from the expectation under the use of isolated lines.

In this chapter, a theory for predicting the inbreeding coefficient of commercial females maintained by the rotational mating with partially isolated sire lines is formulated, and the general effect of migration among sire lines on the inbreeding was theoretically assessed. As a final conclusion of this thesis, an optimum breed structure of the Japanese Black cattle was proposed in the light of the results of pedigree analysis and the formulated theories.

Theory

(1) Theory of group coancestry

Table 6-1. List of main variables and notations used in this chapter.

Variables and Notations	Definition
Structure of sire lines.	
n	Number of sire lines.
N_v	Number of breeding animals of sex v (=m for male or f for female) in each line.
d_v	Migration rate of sex v.
Groups of animals.	
S_{t-i}	Sire line supplying sires to the commercial females in generation t-i, which is referred to as supplier at the generation.
NS_{t-i}	Sire line that is not the supplier at generation t-i, which is referred to as non-supplier at the generation.
$v(i)$	Group of animals of sex v in sire line i before migration.
$V(i)$	Group of animals of sex V (=M for male or F for female) in sire line i after migration.
c	Commercial females.
Probabilities of gene identity by descent.	
$\phi_{x \cdot y,t}$	Group coancestry between groups x and y in generation t.
F_t	Inbreeding coefficient of commercial females in generation t.
F_t^*	Inbreeding coefficient within each line before migration in generation t.
θ_t	Coancestries between two individuals within line before migration in generation t.
α_t	Coancestries between two individuals among lines before migration in generation t.

As in the chapter IV, the group coancestry theory was applied to derive the prediction theory. The main variables and notations used for the derivation are listed in Table 6-1. For example, suppose a group of individuals *x*, which has the parental group *p* and *q*, and the grand parental group *a*, *b*, *c* and *d*, respectively. Let $\phi_{p\cdot q}$ be the group coancestry between the groups *p* and *q*. The expected inbreeding coefficient of individuals in group *x* (F_x) is expressed as

$$F_x = \phi_{p\cdot q} = \frac{1}{4}\left[\phi_{a\cdot c} + \phi_{a\cdot d} + \phi_{b\cdot c} + \phi_{b\cdot d}\right]. \tag{6-1}$$

The group coancestry of group *x* with itself can be considered to be the average pairwise coancestry including reciprocals and self-coancestries (Caballero and Toro 2000) as

$$\phi_{x\cdot x} = \frac{1+F_x}{2N} + \frac{N-1}{N}\bar{\phi}_x, \tag{6-2}$$

where *N* is the number of individuals in group *x*, and $\bar{\phi}_x$ is the average pairwise coancestry among individuals (excluding self-coancestries).

(2) Breeding population

Population structure:

We suppose that the breeding population is subdivided into *n* partially isolated sire lines, each with N_m males and N_f females each generation. Males in the sire lines are not only the breeding animals for the lines but also the sires used in the commercial population. Mating within each sire line is assumed to be at random and the numbers of male and female offspring (which become parents in the next generation) per parent follow independent Poisson distributions within the sex of parent, giving the effective size of a sire line as

$$N_{es} = \frac{4N_m N_f}{N_m + N_f}.$$

Prior to mating, breeding animals are exchanged among the sire lines. As a migration pattern, we assume the island model (Wright 1943), in which each sire line receives a proportion of d_m males and d_f females drawn at random from the other sire lines. The groups of male and female animals in sire line $i\,(=1,2,\cdots,n)$ before and after migration are denoted by $m(i)$ and $f(i)$, and $M(i)$ and $F(i)$, respectively.

Probability of gene identity by descent in the breeding population:

To describe the progress of the gene identity by descent in the breeding population, three coefficients in generation *t*, i.e. the inbreeding coefficient within each sire line (F_t^*), and coancestries between two individuals within and among sire lines before migration (θ_t and α_t, respectively) should be considered (Wang 1997b). Note that when the numbers of male and female offspring follow independent Poisson distributions, there is no need to distinguish the coancestry between individuals according to their sexes, i.e.,

$$\bar{\phi}_{m(i),t} = \bar{\phi}_{f(i),t} = \phi_{m(i)\cdot f(i),t} = \theta_t \qquad \text{(6-3a)}$$

and

$$\phi_{m(i)\cdot m(j),t} = \phi_{f(i)\cdot f(j),t} = \phi_{m(i)\cdot f(j),t} = \alpha_t \qquad \text{(6-3b)}$$

for $i \neq j$ (see Wang (1997b) for more detail).

Let q_{mf} and q'_{mf} be the probabilities that two individuals of different sexes, taken at random from the same sire line and the different sire lines after migration, come from the same sire line before migration, respectively. Similarly, q_{vv} and q'_{vv} are defined as the probabilities that two individuals of the same sex v (=*m* for male and *f* for female), taken at random and without replacement from the same sire line and the

different sire lines after migration, come from the same sire line before migration, respectively. The expression of these probabilities have been already obtained in the previous chapter.

Putting $P_t^* = 1 - F_t^*$, $h_{w,t} = 1 - \theta_t$ and $h_{b,t} = 1 - \alpha_t$, and defining a column vector $\mathbf{s}_t' = \begin{bmatrix} P_t^* & h_{w,t} & h_{b,t} \end{bmatrix}$, the recurrence relation obtained by Wang (1997b) can be expressed as

$$\mathbf{s}_t = \mathbf{T}\mathbf{s}_{t-1}, \tag{6-4}$$

where

$$\mathbf{T} = \begin{pmatrix} 0 & q_{mf} & 1 - q_{mf} \\ \dfrac{1}{2N_{e,s}} & 1 - \dfrac{Q}{4} - \dfrac{q_{mm}}{4N_m} - \dfrac{q_{ff}}{4N_f} & \dfrac{Q}{4} - \dfrac{1 - q_{mm}}{4N_m} - \dfrac{1 - q_{ff}}{4N_f} \\ 0 & 1 - \frac{1}{4}Q' & \frac{1}{4}Q' \end{pmatrix}, \tag{6-5}$$

and

$$Q = 4 - q_{mm} - 2q_{mf} - q_{ff}$$

$$Q' = 4 - q'_{mm} - 2q'_{mf} - q'_{ff}\ .$$

(3) Population of commercial females

Rotational mating system:

Commercial population of females is assumed to be maintained by random mating with sires rotationally supplied from the *n* sire lines before migration. Generations are discrete and proceed with the same interval as in all the sire lines. The population of commercial females is denoted by *c*.

