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—Original—

Phenotype-Based Search of Natural Mutations Related to Hereditary Diseases Existing in a Closed Colony of Mice

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Abstract: We attempted to detect natural mutations existing in the Jcl:ICR closed colony of mice which is maintained by random mating. We used ordinary genetic backcrosses to efficiently detect recessive mutations carried by individual mice in the colony. Crosses of DBA/2 females and ICR males were performed to obtain F_1 mice. Four F_1 females randomly selected from each cross were backcrossed to the male parent. More than thirty backcross progeny were obtained from each F_1 female by several deliveries. Phenotypes of the backcross progeny were observed macroscopically at about one month of age. As a result, 18 (26.1%) of 69 Jcl:ICR males carried 11 recessive mutation(s). Based on the phenotypes, the tentative names were abnormal kidney, aplasia of eyelids/hind limb digits, circling, dwarfism, heterotaxy, hind limb paralysis, hydrocephalus, rigidity (or rigor), testicular hypoplasia, tremor, and wobbling. The genes responsible for aplasia of eyelids/hind limb digits and dwarfism were each carried by two males, the genes responsible for hydrocephalus and testicular hypoplasia were each carried by three males and the gene responsible for wobbling by four males. It was strongly suggested that the genes shared by several males originated from an identical mutated gene. Surprisingly, male No. 43 had the responsible genes of abnormal kidneys and testicular hypoplasia, and No. 79 had those of dwarfism and tremor. The results obtained in this study suggest that breeders need to be aware of the presence of natural mutations in their colonies.

Key words: closed colony, hereditary disease, ICR, mouse, natural mutation

Introduction

It is well-known that several inbred strains used in biomedical research including SJL, SWR (from Swiss Webster mice, origin of ICR) and IQI (from Jcl:ICR) mice, and LEW, WKY and WM/Ms (from Wistar) rats (http://www.informatics.jax.org/external/festing/mouse/STRAINS.shtml), as well as many mouse and rat models for human diseases have been developed from closed colonies. Typical models are NOD/Shi (from ICR) as an IDDM (Insulin-Dependent Diabetes Mellitus) mouse model [16], SHR (from Wistar Kyoto) as a hypertensive rat model [17, 18] and OLETF (from Long-Evans; http://www.informatics.jax.org/external/festing/rat/docs/

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OLETF.shtml) and BB (from Wistar; http://www.informatics.jax.org/external/festing/rat/docs/BB.shtml) as NIDDM (Non-Insulin-Dependent Diabetes Mellitus) rat models.

In the 1970's, the authors attempted to establish inbred strains from the Jcl:ICR closed colony in order to prepare four founder strains to create a multi-cross hybrid (MCH) by four-way cross. The IQI strain mentioned above is one of the four founder strains. During inbreeding, we sometimes found natural mutations related to hereditary diseases. To date, we have reported recessive mutations as follows: *Enpp1*^{ttw} (ectonucleotide pyrophosphatase/phosphodiesterase 1) [9], *Hpd*^{hty} (4-hydroxyphenylpyruvic acid deoxygenase) [13], Tet(5^{12})1Jic (tetrasomy of Chr 12 centromeric region) [12] and *Ttc7*$^{fsn\text{-}Jic}$ (tetratricopeptide repeat domain 7) [26, 27]. Based on the evidence described above, we hypothesized that various natural mutations, which are genetically recessive in most cases, exist in the Jcl:ICR closed colony.

To validate this hypothesis, we started a natural mutation search project in our laboratory in 2002. Mutations are detected by backcrossing, which effectively generates homozygotes of recessive genes. Using this method, we have demonstrated that about 26% of males in the Jcl:ICR colony have recessive mutation(s). In this paper, we describe a strategy for detecting recessive mutations in closed colonies. We describe phenotypes of eleven mutations and mapping data, followed by candidate gene sequencing of six mutations. We also discuss mechanisms of generation and maintenance of natural mutations in a closed colony.

