Genetic Models in Applied Physiology
Invited Review: HXB/BXH rat recombinant inbred strain platform: a newly enhanced tool for cardiovascular, behavioral, and developmental genetics and genomics

Morton P. Printz,1 Martin Jirout,1,2 Rebecca Jaworski,1 Adamu Alemayehu,1 and Vladimir Kren2,3
1Department of Pharmacology, University of California San Diego, La Jolla, California 92093-0636; 2Institute of Biology and Medical Genetics, Charles University, Prague 128 00; and 3Institute of Physiology, Czech Academy of Sciences, Prague, The Czech Republic 142 20

Printz, Morton P., Martin Jirout, Rebecca Jaworski, Adamu Alemayehu, and Vladimir Kren. Invited Review: HXB/BXH rat recombinant inbred strain platform: a newly enhanced tool for cardiovascular, behavioral and developmental genetics and genomics. J Appl Physiol 94: 2510–2522, 2003; 10.1152/japplphysiol.00064.2003.—This review deals with the largest set of rat recombinant inbred (RI) strains and summarizes past and recent accomplishments with this platform for genetic mapping and analyses of divergent and complex traits. This strain, derived by crossing the spontaneously hypertensive rat, SHR/Ola, with a Brown Norway congenic, BN-Lx, carrying polydactyly-luxate syndrome, is referred to as HXB/BXH. The RI strain set has been used for linkage and association studies to identify quantitative trait loci for numerous cardiovascular phenotypes, including arterial pressure, stress-elicited heart rate, and pressor response, and metabolic traits, including insulin resistance, dyslipidemia and glucose handling, and left ventricular hypertrophy. The strain’s utility has been enhanced with development of a new framework marker-based map and strain distribution patterns of polymorphic markers. Quantitative trait loci for behavioral traits mapped include loci for startle motor response and habituation, anxiety and locomotion traits associated with elevated plus maze, and conditioned taste aversion. The polydactyly-luxate syndrome Lx mutation has allowed the study of alleles important to limb development and malformation phenotypes as well as teratogens. The RI strains have guided development of numerous congenic strains to test locus assignments and to study the effect of genetic background. Although these strains were originally developed to aid in studies of rat genetic hypertension and morphogenetic abnormalities, this rodent platform has been shown to be equally powerful for a wide spectrum of traits and endophenotypes. These strains provide a ready and available vehicle for many physiological and pharmacological studies.

quantitative trait loci; gene map; arterial pressure; heart rate; stress response; malformation phenotype

Address for reprint requests and other correspondence: M. P. Printz, Dept. of Pharmacology 0636, Univ. of California San Diego, La Jolla, CA 92093-0636 (E-mail: mprintz@ucsd.edu).
sioned that this rat platform would permit studies of rat genetic hypertension and morphogenetic abnormalities associated with PLS, the strains have turned out to be equally powerful for a wide spectrum of traits and endophenotypes associated with many disorders. With the development of new SDPs, publication of the rat genome sequence (http://www.hgsc.bcm.tmc.edu/projects/rat/assembly.html), and development of maps for rat single nucleotide polymorphisms (see http://snp.cshl.org/), this rat platform has significant future potential for physiological, behavioral, developmental, and pharmacogenomic studies.

DEVELOPMENT OF THE HXB/BXH STRAIN SET

RI and congenic rodent strains constitute an assembly of unique model organisms for genomic research (68, 69). These genetic animal models have been exploited to investigate a wide variety of diseases (33) and complex traits (16). Because of the long-term focus by geneticists on the murine species, this species, and not the rat, has been the choice for RI strain development with several sets of murine RI developed and characterized. Fewer rat RI strain sets have been reported, and the HXB/BXH strain set is, to the best of our knowledge, currently the largest Rattus-based RI strain set. Development of the HXB/BXH strain set commenced in 1982, jointly and cooperatively by Vladimir Kren at the Institute of Biology of Charles University in Prague, Czech Republic, and by Michal Praveneč at the Institute of Physiology of the Czech Academy of Sciences, by using two widely divergent inbred strains, SHR/Ola and BN-Lx/Cub.