The line supplying sires to the commercial females in a given generation is referred to as the supplier at that generation. The sequential numbers 1, 2, …, *n* are given to the suppliers in generations 0, 1, …, *n*-1, respectively. Letting S_{t-i} be the sequential

number of the supplier in generation *t-i*, S_{t-i} could be determined by

$$S_{t-i} = \mathrm{MOD}\left(t-i,\ n\right)+1,$$

where $\mathrm{MOD}\left(x, n\right)$ is the remainder of *x* divided by *n*. Note that, because of the nature of rotational mating, $S_{t-i} = S_{t-i-kn}$ for a given integer number *k*. An arbitrary line that is not the supplier at generation *t-i* is referred to as non-supplier, and is denoted as NS_{t-i}.

Probability of gene identity by descent in the commercial female population:

To obtain the recurrence equation for probability of gene identity by descent in the commercial female population, the three coefficients in generation *t*, i.e., the inbreeding coefficient of commercial females (F_t), the group coancestry between males (before migration in generation *t*) in the supplier in generation *t*-1 and commercial females in generation *t* ($\phi_{m(S_{t-1})\cdot c,t}$), and the group coancestry between males (before migration in generation *t*) in a non-supplier in generation *t*-1 and commercial females in generation *t* ($\phi_{m(NS_{t-1})\cdot c,t}$), should be specified. By the operational rule of coancestry (eq. (6-1)), F_t is simply expressed as

$$F_t = \phi_{m(S_{t-1})\cdot c,t-1}\,. \tag{6-6}$$

Figure 6-1 (a) shows the diagram for deriving the expression of $\phi_{m(S_{t-1})\cdot c,t}$. Applying the operational rule of group coancesty (eq. (6-1)) to the diagram, the expression

$$\phi_{m(S_{t-1})\cdot c,t} = \frac{1}{4}\left[\phi_{M(S_{t-1})\cdot m(S_{t-1})} + \phi_{M(S_{t-1})\cdot c} + \phi_{F(S_{t-1})\cdot m(S_{t-1})} + \phi_{F(S_{t-1})\cdot c}\right]_{t-1} \tag{6-7}$$

can be obtained.

Note that in the parental groups of each sex in the supplier after migration ($M(S_{t-1})$ and $F(S_{t-1})$), non-immigrants and immigrants from an arbitrary sire lines are contained with the proportions of $1-d_v$ and $d_v/\left(n-1\right)$, respectively. Furthermore, applying