Materials and Methods

Mice

Eighty-one male mice randomly selected from the Jcl:ICR closed colony were used in this study. DBA/2JJcl females carrying recessive alleles (*a*, *b*, *C*, and *d*) at four major coat color gene loci were mated with Jcl:ICR males in order to demonstrate the alleles of these loci carried by Jcl:ICR mice. C57BL/6JJcl mice were used to perform gene mapping and to generate congenic strains of the mutations found in this study.

The experimental protocol and design were approved by the Animal Experimentation Committee of Hamamatsu University School of Medicine and performed according to the Guidelines for Animal Experimentation.

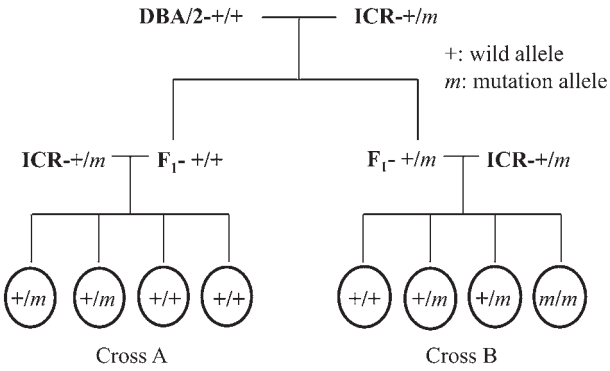


Fig. 1. Principle for finding natural mutations existing in a closed colony. When a mutation allele (*m*) is carried by an ICR male mouse, the probability of transmission to F_1 females is 1/2. F_1 female heterozygous for the mutation allele and an F_1 homozygous for the wild allele are mated with the male parent. The probability of appearance of mice homozygous for the mutation allele in backcross progeny is 1/8 (0/4 in Cross A and 1/4 in Cross B).

Genetic crosses performed to detect recessive mutations

The genetic crosses performed to detect recessive mutations (*m*) are shown in Fig. 1. First, an ICR (+/+ or +/*m*) male mouse was mated with a DBA/2JJcl (+/+) female to obtain F_1 mice. Then, four randomly selected F_1 females (+/+ or +/*m*) were backcrossed to the ICR male parent in order to obtain mice homozygous (*m*/*m*) for recessive mutation. About 30 backcross (BC) mice were obtained from each F_1 female by several deliveries.

Phenotyping

Phenotypes of the backcross mice were observed at about one-month of age. Observation was performed macroscopically and mice showing abnormal phenotype(s) were used for further phenotype analyses and gene mapping.

Gene mapping and candidate gene sequencing

Gene mapping was performed using mice homozygous for mutations in the backcross mice described above, or in F_2 mice obtained by crossing F_1 mice of a male heterozygote (+/*m*) and C57BL/6JJcl females (+/+). Microsatellite DNA markers showing different PCR products (size in bp) between ICR male(s) carrying the

Table 1. Novel autosomal recessive gene mutations found in this study

No. in Fig. 2	Phenotype (tentative name arranged alphabetically)	ICR individuals carrying the mutation	Further genetic analysis of the mutation				Current status of the mutation (generation)
			Methods	Results			
				Chr	Gene symbol	Disease name	
1	Abnormal kidney	43	Gene mapping followed by candidate gene sequencing	2	*Ptgis*	Renal failure	Congenic strain on C57BL/6JJcl background (N12)
2	Aplasia of eyelid/ hind limb digits	52 and 78	Gene mapping followed by candidate gene sequencing	10	*Grip1*	Fraser syndrome	Congenic strain on C57BL/6JJcl background (N11) and C3H/HeNJcl background (N6)
3	Circling	39	n.d.*				
4	Dwarfism	8 and 79	Gene mapping followed by candidate gene sequencing	12	*Tpo*	Hypothyroidism	Congenic strain on C57BL/6JJcl background (N12+F_8)
5	Heterotaxy	62	n.d.				
6	Hind limb paralysis	20	Gene mapping	7	unknown		Congenic strain on C57BL/6JJcl background (N12+F_5)
7	Hydrocephalus	32, 54, and 61	n.d.				
8	Rigidity	48	Gene mapping followed by candidate gene sequencing	10	*Enpp1*	Osteoarthropathy	Congenic strain on C57BL/6JJcl background (N8) and BALB/cByJcl background (N8)
9	Testicular hypoplasia	43, 57, and 68	Gene mapping followed by candidate gene sequencing	15	*Smc1b*	Azoospermia	Congenic strain on C57BL/6JJcl background (N12+F_3)
10	Tremor	79	Gene mapping followed by candidate gene sequencing	3	*Ugt8a*	Demyelinating disease	Congenic strain on C57BL/6JJcl background (N6)
11	Wobbling	25, 30, 63, and 74	n.d.				