Prior studies had led to the development of an inbred Wistar strain, the PD/Cub, from outbred rats that exhibited in 1969 a spontaneous mutation (Lx) predisposing to PLS (7, 34, reviewed in Ref. 65). To better examine the genetic origins of PLS, the trait was transferred onto the genetic background of the normotensive Brown Norway rat (BN/Cub) to form the congenic BN-Lx/Cub (34, 66). The morphological phenotype associated with the Lx mutation was visually apparent, and genetic transfer of this trait could therefore be tracked well before the advent of modern molecular genotyping. However, unknown to the investigators at that time, the PD/Cub carried other traits, documented only recently, such as hypertriglyceridemia (72) and insulin resistance (65), which have enhanced the experimental utility of the HXB/BXH strain set for human diseases. The original BN rat, Rattus norvegicus, was inbred from wild rats captured in the United States in 1917. Breeding pairs of the inbred BN strain were transferred to the Institute of Biology at Charles University (Cub) in 1964 and brother-sister bred to maintain homozygosity. The genetic homogeneity of the BN/Cub inbred strain was verified by total genome scan (66). Transfer of the PLS trait from PD/Cub onto the genetic background of the BN yielded the BN-Lx/Cub congenic strain, a model of genetic determination of PLS coded for by a major gene, Lx, the phenotypic manifestation of which was strongly influenced by minor modifying genes from the genetic background on which it expresses itself. The Lx mutation was subsequently mapped to a differential segment of chromosome 8 of PD origin in close linkage with other genes important to the widespread utility of the RI strains, specifically, Drd2, Ncam, and these apolipoprotein clusters: Apoa1, Apoa4, Apoc3, and Apoa5 (40, 43, 64, 66).

The SHR strain originated from an outbred Wistar colony trait selected by Okamoto and Aoki (46) for spontaneous development of hypertension as young adults and inbred to form the SHR/Kyoto as a potential genetic model of human essential hypertension. Breeder pairs were transferred to Hanover, Germany, and subsequently to Prague as the SHR/Ola. The selection of the SHR/Ola for the new HXB/BXH strain set was deliberate, to enable future studies of the genetic basis of this rodent model of human essential hypertension. The selection of the BN-Lx was also deliberate because the BN is distantly related to albino Wistar rats, which optimize the frequency of genetic polymorphisms between the strains, facilitating genetic mapping. In addition, carrying the PLS mutation permitted segregation analyses of this trait and studies of the influence of varying genetic backgrounds. Finally, BN and BN-Lx are normotensive strains that permit mapping of alleles for the normal control of arterial pressure. Because the two overt traits, hypertension and PLS, were considered to be independent, there was minimal concern at the time of trait interference and that assumption has largely been borne out. At the time that the intercross was begun, little information existed regarding other traits contained within the progenitor's genomes; however, we now know that polymorphisms in genes involved in metabolic disturbances, behavior, developmental processes, and response to toxico- and teratogenic stimuli were all contained within the genomes of the progenitors. As discussed below, the assembly and segregation of genes led to strain-dependent trait expression even when no significant differences existed between the progenitors.

The construction of RI strains is direct and well described (68): two highly inbred progenitor strains, as genetically distant as possible, are mated to produce F2 hybrids. Any F2 hybrid individual will carry a unique combination of genes because of both the independent segregation of maternal and paternal chromosomes and recombinations between homologous chromosomes during gametogenesis in F1 hybrids. Independent segregation of maternal and paternal chromosomes and recombination events during meiosis result in a unique and practically irreproducible combination of genes in any F2 individual. Subsequent inbreeding of randomly chosen pairs of F2 animals and brother-sister mating for at least 20 generations yield individual, relatively homozygous RI strains. These individual strains carry a pattern of unique paternal-maternal gene combinations, similar to the F2 animal. In addition, development of the HXB/BXH strain set utilized gender reciprocal crossing, which provided two sets of strains differing in the source of mitochondrial DNA and the Y chromosome. RI strains have several
advantages over single-generation intercross or backcross progeny: 1) being inbred they exhibit homozygosity at all loci; 2) all individuals of a particular RI strain are identical replicas so that studies may be replicated, permitting verification of any experimental result by independent testing of genetically identical animals; 3) phenotyping and genotyping data are cumulative; and 4) studies may be conducted during development, preand postnatal, as discussed below and shown in studies of target organ damage from chronic pressure or volume overload (29).