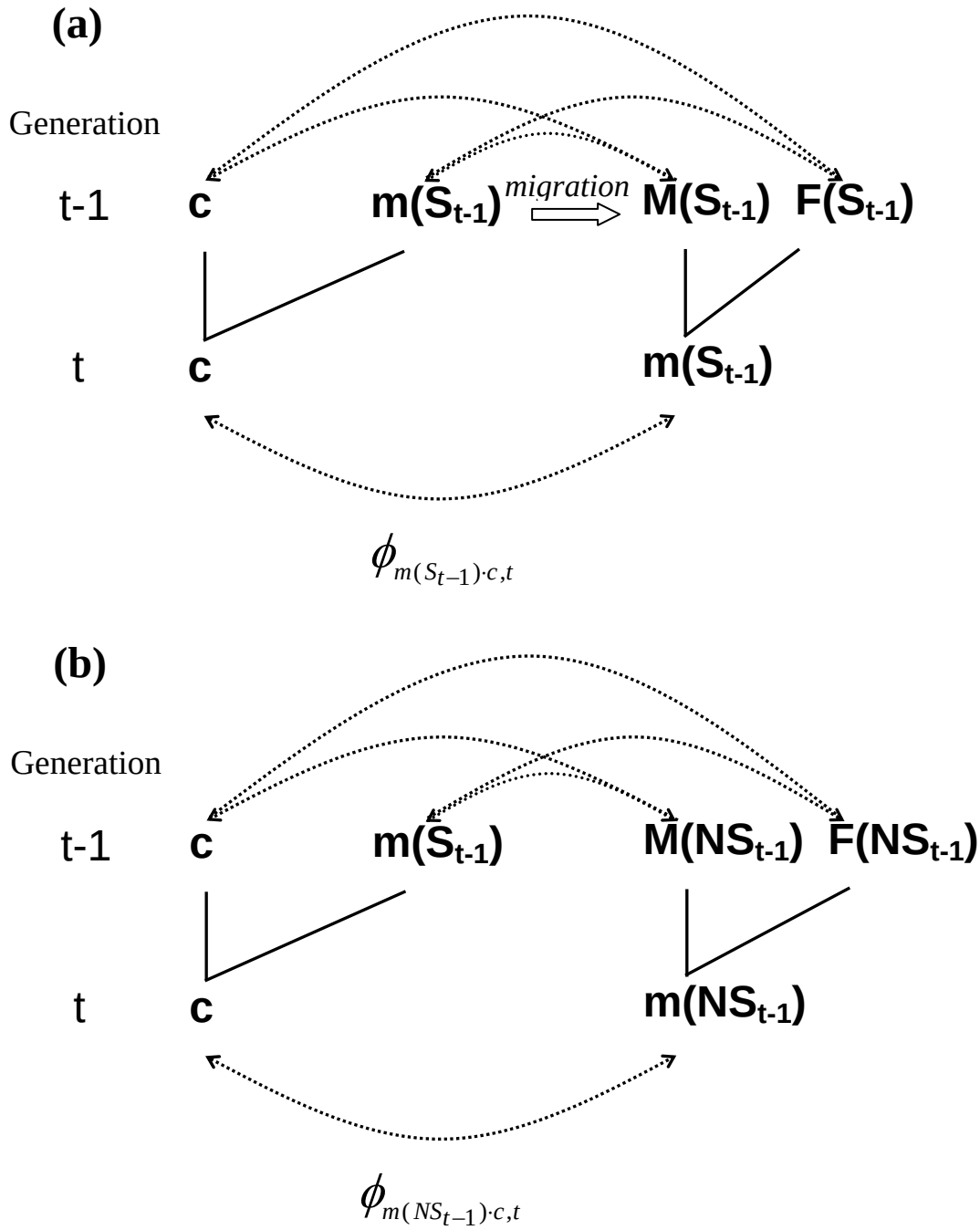


Figure 6-1. Pedigree diagrams to derive the group coancestry (in generation *t*) between the commercial females and (a) males of the supplier in generation *t*-1 ($\phi_{m(S_{t-1})\cdot c,t}$), and (b) males of an arbitrary non-supplier in generation *t*-1 ($\phi_{m(NS_{t-1})\cdot c,t}$). The group coancestries relevant to $\phi_{m(S_{t-1})\cdot c,t}$ and $\phi_{m(NS_{t-1})\cdot c,t}$ are shown by dashed curves with arrows of both derection.

the operational rule (eq.(6-2)) and noting that the relations (6-3) hold in our model and for the same reason, $\phi_{m(i)\cdot c,t-1} = \phi_{f(i)\cdot c,t-1}$, the four group coancesties on the right hand of eq. (6-7) can be expressed as

$$\phi_{M(S_{t-1})\cdot m(S_{t-1}),t-1} = (1-d_m)\left[\frac{1+F^*_{t-1}}{2N_m} + \frac{N_m-1}{N_m}\theta_{t-1}\right] + d_m\alpha_{t-1}$$

$$\phi_{M(S_{t-1})\cdot c,t-1} = (1-d_m)\phi_{m(S_{t-1})\cdot c,t-1} + \frac{d_m}{n-1}\sum_{i\neq S_{t-1}}^{n}\phi_{m(i)\cdot c,t-1}$$

$$\phi_{F(S_{t-1})\cdot m(S_{t-1}),t-1} = (1-d_f)\theta_{t-1} + d_f\alpha_{t-1}$$

$$\phi_{F(S_{t-1})\cdot c,t-1} = (1-d_f)\phi_{m(S_{t-1})\cdot c,t-1} + \frac{d_f}{n-1}\sum_{i\neq S_{t-1}}^{n}\phi_{m(i)\cdot c,t-1}\,.$$

Substituting these expressions into (6-7) gives

$$\phi_{m(S_{t-1})\cdot c,t} = \left[r\phi_{m(S_{t-1})\cdot c} + s\sum_{i\neq S_{t-1}}^{n}\phi_{m(i)\cdot c} + xF^* + y\theta + z\alpha\right]_{t-1} + x\,, \qquad \text{(6-8)}$$

where $r = \dfrac{2-d_m-d_f}{4}$, $s = \dfrac{d_m+d_f}{4(n-1)}$, $x = \dfrac{1-d_m}{8N_m}$, $y = \dfrac{(1-d_f)N_m + (1-d_m)(N_m-1)}{4N_m}$,

and $z = \dfrac{d_m+d_f}{4}$.

Applying the similar argument to Figure 6-1(b), the expression of $\phi_{m(NS_{t-1})\cdot c,t}$ can be obtained as

$$\phi_{m(NS_{t-1})\cdot c,t} = \left[r\phi_{m(NS_{t-1})\cdot c} + s\sum_{i\neq NS_{t-1}}^{n}\phi_{m(i)\cdot c} + uF^* + v\theta + w\alpha\right]_{t-1} + u\,, \qquad \text{(6-9)}$$

where $u = \dfrac{d_m}{8(n-1)N_m}$, $v = \dfrac{d_m(N_m-1)+d_fN_m}{4(n-1)N_m}$, and $w = \dfrac{2(n-1)-(d_m+d_f)}{4(n-1)}$.

Recurrence equations in vector-matrix form:

Putting $P_t = 1 - F_t$ and $h_{m(i)\cdot c,t} = 1 - \phi_{m(i)\cdot c,t}$, and defining a column vector $\mathbf{h}_t$ and a matrix $\mathbf{G}$ with order $(n+4)\times(n+4)$ as

$$\mathbf{h}_t' = \begin{bmatrix} P_t & h_{m(S_{t-1})\cdot c,t} & h_{m(S_t)\cdot c,t} & \cdots & h_{m(S_{t+n-2})\cdot c,t} & P_t^* & h_{w,t} & h_{b,t} \end{bmatrix}$$

and

$$\mathbf{G} = \left[\begin{array}{c|ccccc} 0 & 0 & 1 & 0 & \cdots & 0 \\ \hline 0 & & & & & \\ \vdots & & \mathbf{U} & & & \mathbf{V} \\ 0 & & \mathbf{O} & & & \mathbf{T} \end{array}\right], \tag{6-10}$$

eqs (6-4) and (6-6), (6-8), and (6-9) can be combined as

$$\mathbf{h}_t = \mathbf{G}\mathbf{h}_{t-1}, \tag{6-11}$$

where $\mathbf{T}$ is the matrix defined by eq (6-5), $\mathbf{O}$ is the null matrix with order $3\times n$,

$$\mathbf{U} = \begin{bmatrix} s & r & s & \cdots & s \\ s & s & r & \ddots & \vdots \\ \vdots & \ddots & s & \ddots & s \\ s & \ddots & \ddots & \ddots & r \\ r & s & \cdots & s & s \end{bmatrix}_{n\times n} \quad \text{and} \quad \mathbf{V} = \begin{bmatrix} x & y & z \\ u & v & w \\ \vdots & \vdots & \vdots \\ u & v & w \end{bmatrix}_{n\times 3}.$$

Note that $\mathbf{U}$ is a circular matrix describing the periodic nature of rotational mating such as $S_{t-1} = S_{t+n-1}$.