*n.d.: not done.

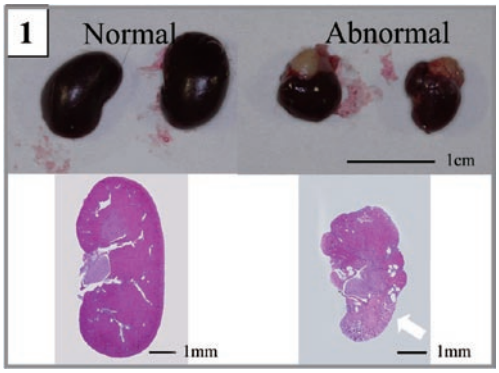
mutation and DBA/2JJcl or C57BL/6JJcl female(s) were selected on each autosome. In addition to MIT microsatellite markers, we used newly developed Ham (code for Hamamatsu University School of Medicine) markers in order to narrow down the positions of candidate genes on the chromosome. Candidate gene sequencing was performed for the most suggestive genes between the microsatellite markers closely linked to the mutations. PCR techniques used in gene mapping and candidate gene sequencing are described elsewhere [26, 28, 29].

Results

Alleles of coat color genes, a, b, and d loci, observed in Jcl:ICR mice

Of 69 DBA/2 females mated with Jcl:ICR males, 67 females delivered agouti F_1 mice and two females delivered both agouti and black mice. The genotype of agouti F_1 mice is considered to be *A*/*a*, *B*/*b*, and *D*/*d*, and that of black F_1 mice *a*/*a*, *B*/*b*, and *D*/*d*. Therefore, genotypes of 97.1% of Jcl:ICR mice are estimated to be *A*/*A*, *B*/*B*, and *D*/*D*, and 2.9% are *A*/*a*, *B*/*B*, and *D*/*D*. Thus, two coat color gene loci, *b* and *d*, are monomorphic, but the *a* locus is polymorphic with two alleles, *A* (0.985) and *a* (0.015).