The derivation of the HXB/BXH set started with gender-reciprocal crosses between SHR/Ola and BN-Lx/Cub. The HXB set (H representing SHR and of female gender, B representing male BN-Lx) was bred at the Czech Academy of Sciences (Ipcv) so that all descendants carry mitochondrial DNA of SHR origin with the Y chromosome (RNOY) from the male BN-Lx/Cub strain. Originally, brother-sister inbreeding developed 26 HXB/Ipcv strains; however, five strains (HXB/Ipcv 9, 14, 16, 19, and 30) were lost after inbreeding due to poor breeding performance. The BXH set was derived at Charles University from female BN-Lx/Cub and male SHR/Ola so that the BXH set carries mitochondrial DNA of BN origin, whereas males carry the Y chromosome from SHR/Ola. Altogether, 32 RI strains presently exist that are all well beyond 50 generations of inbreeding, 21 HXB/Ipcv, and 11 BXH/Cub, with DNA preserved from all of the original 36 strains for map construction.

The original colonies have remained in Prague, whereas a second, pathogen-free colony has been established through embryo rederivation in La Jolla at the University of California, San Diego (40). Both colonies, Prague and La Jolla, are checked by genotyping and phenotype analyses. Frozen embryos are being preserved in both Prague and La Jolla to prevent loss through aberrant breeding, infection, genetic contamination, or disaster. In addition to the two sets of RI strains, there continues to be active congenic (39) and transgenic (56) strain development to test putative QTL and to explore complex phenotypes. Within the past 6 yr, a complementary set of 10 RI strains (known as PXO) has been developed with SHR.Lx and BXH2 strains as progenitors (see DEVELOPMENTAL TRAITS WITHIN THE HXB/BXH AND PXO PLATFORMS; Ref. 37). This set carries many of the disease alleles common to the HXB/BXH but with a diminished portion of BN/Cub genetic background and with homozygosity of the Lx locus, facilitating further analyses of alleles that modify expression of PLS. The overt physical characteristics of the HXB/BXH RI strain set are detailed in Table 1.

### Table 1. Descriptors of the HXB/BXH R1 strain set: coat color and limb phenotypes

<table>
<thead>
<tr>
<th>Strain</th>
<th>Coat color</th>
<th>FFD</th>
<th>PLS</th>
<th>HFT</th>
<th>HLEG</th>
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<td>NORM</td>
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<td>PLD</td>
<td>LUX</td>
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<td>PLD</td>
<td>LUX</td>
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<td>PLD</td>
<td>PLD</td>
<td>LUX</td>
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FFD, front feet digits; HFT, hind feet toes; HLEG, hind leg (zeugopod); ND on front feet, 4 triphalangeal digits and rudimentarily developed diphalangeal first digit; ND on hind feet, 4 triphalangeal toes and diphalangeal halux; PLD, polyphalangy up to polydactyly on front and hind feet; OLD, oligodactyly, 4 triphalangeal toes with first halux lacking; LUX, luxate appearance due to zeugopod affliction (attenuation of tibia up to tibial hemimely, thickening of the fibula and bunting of both bones), note that the more affliction the more expressed luxoid appearance; PLS, polydactyly luxate phenotype.

### HXB/BXH GENETIC MAP AND SDPs OF POLYMORPHIC MARKERS

The power and precision of meiotic mapping of alleles for complex traits depend on the accuracy of the genetic map for the species and strain under study and on the specific traits and models selected. One of the most important attributes of RI strains is that genotyping (like phenotyping) is cumulative (68), thereby facilitating both the initial construction and subsequent refinement of SDPs of polymorphic markers and
traits. The former can facilitate locus mapping, whereas the latter can improve statistical measures of traits. However, errors present in the cumulative data set can rapidly attenuate mapping power and/or bias linkage results. Commencing in 1986, the original genetic characterization of this RI strain set, essential to QTL mapping, was gradually developed (52–55). The application of minisatellite markers, amplified fragment length polymorphism markers (47), and microsatellite polymorphisms (51, 54) rapidly expanded the SDPs and permitted identification of many putative QTL. However, the map was still incomplete, and its power was weakened due to unlinked markers on many chromosomes and the fact that most markers were not commercially available and their map positions were not defined with high accuracy to a single reference map.