Rotational mating with a partial set of sire lines:

Rotational mating will have practical merit in its application to the commercial female population, because it does not require systematic controls of mating over the population. For example, if five sire lines (say A, B, C, D and E) are available, some farmers could rotationally use the four lines A, B, C and D, while other could adopt the mating with the four lines B, C, D and E.

Suppose a situation where n sire lines are available and a group of commercial females are maintained by the rotational mating with $n'(<n)$ sire lines. The

inbreeding coefficient of the commercial females can be predicted by replacing the submatrix $\mathbf{U}$ in eq (6-10) by $\mathbf{W}$ defined as

$$\mathbf{W} = \begin{bmatrix} \mathbf{U}_{n'\times n'} & s & \cdots & s & s \\ s & r & s & \cdots & s \\ \vdots & s & r & \ddots & \vdots \\ s & \vdots & \ddots & \ddots & s \\ s & s & \cdots & s & r \end{bmatrix}_{n\times n} .$$

Asymptotic rate of inbreeding of commercial females:

In chapter IV, it was proved that when each sire line is completely closed ($d_m = d_f = 0$), the inbreeding coefficient of commercial females asymptotically approaches to an intermediate value ($0 \le F_\infty \le 1$). However, when migration is allowed among the sire lines, the inbreeding coefficient of commercial females asymptotically approaches to unity. The asymptotic rate of inbreeding ($\Delta F = \lim_{t\to\infty}\left(F_t - F_{t-1}\right)/\left(1 - F_{t-1}\right)$) is obtained from the largest eigenvalue (λ) of $\mathbf{G}$ as $\Delta F = 1 - \lambda$.

(4) Numerical computations and an application

To evaluate the efficiency of the proposed mating system, numerical computations with eq (6-11) were carried out for various combinations of the number of sire lines (n=2~5), number of males in each sire line (N_m=2~10) and male migration rate (d_m=0~0.5). In all the computations, the female migration rate was set to zero ($d_f = 0$). Since preliminary analysis showed that the number of females in each sire line has a minor effect on results, N_f was also fixed to 200. In addition, for a fixed total size of breeding population, the effects of the number of sire lines on the inbreeding coefficients of the breeding and commercial populations were examined.

In the previous chapter, management plan for the five hypothetical sire lines (n=5), constructed from each of the five regional subpopulations in the Japanese Black, was examined. Here, I reexamined the application of the proposed plan in terms of the amount of inbreeding in the commercial females. Commercial females were assumed to be maintained by rotational mating with $n' = 3$, 4 and 5 sire lines chosen from the five available lines. Computations with N_m=10 and $d_m = 0$, 0.1 and 0.2 were carried out over 10 generations (approximately 100 years in this breed). In addition to the inbreeding coefficient, the total genetic diversity retained in the set of the five lines in generation 10, and the proportion of genes in a line in generation 10 that have descended from the line in generation 0 (GD_{10} and m_{10}, respectively) were also computed (for the computational procedures of these parameters, see chapter V). The latter is an indicator of the maintainability of the initial genetic differentiation among the lines. In the previous chapter, the management with $N_m \cong 10$ and $d_m \cong 0.1$ was suggested to be an optimum plan for the maintenance of the set of the five sire lines, which satisfies $\Delta F^* \leq 1\%$, $GD_{10} \geq 0.97$ and $m_{10} \geq 0.5$, simultaneously.

Results and discussion

In chapter IV, I showed that with completely closed sire lines ($d_m = d_f = 0$), the inbreeding coefficient of commercial females asymptotically approaches an intermediate value $F_\infty = 1/\left(2^n - 1\right)$. However, when migration is allowed among sire lines, the asymptotic inbreeding coefficient reaches unity ($F_\infty = 1$) irrespective of the number of sire lines (n). In Figure 6-2, the evolution of inbreeding in the commercial females over initial 20 generations is exemplified, for the cases of $n = 3$, $N_m = 2$ and various rates of male migration among the sire lines (d_m=0~0.5). The migration among the sire lines substantially increases the initial inbreeding in the commercial females, and for all the computed cases the inbreeding coefficient in generation 20 is already beyond the asymptotic value ($F_\infty = 14.3\%$) expected under $d_m = 0$. Under $d_m = 0.1$, 0.2, 0.3, 0.4 and 0.5, the asymptotic rate of inbreeding are predicted as 1.51, 1.71, 1.78, 1.82 and 1.84%, respectively.

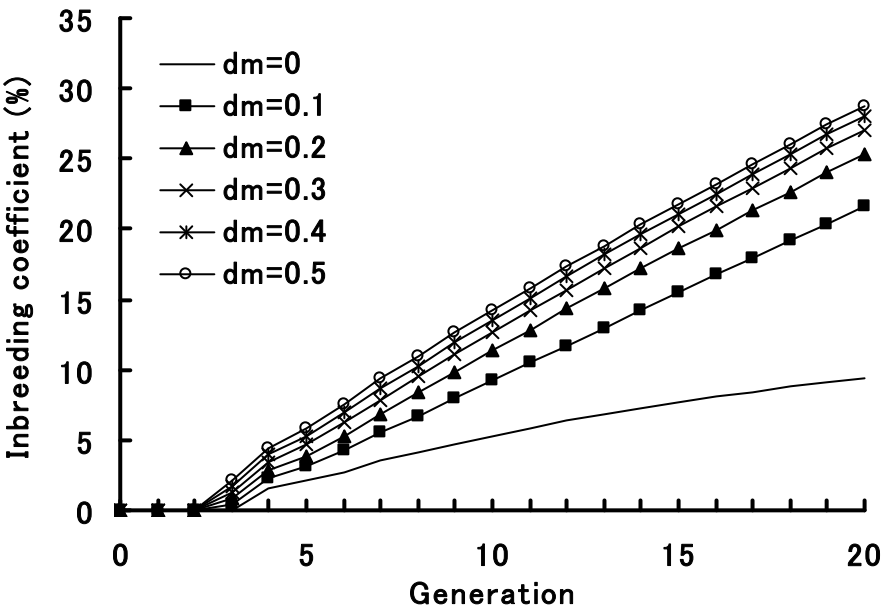


Figure 6-2. Inbreeding coefficient of the commercial females under rotational mating using n=3 sire lines with the sizes of N_m=2 males and N_f =200 females.
d_m=migration rate of males

