

Genetic and phenotypic characterization of a Japanese wild-derived DOB/Oda rat strain

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Abstract

Wild-derived rat strains can provide novel genome resources that are not available in standard laboratory strains. Genetic backgrounds of wild-derived strains can facilitate effective genetic linkage analyses and often modulate the expression of mutant phenotypes. Here we describe the development and characterization of a new inbred rat strain DOB/Oda from wild rats (*Rattus norvegicus*) captured in Shitara, Aichi, Japan. Phenotype analysis of 109 parameters revealed that the DOB/Oda rats had small body weight, preference for darkness, and high locomotor activity compared with the rat strains in the National Bio Resource Project for the Rat (NBRP-Rat) database. Genome analysis with 357 SSLP markers identified DOB/Oda-specific alleles in 70 markers. Percentage of SSLP markers that showed polymorphism between the DOB/Oda strain and any of 132 laboratory strains from NBRP-Rat varied from 89% to 95%. The polymorphic rate (average of the values of the percentage) for the DOB/Oda strain was 91.6%, much higher than the rates for available wild-derived strains such as the Brown Norway rat. A phylogenetic tree constructed with DOB/Oda and all the strains in NBRP-Rat, showed that the DOB/Oda strain localized within the wild rat groups, apparently separate from the laboratory strains. Together, these findings indicated that the DOB/Oda rat has a unique genome that is not available in the laboratory strains.

Therefore, the new DOB/Oda strain will provide an important genome resource that will be useful for designing genetic experiments and for the discovery of genes that modulate mutant phenotypes.

Introduction

There has been an extraordinary increase in rat genomic resources over the past decade.

Rat genome sequences and genetic markers are now available and new methodologies for high-throughput genotyping and expression profiling have been developed (Aitman et al. 2008). In addition, database services that integrate genomic information and provide tools for data mining and analysis have been established (Dwinell et al. 2009).

These infrastructure developments have allowed disease genes, not only for monogenic traits but also for more complex traits, to be positionally cloned (Aitman et al. 2008; Aitman et al. 1999; Kuramoto et al. 2011).

Recently, gene knockout rat models have been generated using technologies such as embryonic stem cells, zinc-finger nuclease (ZFN), and transcription activator-like effector nuclease (TALEN) (Kawamata and Ochiya 2010; Mashimo et al. 2010; Tesson et al.). These technologies are likely to accelerate the generation of gene knockout rats for disease models and for functional annotation (Jacob et al. 2010). The rat model is an excellent experimental system in which to study genotype-phenotype correlations in both forward and reverse genetic approaches.

It is well known that rat mutant or disease phenotypes are often altered when the genetic

background of the causative gene underlying the phenotype is changed (Kuramoto et al. 1998; O'Meara et al. 2012; Yokoi et al. 2007). Thus, rat strains that share common mutations with different genetic backgrounds are good resources that can be used to find other causative genes underlying a mutant phenotype as well as modifier genes of the phenotype. Because gene disruption with the ZFN or TALEN technologies can be applied to the majority of available rat strains, it is expected that a repertoire of inbred strains with a particular mutation can be developed much easier than with the breeding of traditional congenic strains.

To identify effectively the genes that modulate a mutant phenotype, a rat strain with a genetic background that is very different from that of the commonly used laboratory strains is required. Because most available rat strains are derived from or are descendants of Wistar albino rats (Kuramoto et al. 2012), captured wild rats are considered as a good genetic source of genes that are not available in the laboratory strains.

The Brown Norway (BN) rat was the first wild-derived strain. It was derived from wild rats captured in the vicinity of Philadelphia, USA and various BN substrains are now available. One of the substrains was used to generate the draft sequence of the rat

genome (Gibbs et al. 2004). Mitake rats were derived from a cross between wild rats captured in Mitake, Gifu, Japan (Ohno et al. 1994). Five strains MITA, MITB, MITC, MITD, and MITE, were developed, but they are all now extinct.

Several rat strains have been developed from hybrids obtained from crosses between laboratory rats and wild rats; they include Long-Evans (LE), Ishibashi (IS), and NIG-III rats. The LE rats are descendants of a cross (made in about 1915) between several white female Wistar rats and a wild grey male caught in Berkeley, USA (Bennett 1991). The IS rats are descendants of a cross (made in about 1975) between female Wistar rats and wild grey male rats caught in the Azabu University, Sagamihara, Kanagawa, Japan. The NIG-III rats are descendants of a cross (made in 1956) between Castle's black rats and a wild rat trapped in Mishima, Shizuoka, Japan.