With completion of the rat map (70) with high marker position reliability (framework markers with LODs >3), a reference rat map was available that could facilitate a reanalysis of the RI strain set. This effort was undertaken in La Jolla and Prague, and the resulting linkage map for the 20 autosomes was developed by Jirout et al. (32) with the use of 245 microsatellite markers, predominantly framework, yielding 8,726 individual genotypes. The map spans a total length of 1,789 cM, which is in good agreement with the reference (70) and other published maps. This new map covers the rat genome contiguously and completely with an average intermarker distance of ~8.0 cM. The map for chromosome 1 is provided (Fig. 1A), illustrating linkage of all markers; Fig. 1B provides a sampling of the framework marker-based SDPs (32). The original (55) and new (32) table of SDPs may be accessed at http://www.ratmap.gen.gu.se.

With the new contiguous map and SDPs, an opportunity was available to analyze the genetic composition of the strains important to QTL mapping (32). A genetic similarity matrix indicated that the progenitors' polymorphic microsatellite markers are evenly distributed over all but 4 of the available 31 strains: strains BXH8 and BXH08, which are genotypically similar due to derivation commencing several generations after the F2 cross, and strains HXB3 and HXB15 (similarity ~78%) for unknown reasons (32). The evidence for nonsyntenic association across the strains was also assessed by Jirout et al. (32) as nonsyntenic loci showing allelic association may lead to false-positive signals (73). Three examples of two different chromosomal areas with identical sequence were identified, as well as three chromosome pairs with mirror image in sequence. The former could lead to multiple QTL assignment, whereas the latter could also yield an apparently opposite allelic influence. With these analyses, the new SDPs provide enhanced validity for QTL identification. In pilot studies (Jirout and Printz, unpublished observations), the new SDPs significantly decrease the number of strains needed to map loci (significant or highly significant LOD values), provided that the trait measurements are accurate with relatively low variance.

CARDIOVASCULAR AND METABOLIC TRAITS WITHIN THE HXB/BXH PLATFORM

Arterial pressure. It would be expected that trait measures of arterial pressure would be continuously distributed across the HXB/BXH RI strains because genetic hypertension is a complex multigenic disorder and the SHR/Ola was one of the progenitors. Systemic arterial pressure, although seemingly easy to measure, is a very challenging phenotype as it is environment sensitive (both dietary and through behavioral influences), reflects the controlling influence of a large number of complex integrated systems, exhibits diurnal variation, and is sensitive to many physical characteristics of the animals, including body mass (58). Although arterial pressure and hypertension were early traits in mapping efforts, the results were far from convincing. We now know that one problem was due to the available linkage map and SDPs (Jirout and Printz, unpublished observations). Nevertheless, there were successes, and these early results on the genetics of hypertension and other risk factors for cardiovascular disease with the use of the HXB/BXH strain set, along with proof of locus identification through congenic development, have recently been addressed (41, 42, 50). For this reason, we will focus here only on recent and unpublished findings.

Initial arterial pressure phenotyping of progenitors and RI strains by Kunes and colleagues (45, 52) documented a continuous distribution of systolic and diastolic arterial pressures among the RI strains, suggestive of multigenic influences on the traits. On the basis of this initial phenotyping and with the use of the older SDPs, linkage and association analyses suggested several putative arterial pressure loci, specifically on chromosomes 1 (associated with marker Rt6), 2 (associated with D2N35), 4 (associated with marker Il6), 19 (associated with marker D19Mit7), and 20 (associated with marker D20Utr1) (37). These analyses were not optimized owing to uncertainties in the map, the use of acute arterial pressure measurements, and environmental factors on arterial pressure; nevertheless, through the use of a maximum number of strains, associations were identified. Although the exact positions of the loci remain to be determined, many of the chromosomal assignments drew subsequent confirmation through congenic strain development where (large) chromosomal segments from the BN or BN-Lx were transferred onto the SHR genetic background. However, it has also become clear that each of these chromosomes likely contain multiple arterial pressure loci, as has been shown recently for chromosomes 1 (28), 2 (4), and 10 (27). Furthermore, in the case of chromosome 2 (4), both pressor and depressor loci are close in proximity to one another.