Figures 6-3 (A)-(D) show the effects of the number of males (N_m) in each sire line and male migration rate (d_m) on the inbreeding coefficient of commercial females in generation 20, for the cases of $n = 2$, 3, 4, and 5, respectively. For given *n* and N_m, a small amount of male migration (say $d_m < 0.1$) substantially inflates the inbreeding coefficient of commercial females, but the effect is diminished as d_m becomes larger. Comparison of Figures 6-3 (A)-(D) reveals that an increase of *n* effectively reduces the inbreeding of commercial females. When four or five sire lines are available, the inbreeding coefficient in generation 20 is suppressed below 20% (or approximately the rate of inbreeding is kept below 1%) under most of the investigated combinations of N_m and d_m.

When the breeding population has an undivided structure at the initiation of rotational mating, it will raise a question of how many sire lines should be established. To illustrate this problem, we supposed a situation where the breeding population has a fixed size of 60 males and 480 females ($T_m = 60$ and $T_f = 480$), and *n* sire lines each with $N_m = T_m / n$ and $N_f = T_f / n$ are constructed. Figures 6-4 (A) and (B) show the inbreeding coefficients in the sire lines and commercial populations in generation 20, respectively. The inbreeding in the sire lines is inflated by an increase in *n*, since it is accompanied by an decrease in the size of each sire line (Fig. 6-4 (A)). A small amount of migration among the sire lines effectively reduces the increase of inbreeding, but the effect is rapidly diminished as the migration rate becomes larger. With a high rate of migration (say $d_m > 0.4$), the inbreeding coefficient tends to converge to a single value regardless of the number of sire lines, since the lines essentially behave as a single population. In contrast, the increase in *n* and d_m have reversal effects on the inbreeding coefficient of commercial females (Fig. 6-4 (B)). As illustrated by this example, the optimum structure of breeding population should be empirically found so