To date, the BN is the only pure wild-derived strain that is available. The polymorphic rate for 357 simple sequence length polymorphism (SSLP) markers between the BN strain and any other strain ranges from 78% to 92% (Mashimo et al. 2006), which is not higher than the rate for the intra-species wild-derived mouse strains (Abe et al. 2004). Thus, to expand the rat genetic resources it is important to develop new wild-derived rat strains.

In this study, we describe the development of the DOB/Oda rat strain from wild-captured rats (*Rattus norvegicus*) and examine their genetic and phenotypic characteristics.

Materials and methods

Capture of wild rats

To capture wild rats (*R. norvegicus*), we set rat traps in the goat house of the Experimental Station of Highland Animal Production (present name, Shitara Field), Graduate School of Bioagricultural Sciences, Nagoya University, Japan in February 2000. This institute is located in Shitara-cho, Aichi Prefecture, Japan, about 70 km east of Nagoya.

Development of the DOB/Oda rat strain

We crossed three males to four females of the wild-captured rats and obtained F1 hybrids in March 2000. Brother-sister mating with F1 hybrids was started to establish an inbred strain, tentatively named DOB after “dobu-nezumi”, a Japanese word which means the rat. At the F14 generation, the microbiological cleaning of the DOB strain was carried out by Caesarean section in the Central Institute for Experimental Animals, Kawasaki, Japan in June 2006. The colony of DOB rats (F15) was transferred to the Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University in October 2007. The brother-sister mating was undertaken at every generation during the inbreeding process. Inbreeding reached the F20 generation in June 2009 and the inbred

strain DOB/Oda was established. The DOB/Oda shows enough tameness, so we can touch and handle them without protection of our hands from their aggression. In December 2012, the inbreeding generation of the DOB/Oda strain was F27. A history of the development of the DOB/Oda strain is summarized in **Fig. 1**.

Breeding performance

To maintain the DOB/Oda rat strain, the routine mating of brother-sister pairs at 2 months of age was undertaken. The rats were kept in a 14h-10h light-dark cycle, at $25 \pm 2^\circ\text{C}$ and $50 \pm 10\%$ humidity and fed a standard diet (F2, Funabashi, Japan) and tap water. At each generation, the number of paired rats and the number of weaned offspring were recorded. As references, the breeding performances of the F344/Stm and LE/Stm were examined.

Phenotyping

The F20 generation of male (n=6) and female (n=6) DOB/Oda rats at eight weeks of age were examined for 109 phenotypic parameters as described previously (Mashimo et al. 2005). The parameters belonged to seven categories: functional observational battery (neurobehavioral), behavior studies, blood pressure, biochemical blood tests, hematology, urology, and anatomy.

Genetic profile

Genetic profiles of the DOB/Oda rats (F28 generation) were made using the 357 SSLP markers reported previously (Mashimo et al. 2006). As reference DNA from wild rats, we used the DNA from three wild-derived Mitake strains (MITB, MITC, and MITE) (Ohno et al. 1994), six rats captured in Germany (B6, G3, HR1, HR2, RU4, and RU5) (Voigt et al. 2000), four rats captured in Xian, China, and six rats captured in Obihiro, Hokkaido, Japan. The polymorphic rate for the DOB/Oda strain was calculated as follows. First, the percentage of the SSLP markers that showed polymorphism between the DOB/Oda and any of 132 laboratory rat strains from the NBRP-Rat was obtained. Then, the average of the values of the percentage was calculated. This value was defined as the polymorphic rate for the DOB/Oda strain. Such procedure was made for each strain.

Phylogenetic tree

A phylogenetic tree for the DOB/Oda rat and other strains from the NBRP-Rat database was obtained using a heuristic search for maximum parsimony implemented in the PAUP 4.0b10 as described previously (Mashimo et al. 2006). TreeView software was used to display the radial tree.

Results

Breeding performance of the DOB/Oda rats

We obtained 650 weaned offspring from 141 mated pairs from the F20 to F30 generations of DOB/Oda rats. Some females failed to become pregnant and some killed their offspring. The average number of weaned rats from a brother-sister mating was 4.7 ± 0.8 . This value was not significantly different from the value for the LE/Stm rats (5.6 ± 1.5), but was significantly lower than the value for the F344/Stm rats (12.1 ± 0.9). This finding indicated that the breeding performance the DOB/Oda was relatively low, but it was still sufficient to obtain enough progeny to maintain the DOB/Oda rats as an inbred strain.