Mutations obtained by backcrossing and their


1
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Abnormal
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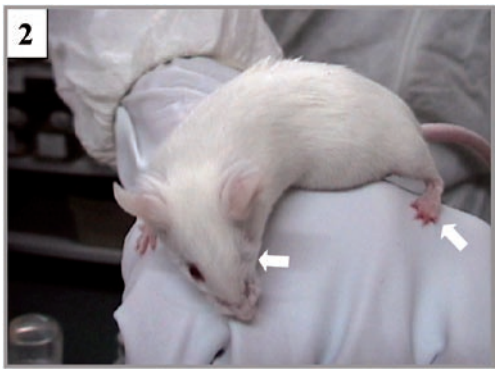
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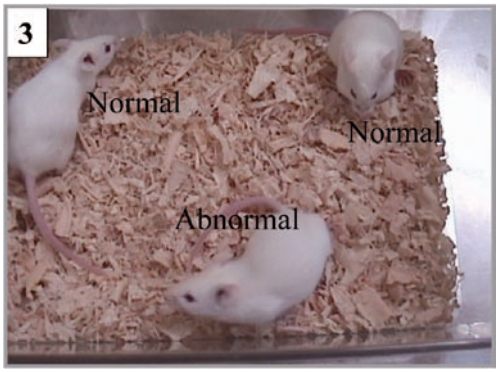
3
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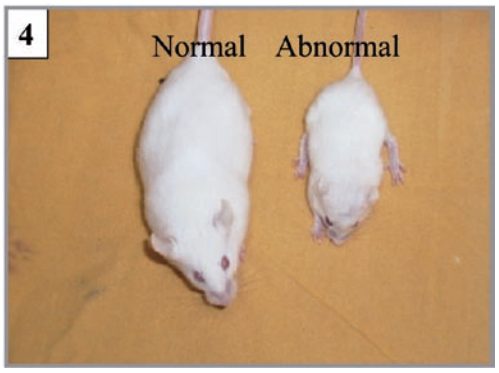
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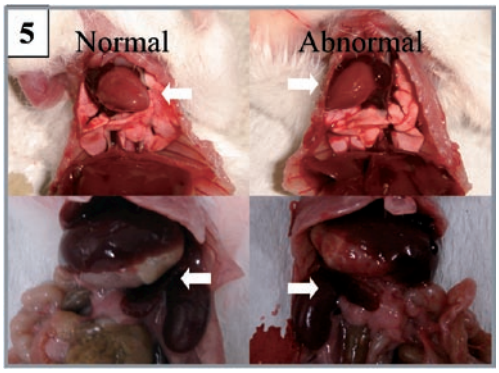
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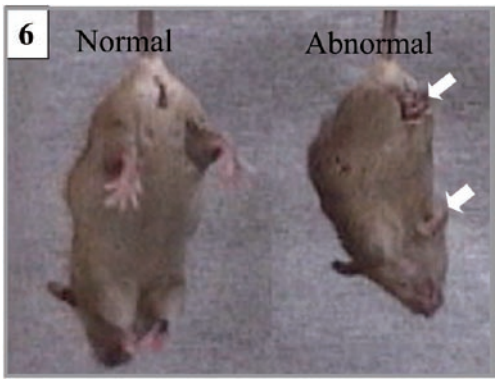
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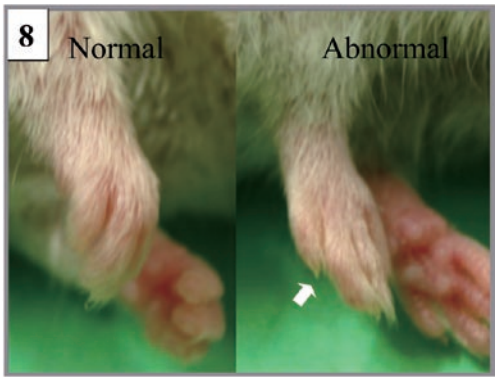
8
Normal
Abnormal