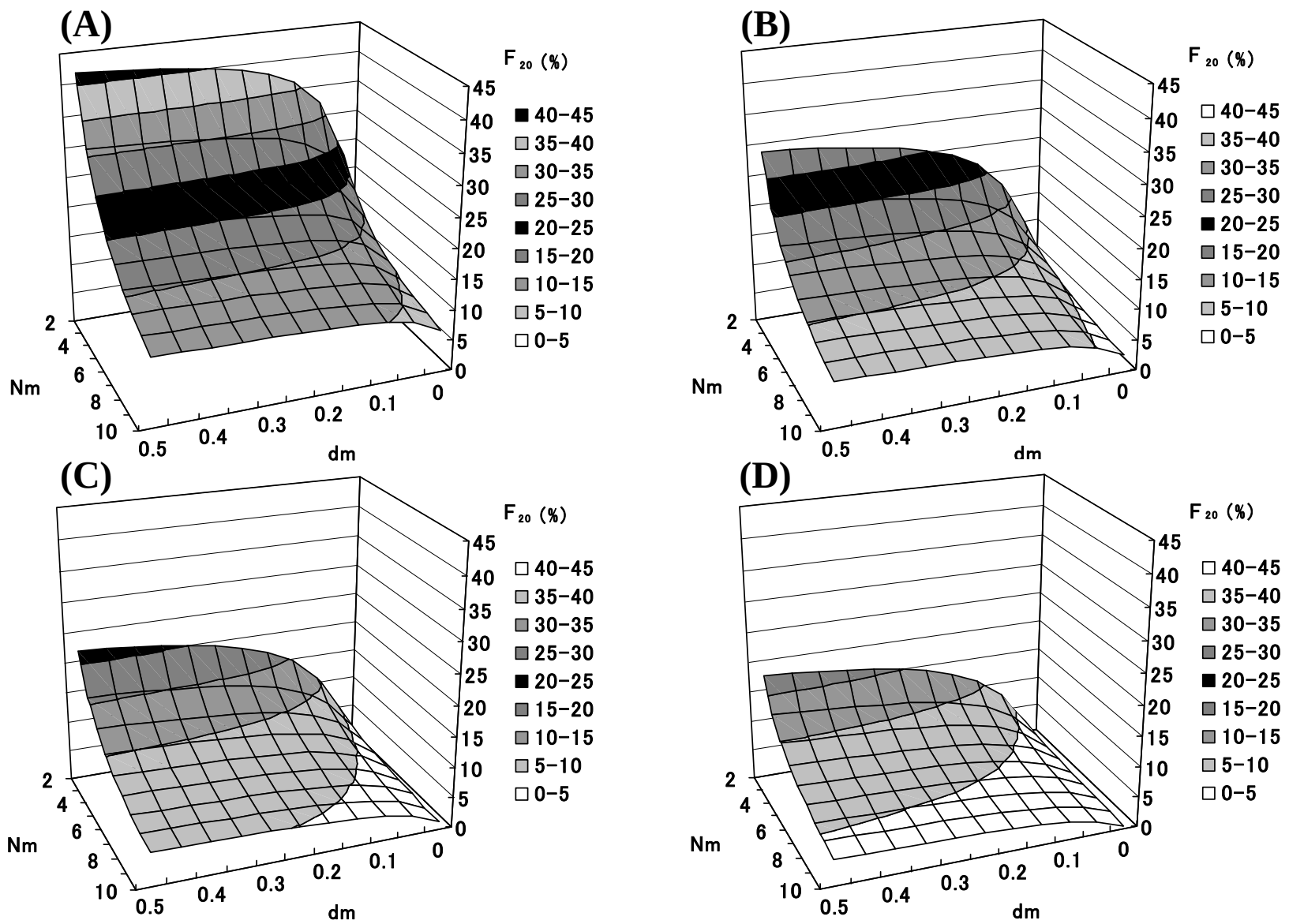


Figure 6-3. Effects of the number of males (N_m) in each sire line and male migration rate (d_m) on the inbreeding coefficient of commercial females in generation 20, for the cases of (A) *n*=2, (B) *n*=3, (C) *n*=4, and (D) *n*=5.

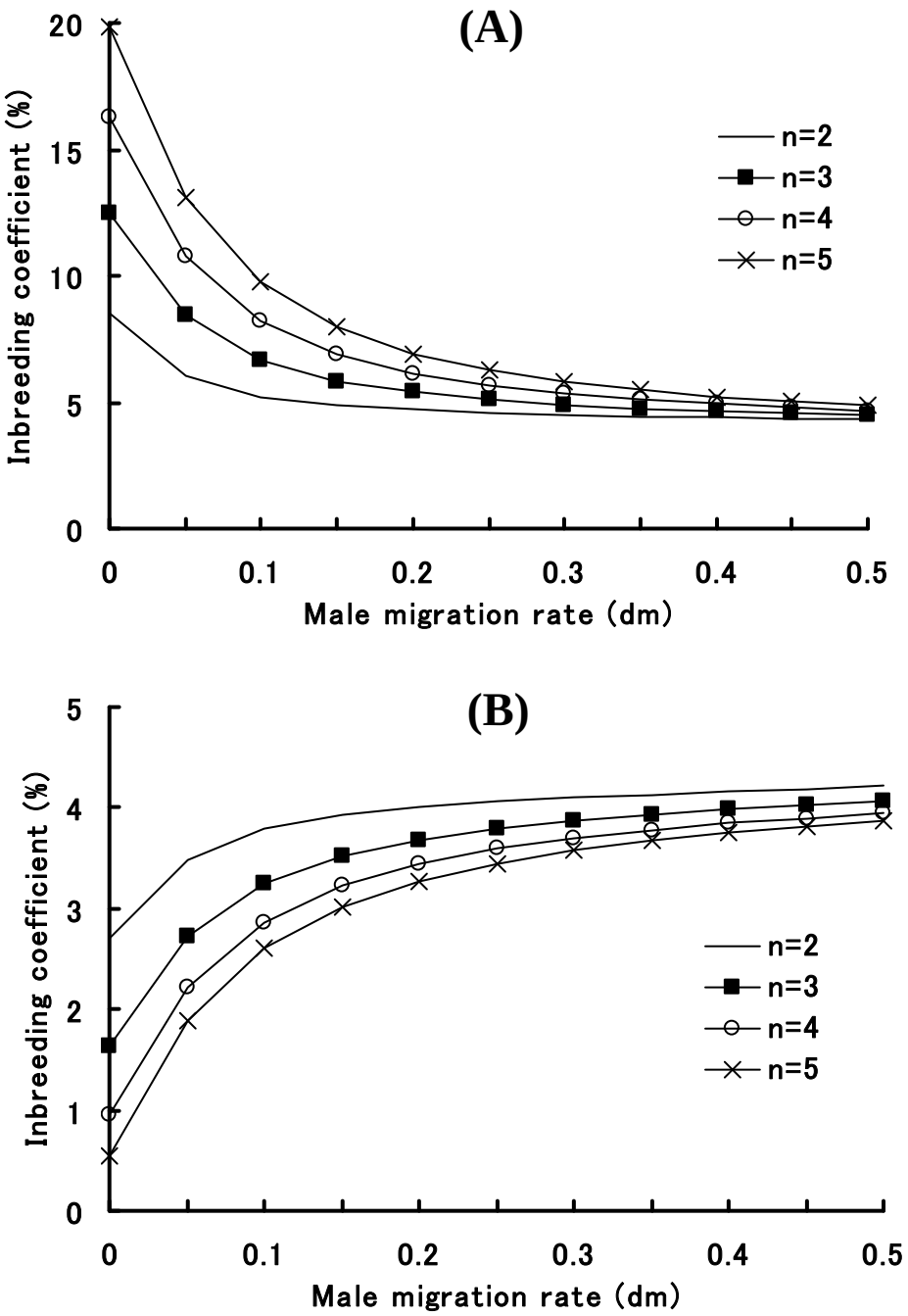


Figure 6-4. Inbreeding coefficients in the (A) sire lines and (B) commercial females in generation 20, using *n* sire lines subdivided from a breeding population with a fixed size of 60 males and 480 females.

as to balance the conflicting effects on the inbreeding in the breeding and commercial populations.

The results of an application of rotational mating with the five hypothetical sire lines of the Japanese Black are summarized in Table 6-2. When the sire lines are completely closed ($d_m = 0$), the inbreeding coefficient of commercial females is minimized, but the criterion for the rate of inbreeding in each sire line ($\Delta F^* \leq 1\%$) is violated. Under the optimum rate of male migration ($d_m = 0.1$) defined in chapter V, the inbreeding coefficient of commercial females is inflated compared to the case of $d_m = 0$, but the inbreeding in generation 10 is suppressed to a negligible level, irrespective of the number of sire lines (n') used in the commercial population. An excess of migration ($d_m = 0.2$) leads to a rapid decay of the genetic variability among the sire lines, as seen from the lower value of m_{10}. These results suggest that the optimum management plan of the set of sire lines defined in chapter V could also give a successful solution for the inbreeding problem of the commercial females.