Phenotypic characteristics of the DOB/Oda rats

The mean values of each phenotypic parameter obtained for the DOB/Oda rats were compared with the same parameters for the rat strains in the NBRP-Rat database (165 strains for male and 37 strains for female). The 109 parameters consist of 76 quantitative and 33 qualitative measurements. For the quantitative phenotypic parameters, the strains were ranked according to the value of each parameter, and a “strain ranking” for a selected parameter was obtained. All the data for the DOB/Oda

rats are available from the phenome project pages on the NBRP-Rat web site (<http://www.anim.med.kyoto-u.ac.jp/nbr/phenome.aspx>). Here, we focused on the parameters with the highest or lowest values in the strain rankings.

The most striking phenotype of the DOB/Oda rats was low body weight (**Table 1**). At 5, 6, and 10 weeks of age, the female DOB/Oda rats had the lowest body weight among the 38 female strains (the 37 strains from NBRP-Rat plus the DOB/Oda strain). At 10 weeks of age, the male DOB/Oda rats had the lowest body weight among the 166 male strains (the 165 strains from NBRP-Rat plus the DOB/Oda strain), although at 6 weeks, their body weight was the second lowest.

At 9 weeks of age, the male DOB/Oda rats had the highest heart rate among the 166 strains and female DOB/Oda rats had the third highest heart rate among the 38 strains (**Table 1**). For the 30-min locomotor activity, female DOB/Oda rats were ranked the highest and male rats was ranked 13th among the other male strains (**Table 1**).

In a step-through-type passive avoidance test, we measured the “training time” for which the rats stayed in the light box; rats were exposed to an 8-mA electric foot shock when they entered the dark box. After 24 hours, we measured the “retention time” of the trained rats. Male DOB/Oda rats had the lowest training time among the 166 strains and

the female rats had the second lowest training time among the 38 strains (**Table 1**). Although the retention time of the trained male DOB/Oda rats was more than 300 seconds, that of female rats was 49.0 ± 33.4 seconds, which was the lowest ranked times among the examined 38 strains.

Genetic profile of the DOB/Oda rats

We used 357 SSLP markers to build a genetic profile of the DOB/Oda strain and identified novel alleles that were specific to the DOB/Oda strain in 70 (19.6%) of the SSLP markers.

Percentage of SSLP markers that show polymorphism between DOB rat strain and any of 132 laboratory rat strains from the NBRP-Rat database varied from 89% for DMY (demyelination) to 95% for ICR (Ihara cataract rat) strains, with the polymorphic rate (an average of the values of the percentage) of 91.6 (**Table 2**). This value is very similar to the rates reported for the Mitake strains, although these strains are now extinct, and higher than the polymorphic rates for the wild-derived laboratory rat strains, BN, IS, and NIG-III strains.

Phylogenic tree including the DOB/Oda rats

Using the genetic profile derived using the 357 SSLP marker set, we constructed a

phylogenetic tree that included 132 laboratory rat strains, the DOB/Oda strain, three Mitake strains, six wild rats captured in Obihiro, Japan, four wild rats captured in Xian, China, and six wild rats captured in Germany (**Fig. 2**). The laboratory rat strains formed a large cluster, while the wild rat strains were grouped according to the place where they were captured and variations among the groups were large. Of the 132 laboratory rat strains, the BN strains clustered closest to the wild rat groups. The DOB/Oda strain was localized within the wild rat groups, apparently separate from the existing laboratory strains.

Discussion

The DOB/Oda rats showed several unique phenotypes. First, both sexes had the lowest body weights among the examined strains. Although food intake was not determined, other environmental factors during the experiments were constant. Thus, it is likely that low body weight is determined genetically in the DOB/Oda rat.

Second, the DOB/Oda rats showed strikingly short stay times in the light box in the training trials of the passive avoidance test. Rats instinctively prefer darkened areas to illuminated areas. Antianxiety agents such as the benzodiazepines have been shown to increase durations of stay and exploratory behavior in the illuminated area, while anxiety agent decrease them (Chaouloff et al. 1997). Thus, the DOB/Oda strain appeared to feel more anxiety than other laboratory strains when exposed to a new environment.

Last, the DOB/Oda rats showed comparatively high locomotor activity in the open field test, suggesting high spontaneous motor activity. Together, these findings suggest that the DOB/Oda rats may have inherited some of the characteristics of wild rats, including low body weight, high activity, and preference for darkness.

With such unique phenotypes, the DOB/Oda rats could be used in research fields such as physiology, pharmacology, neurology, and behavioral genetics.