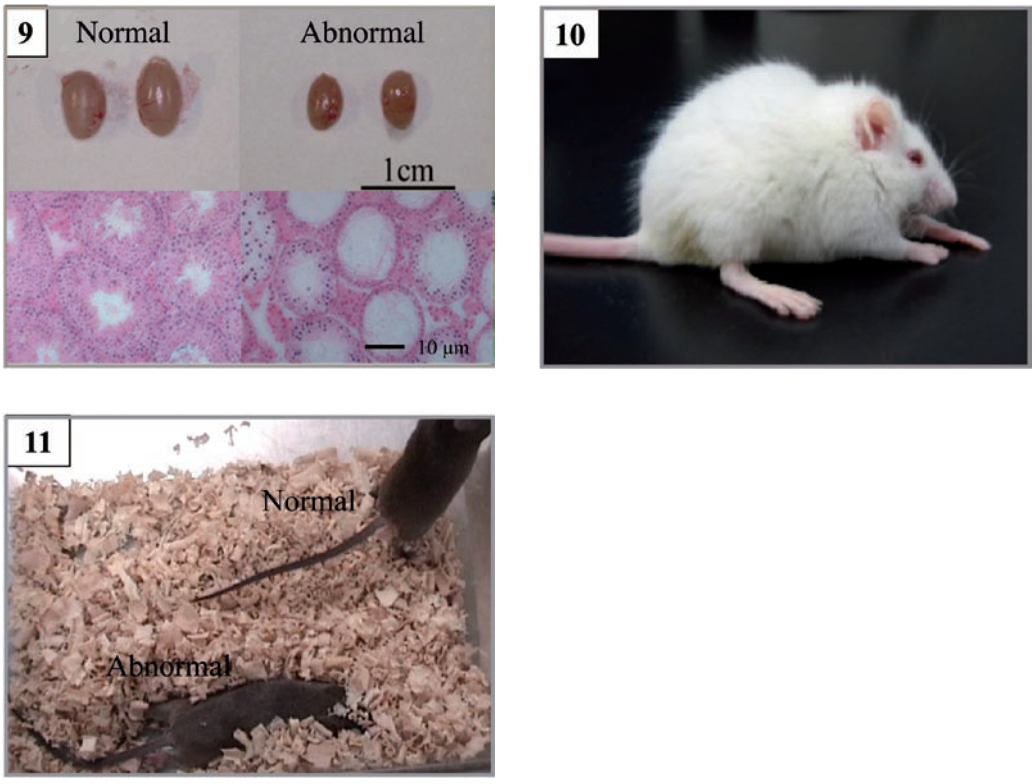

Fig. 2. Mutant mice obtained from the Jcl:ICR closed colony. White arrows indicate typical phenotype(s) of the abnormalities. 1) Abnormal kidney (about 1-mo-old male mouse): abnormal kidneys are smaller and more irregular than normal kidneys [right upper] and histology of the abnormal kidney stained with hematoxylin-eosin showed necrosis, calcification and fibrosis [right lower]. 2) Aplasia of eyelid/hind limb digits (about 2-mo-old female mouse): left eyelid and hind limb digits indicated by white arrows show aplasia. 3) Circling (about 2-mo-old littermates): since the abnormal mouse revolves frequently clockwise, the head leans slightly toward the right and is blurred. 4) Dwarfism (about 1-mo-old littermates): the abnormal mice show lower body weights compared with normal littermates. 5) Heterotaxy (about 1-mo-old male littermates): all organs of the abnormal mouse are in reverse compared with those of the normal mouse. 6) Hind limb paralysis (about 2-mo-old female littermates): abnormal mice show crouching at the fore and hind limbs. 7) Hydrocephalus (1-mo-old male mouse): the parietal region of the head is swollen as indicated by the white arrow. 8) Rigidity (about 1-mo-old male littermates): abnormal mice show rigidity due to ossification of all joints. 9) Testicular hypoplasia (about 1-mo-old male littermates): abnormal testes [right upper] are smaller than normal testes [left upper]. Histology of the abnormal testis stained with hematoxylin-eosin shows clear aspermatogenesis. 10) Tremor (about 1-mo-old male mouse): abnormal mice usually lie down due to tremor and, 11) Wobbling (about 1-mo-old littermates): abnormal mice are eager to burrow in the bedding.

frequencies

We observed eleven mutations in backcross mice obtained from 18 (26.1%) out of 69 Jcl:ICR males as shown in Table 1. Abnormalities were tentatively named immediately after finding them. Typical phenotype(s) of each mutation are shown in Fig. 2. Aplasia of eyelid/hind limb digits (list No. 2), dwarfism (list No. 4), hydrocephalus (list No. 7), testicular hypoplasia (list No. 9), and wobbling (list No. 11) were carried by two (ICR Nos., 52 and 78), two (ICR Nos., 8 and 79), three (ICR Nos., 32, 54, and 61), three (ICR Nos., 43, 57, and 68), and four (ICR Nos., 25, 30, 63, and 74) males, respectively. ICR No. 43 had two mutations, abnormal kidney and testicular hypoplasia, and No. 79 had two mutations, dwarfism and tremor.

Circling (list No. 3) and heterotaxy (list No. 5) were observed only once in backcross mice of ICR No. 39 and ICR No. 62, respectively. Frequencies of hydrocephalus

(list No. 7) and wobbling (list No. 11) in the backcross mice were extremely low. Frequencies of abnormal kidney (list No. 1), aplasia of eyelid/hind limb digits (list No. 2), dwarfism (list No. 4), hind limb paralysis (list No. 6), rigidity (list No. 8), testicular hypoplasia (list No. 9), and tremor (list No. 10) in the backcross mice agreed with the expected frequencies (1/4) shown in Fig. 1.

Gene mapping of the newly identified mutations

According to the mode of inheritance observed during genetic crosses and gene mapping, seven mutations, namely abnormal kidney (list No. 1), aplasia of eyelid/hind limb digits (list No. 2), dwarfism (list No. 4), hind limb paralysis (list No. 6), rigidity (list No. 8), testicular hypoplasia (list No. 9), and tremor (list No. 10), were apparently autosomal recessive genes. As shown in the current status of the mutations (Table 1), we have developed congenic strains of these mutations using C57BL/6JJcl.