Throughout the present study, the island model has been assumed as a migration pattern. The migration pattern in actual livestock populations will more or less departure from this idealized model. For example, the male migration among the five regional subpopulations in the Japanese Black shows an asymmetrical pattern (see chapter V). According to the migration matrix defined by Hedrick (1982), I predicted that when this asymmetrical pattern is persisted over generations, the genetic variability among the subpopulations will be seriously decayed within a few generations. The decay will largely reduce the merit of the utilization of sire lines constructed from the different subpopulations. For an efficient use of genetic diversity among sire lines, the management of migration to avoid an extreme asymmetrical pattern has an essential importance.

Table 6-2. Application of rotational mating to the population of Japanese Black cattle. Five sire lines (n=5) each with the size of N_m=10 males and N_f =200 females are assumed to be available for rotational mating.

	Breeding population				Commercial females					
					$n'=3$ [a]		$n'=4$ [a]		$n'=5$ [a]	
d_m [b]	$\Delta F^*(\%)$ [c]	$F_{10}^*(\%)$	GD_{10}	m_{10}	$F_5(\%)$	$F_{10}(\%)$	$F_5(\%)$	$F_{10}(\%)$	$F_5(\%)$	$F_{10}(\%)$
0	1.17	11.1	0.975	1.00	0.47	1.33	0.16	0.55	0.00	0.23
0.1	0.74	7.1	0.975	0.62	0.54	1.62	0.29	1.11	0.17	0.90
0.2	0.52	5.1	0.975	0.41	0.60	1.78	0.42	1.44	0.31	1.31

[a] n' : Number of sire lines used in the rotational mating.

[b] d_m : Male migration rate

[c] ΔF^* : Rate of inbreeding averaged over the initial 10 generations.

Although the hierarchical population structure has been assumed through the last three chapters, the hierarchical structure has been found in many livestock breeds (for a review, see Vu Tien Khang, 1983). The subdivision in the upper level of the hierarchy (breeding population) has been also reported in many breeds (e.g. Sölkner et al., 1998; Goyache et al. 2003). For these breeds, the mating system proposed in this thesis could be a guideline to the conservation of genetic diversity in the breeding population and the suppression of inbreeding in the commercial population.

GENERAL DISCUSSION

Currently, approximately 40 domesticated mammalian and avian species, including about 5,000 breeds, are utilized to meet demands of human being for food, clothing, draught power, and manure, and to satisfy various cultural, religious, and recreational purposes. However, because of rapid changes in production systems, market forces, and of indiscriminate crossbreeding, giving clear priorities on economical efficiency, substantial numbers of livestock breeds have been extinct in the last five decades (Barker 2001). It is also suggested that approximately 30% of all livestock breeds are at risk of extinction nowadays (FAO 1995). In assuring potential abilities to address the possible future changes in production conditions, an importance of conservation of minor breeds, especially in developing countries, as genetic resources has been recently recognized.

In analogy with the world situation described above, the production system of Japanese Black cattle has been influenced by international affairs. Since the liberalization of beef import restriction in 1991 more emphasis has been put on the production of high-quality beef to compete with the economical imported beef. For this purpose, genetic evaluation system with BLUP methodology under animal model was initiated in the same year. Although carcass traits have been remarkably improved during the last decade, the increase of inbreeding rate and decay of genetic variability are recently increasing problems. To investigate the genetic diversity and genetic structure of the breed, pedigree analyses were carried out in the first three chapters in this thesis.

In chapter I, the impacts of the initiation of genetic evaluation system with BLUP methodology on genetic structure and diversity of the breed were assessed, in terms of

the amount of inbreeding and effective population size. Although the breed maintained the effective size of approximately 30 until 1990, the harmonic mean between 1991 and 2000 was only 20.3, which is critically below a standard level for domestic animal population ($N_e \approx 100$; see Wright 1977; Pirchner 1983).

To draw more useful information from pedigree data, new analytical methods proposed in the field of captive management of endangered species was applied in chapter II. The behaviors of the effective numbers of ancestors and the two indices of genetic diversity strongly suggested that the major cause of the reduced diversity was the bottleneck effect due to the intensive use of a few prominent sires. Due to the preference of the high growth ability, the genetic contribution of Tottori prefecture predominated until late 1970s. However, according to the shift of the breeding objective to meat quality from late 1970s (Wagyu Registry Association 1992), Hyogo prefecture had become to contribute predominately. Because of the spread of genes originated from founders in Hyogo prefecture, contributions of the other traditional subpopulations (i.e. Shimane, Okayama, and Hiroshima prefectures) were drastically reduced.

Results obtained by multivariate analyses in chapter III clearly showed the disappearance of genetic subdivision in the breed. Although the breed in 1980s was genetically subdivided into several groups according to the direct relationships between the subpopulations in Chugoku district (Nomura 1988; Nomura and Sasaki 1988), most of the subpopulations investigated in this chapter formed a single predominant group, indicating that the genetic homogeneity had progressively proceeded in the breed. Since subpopulation of Hyogo prefecture joined to the group in the earliest stage (Fig. 3-3), it was strongly inferred that the disappearance of the subdivided structure was primarily caused by the nation-wide spread of Hyogo founder genes. On the other

hand, the other four traditional subpopulations (i.e. Tottori, Shimane, Okayama and Hiroshima prefectures) were separated from the group, suggesting that their unique genetic compositions are still maintained by their own breeding policies.

From the series of pedigree analyses, it is concluded that the nation-wide use of a limited sires originated from founders in Hyogo prefecture was the major cause for the disappearance of subdivided structure and the reduction of genetic diversity in the breed. As a strategy for the recovery of the genetic diversity, the imposition of an upper limit for the use of AI semen per sire may have an immediate effect. However, it would be difficult to implement this strategy in the population of Japanese Black cattle, because choice of sires to mate with reproductive females largely depends on individual farmers' decisions.