The DOB rats also showed some unique genetic features. Among the 357 SSLP markers we examined, number of markers that showed polymorphism between the DOB/Oda and any of 168 laboratory strains varied 314 (88%) to 340 (95 %). As for the BN rat, which was derived from wild-captured rats, number of SSLP markers that showed polymorphism against any of 168 strains varied 250 (70 %) to 335 (94 %). The 168 laboratory strains included almost all the available strains; however, two recently-established strains of wild-derived rats artificially selected for tameness and defensive aggression towards humans are not included (Albert et al. 2008). Although we have not yet analyzed the genome of these strains, it is likely that, like the DOB rats, they too will show remarkably high polymorphic rate compared with the rates for the other laboratory rat strains. We have shown that the DOB/Oda strain has enough breeding performance to be maintained as an inbred strain; therefore, we propose that the DOB/Oda strain can become a prominent bioresource for genetic linkage analyses and for the identification of the causative genes in rat models of human diseases. Recently, we identified the causative gene for cataract development using a DOB/Oda

rat as a parental strain in the linkage analysis (Watanabe et al. 2012).

It was suggested that the ZFN and TALEN technologies could be powerful tools to disrupt gene functions in rats (Mashimo et al. 2010). To make gene knockout rats, the RNAs that encode ZFN and TALEN, which recognize and digest the specific site of a target gene, are microinjected to rat embryos. When these technologies are applied to the embryos of different rat strains, a repertoire of knockout rat strains in which the target gene is disrupted against the different genetic backgrounds, are efficiently generated. Because the DOB/Oda strain has a genetic background that is extremely different from other laboratory strains, it could contribute to the identification of modifiers of the knockout gene.

In summary, the genome of the wild-derived DOB/Oda rat strain is apparently different from the genomes of the existing laboratory strains. Thus, the DOB/Oda rat will be useful as a parental strain in genetic linkage analyses and will provide an important genome resource for use in the discovery of genes that modulate mutant phenotypes.

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Tables

Table 1 Parameters for which DOB/Oda rats had the highest or lowest values among the strains characterized by the NBRP-Rat

Parameter ^a	Male			Female		
	All rat strains			All rat strains		
	examined (n=166)	DOB/Oda	Ranking ^b	examined (n=38)	DOB/Oda	Ranking ^b
Body weight at 5 weeks of age (g)	103.7 ± 23.2	72.9 ± 0.9	153	100.0 ± 23.2	57.8 ± 3.1	38
Body weight at 6 weeks of age (g)	142.2 ± 30.7	84.9 ± 2.5	165	118.5 ± 30.2	63.2 ± 2.7	38
Body weight at 10 weeks of age (g)	248.5 ± 54.7	132.8 ± 8.4	166	164.9 ± 40.6	79.3 ± 5.1	38
Hear rate (beats/min)	437.8 ± 42.6	539 ± 104	1	472.6 ± 37	532 ± 71	3
Locomotor activity for 30 min	6646 ± 1301	8373 ± 741	13	6362 ± 1389	866 ± 1224	1

Passive avoidance test training time (s)	28.2 ± 20.2	4.3 ± 2.8	166	25.9 ± 15.4	8.3 ± 12.1	37
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^aData obtained from the NBRP-Rat web site (<http://www.anim.med.kyoto-u.ac.jp/nbr/phenome/>).

^bRanked in descending order.

Table 2 Polymorphic rates for wild-derived rat strains

Strain ^a	Polymorphic rates (%) ^b
DOB/Oda	91.6
MITB (extinct)	90.7
MITC (extinct)	90.7
MITE (extinct)	92.0
BN	80.3
IS/Kyo	84.0
NIG-III/Hok	76.3

^aThe three Mitake strains are extinct and only their genomic DNA was available.

^bPolymorphic rate is defined as the average of percentage values of the SSLP markers that show polymorphism between the corresponding strain and the remaining strains.

Because the BN, IS, and NIG-III strains were included in the 132 laboratory strains, the polymorphic rates were calculated against the other 131 strains.

Figure legends

Fig. 1 Development of the DOB/Oda strain

A History of the development of the DOB/Oda strain. In 2000, several wild rats were captured in the Shitara area of Aichi, Japan. These rats are the ancestors of the DOB/Oda inbred strain. Following microbiological cleaning in 2006, the F14 breeding colony was transferred to Kyoto University where it was maintained in a specific pathogen-free condition. In 2009, the inbreeding generation was F20 and the DOB/Oda inbred strain was established. **B** External appearance of the DOB/Oda rat at 10 weeks of age.