Gene mapping and candidate gene sequencing were successfully performed and revealed that abnormal kidney (list No. 1) is controlled by *Ptgis* (prostaglandin I2 (prostacyclin) synthase, Chr 2), aplasia of eyelid/hind limb digits (list No. 2) by *Grip1* (glutamate receptor interacting protein 1, at 77.0cM on Chr 10), dwarfism (list No. 3) by *Tpo* (thyroid peroxidase, at 15cM on Chr 12), rigidity (list No. 8) by *Enpp1* (ectonucleotide pyrophosphatase/phosphodiesterase 1, at 19cM on Chr 10), testicular hypoplasia (list No. 9) by *Smc1b* (structural maintenance of chromosomes 1B, Chr 15), and tremor (list No. 10) by *Ugt8a* (UDP galactosyltransferase 8A, Chr 3).

Discussion

In this study, it was revealed that 18 (about 26%) of 69 Jcl;ICR male mice carried one to three mutations. If phenotypes of backcross progeny were studied in detail, not only single gene diseases but also polygenic diseases might be found. Therefore, we may underestimate the frequency of natural mutations existing in the Jcl:ICR colony.

In six out of eleven natural mutations found in this study, *Tpo* [28] was the first report of this mutation. Our *Ptgis*, *Ugt8a*, and *Smc1b* [29] are also first reports, but the knock-out mice have already been reported in [31], [1, 7], and [21], respectively. Since the *Enpp1* and *Grip1* mutations have already been reported in [9, 10] and [2, 30], respectively, our mutations are second reports.

The gene mutations responsible for heterotaxy were reported in mice [11] and human [19]. Gene knock-out mice have revealed that the heterotaxy phenotype seems to be controlled by the mouse hepatocyte nuclear factor/forkhead homolog 4 gene mutation [4] and gene expressions [15]. Hydrocephalus is an old mutation and two old gene loci, *hy1* [5, 22] and *hy2* [6, 32] are known. However, chromosome mapping of these loci has never been successful. In fact, there is no information on them in the MGI database. Frequencies of heterotaxy and hydrocephalus were extremely low in this study. It is possible that the genetic background of Jcl:ICR may be genetically resistant to expression of these mutations. Thus, there are opportunities for natural mutations existing in closed colonies to be the first reported mutation of a novel gene.

The results obtained in this study strongly suggest that natural mutations will have occurred not only in mouse colonies but also in rat and other laboratory animal colonies. Shibayama *et al*. reported a spontaneous dwarf rat originating from a Wistar Imamichi closed colony [23]. Suzuki *et al*. reported a novel gene of rat dwarfism from Wistar Imamichi rats [25]. It is likely that these dwarfism mutations are genetically identical and that they have been maintained in the colonies for a long time.

Other mouse colonies have mutations differing from those in the Jcl:ICR colony as follows: *pma* (peroneal muscular atrophy) (from a CF1 closed colony, http://www.informatics.jax.org/external/festing/mouse/docs/CF1.shtml) [8, 14] and $Ttc7^{fsn\text{-}hea}$ (from CF1) [24]. These mutations have accidently appeared during breeding without any systematic crosses to look for mutations. Therefore, it is obvious that the incidence of mice homozygous for some recessive mutations is extremely rare.

Generally, a closed colony is maintained by a rotation system [20] or similar mating system to suppress any increase of the inbreeding coefficient in the colonies. Since inbreeding such a brother-sister mating happens

rarely, the probability of becoming homozygous for identical mutated genes is extremely low. Once recessive mutations spontaneously occur in a colony, they are dispersed and drift in the colony.

Finally, we propose that we should pay special attention to natural mutations existing in closed colonies, because they could result in confusing results in biomedical studies such as toxicology. Breeders should consider searching for natural mutations in their own colonies and report the results to users immediately after finding any mutations. On the other hand, scientists should communicate frequently with breeders and utilize the information obtained for their studies.

Acknowledgments

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