In this thesis, a management plan of the genetic diversity was proposed on the basis of the two concepts. The first concept was "diversification of breeding objectives". The current genetic evaluation project has been limited to carcass traits, and the improvement of the other essential traits for beef production, such as reproductive performance, maternal ability, and feed utilization efficiency, has been less emphasized. Although the current economic values of these traits in the markets would be relatively low, recognition of their importance would contribute to the preservation of diverse genes in the whole breed. The second concept was "reconstruction of genetically subdivided structure". As reviewed in chapter III, several simulation and theoretical studies have confirmed the advantage of subdivided structure for the maintenance of genetic variability in the population. The breed history of Japanese Black cattle also verifies the advantage of population subdivision, because the rapid adaptation to the past changes in breeding objectives seems to largely owe to the genetic diversity maintained among the subpopulations from the foundation of the breed.

In addition to these concepts, the hierarchical structure of the breed was also taken into account in designing the management plan of genetic diversity. In chapter IV, as a mating system to reduce inbreeding in the lower level of hierarchy (commercial population), the efficiency of the rotational mating was theoretically examined by assuming that females in the commercial population are rotationally mated with males supplied from several closed sire lines in the upper level (breeding population). Numerical computations showed that the critical factor for determining the inbreeding is the number of sire lines, and that size of each line has a marginal effect. If $n \geq 4$ sire lines are available, short- and long-term inbreeding of the commercial females could be restricted to the lower level, irrespective of the effective size of each line.

In chapter V, the optimum structure of the breeding population was assessed on the assumption that five lines are constructed from each of the five subpopulations of prefectures in Chugoku district, which have played important roles as breeding stock suppliers. The optimum male migration rate among the lines and the required male numbers were determined so as to simultaneously satisfy the following three conditions: (1) The rate of inbreeding in each line is below 0.01 per generation. (2) At least 97% of the initial genetic diversity is preserved after 10 generations. (3) More than 50% of genes in an initial line is retained in the line after 10 generations. It was found that in each line, approximately one breeding male should be selected per year, and one breeding male should be exchanged among the lines per generation, to satisfy the three conditions.

In the final chapter (chapter VI), an optimum structure of the entire breed was defined by combining the theories formulated in the previous two chapters. Numerical computations showed that the optimum management plan of the breeding population defined in chapter V could also give a successful rotational mating for the maintenance

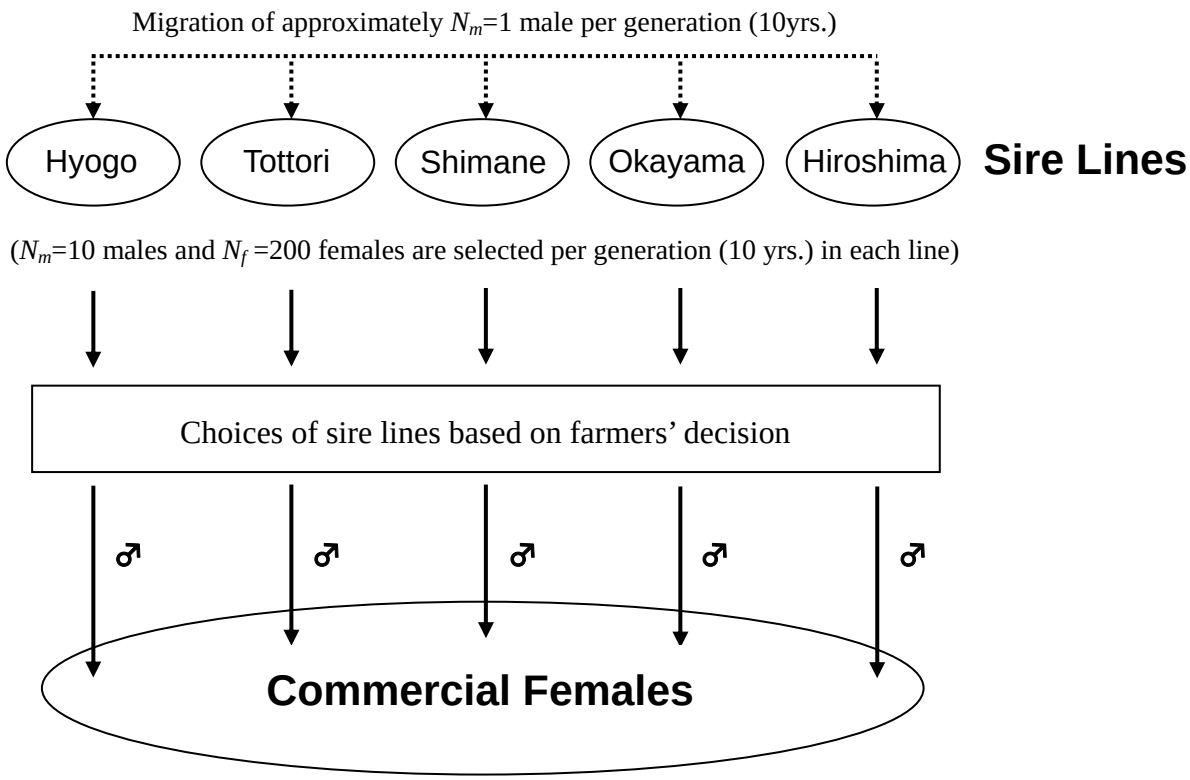


Figure G-1. Illustration of the optimum population structure proposed in this thesis.

of commercial females. Figure G-1 illustrates the optimum population structure of the breed, which is depicted from the examinations in the last three chapters.

The hierarchical population structure as assumed in this thesis has been found in many livestock breeds. The subdivision of the upper level of hierarchy (breeding population) has been also reported in many breeds. For these breeds, the population structure illustrated in Fig. G-1 could be a general guideline to the conservation of genetic diversity in the breeding population and the suppression of inbreeding in the commercial population.

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