Fig. 2 Phylogenic tree including the DOB/Oda rats

This phylogenic tree includes the DOB/Oda strain, 19 wild-captured rats, and 132 laboratory strains. To make it easy to see names of strains, substrain names were omitted in ACI, BN, F344, and SHR substrains. The DOB/Oda strain localized within the wild rat groups, apparently separate from the existing laboratory rat strains.

A

2000/2	P	wild rats were captured in Shitara, Aichi, Japan
2000/3	F1	
2006/6	F14	microbiological cleaning
2007/7	F15	colony was transferred to Kyoto University
2009/6	F20	inbreeding reached to 20 generations
2012/12	F27	inbreeding reached to 27 generations

B



Fig. 1

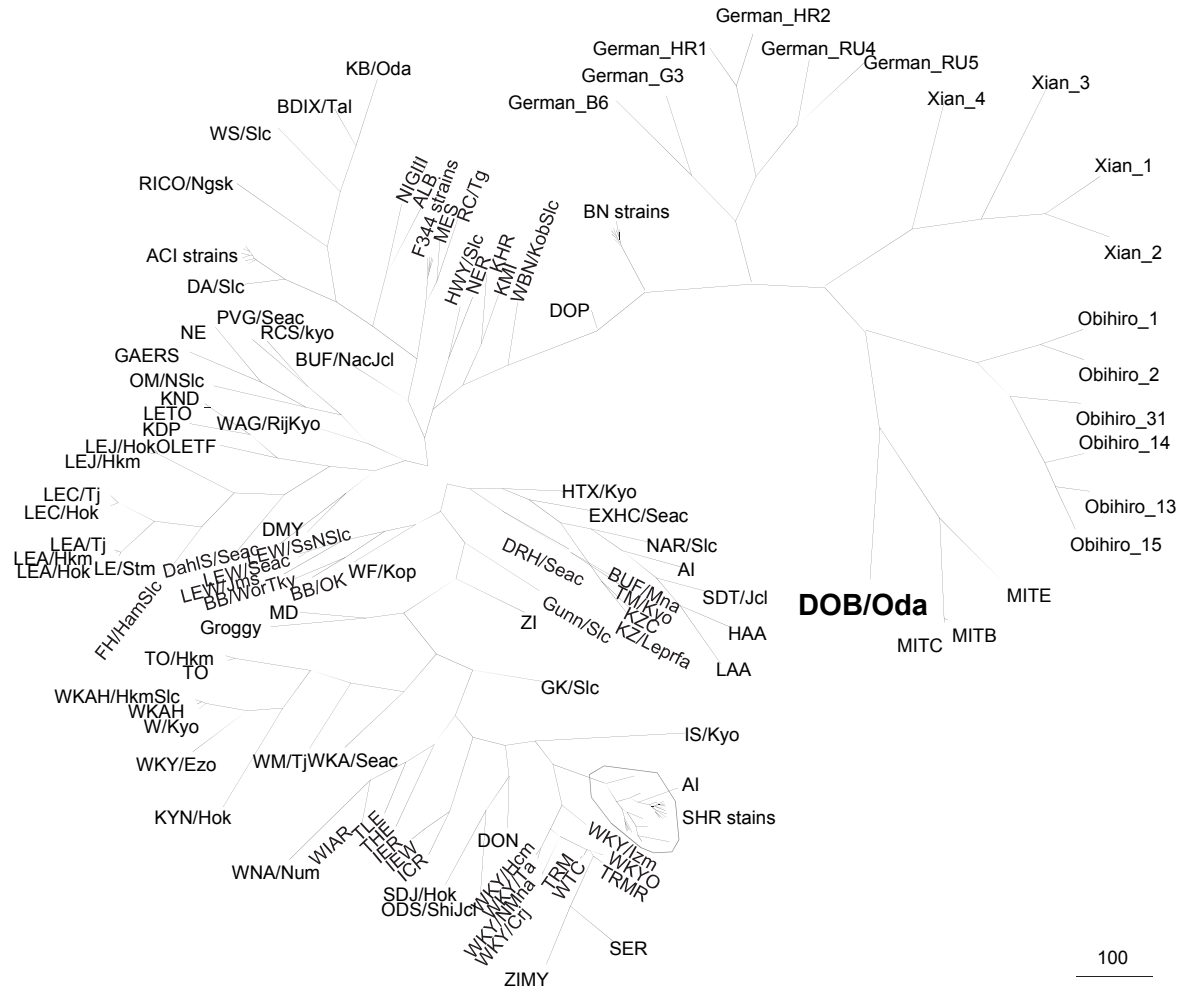


Fig. 2