To minimize environmental influences on arterial pressure measurements and to examine the effects of diurnal variation and dietary NaCl loading, we undertook to reanalyze all the RI strains in La Jolla using radiotelemetry (58). In this study, which was modeled after a typical clinical paradigm, samplings of systolic
pressure, diastolic pressure, heart rate, and activity were obtained over a 5-s window, every 5 min, 24 h, for 12 wk. The first 6 wk consisted of “baseline” measures with normal dietary NaCl intake, weeks 7 through 9 consisted of an 8% NaCl intake, whereas weeks 10 through 12 was a return to baseline salt intake. Over the entire 12 wk, rats were maintained in their home cages in a specially instrumented vivarium room with human interruption only to the extent of normal vivarium room operation. The data set, which largely occupies one server, is presently undergoing linkage analysis and mining; however, much new and exciting information has already been obtained. First, confirming the findings of Pravenec et al. (52), a continuous strain distribution of arterial pressures was evident with BN-Lx lowest and SHR/Ola highest (Fig. 2). However, not evident in Fig. 2 is that the arterial pressures measured by radiotelemetry were significantly lower than those measured by direct catheterization (45, 52), likely reflecting the fact that the latter measurements were taken under stressful conditions. There is, nevertheless, a rank-order correlation between the two measurements. Second, the strain order distributions for systolic and diastolic pressures are not identical, indicating that different genes are likely influencing the two measures, a not unexpected result given that a multiplicity of physiological systems control these two measures of arterial pressure. Third, some strains exhibit marked diurnal rhythms of arterial pressure or heart rate, analogous to human “dippers,” whereas other strains exhibit minimal rhythm analogous to human “nondippers” (Fig. 2). Note that in the measure of systolic pressure the two progenitors constitute the extremes of the trait-strain distribution, as would be predicted. Fourth, the arterial pressure of some strains is markedly sensitive to dietary intake levels of NaCl (analogous to salt sensitivity), whereas other strains are quite resistant (salt insensitive). We documented that the systolic pressure of the BN-Lx was salt sensitive, whereas diastolic pressure was far less salt sensitive. Although normotensive, during high salt intake, other RI strains exhibited lower systolic pressure than the BN-Lx. The SHR/Ola remains the strain with highest arterial pressures during both normal and high salt intake. Although the progenitors tend to be the extremes in trait-strain distributions, transgressive variation was evident for other cardiovascular traits. These results indicate that genetic determination of arterial pressure exhibits multigenic complexity, with influences from both the environment and the genetic background of the strain, constituting significant confounds for genomics of arterial pressure.

It has been argued (44) that the use of inbred congenic strains will facilitate identification of alleles that control arterial pressure and likely contribute to the hypertension syndrome. It is certainly correct that congenic development is necessary for testing of putative QTLs and to facilitate identification of candidate genes; however, studies conducted with the HXB/BXH RI strain set by our laboratories and others would argue that gene-trait relationships are disrupted when the locus under test is placed onto an artificial, uniform genetic background. In such a case, an entirely new form of genetic hypertension may have been created, while some endogenous genetic forms may have been lost. We would propose that RI strains, varying normally in genetic background, rather than congenic strains, offer the best opportunity for identifying those alleles that will more likely have maximal influence on resting and active arterial pressures in the population and thereby would likely be significant contributors to genetic hypertension.

Metabolic traits. In 1996, Bottger et al. (17) reported their linkage study with the HXB/BXH RI strains in which QTLs for cholesterol and phospholipid phenotypes were identified. Using a genome scan of 534 polymorphic markers and high-cholesterol diets, they reported a locus on chromosome 19 for HDL2 cholesterol. Subsequent congenic studies identified suggestive loci on chromosomes 4 and 8 for serum triglycerides (66). Since then, there has been a flurry of papers examining several metabolic traits with the use of the RI strains and/or congenics derived from them. As discussed (66), it is now clear that the origins of these cardiovascularly important metabolic phenotypes derive from all three progenitors: PD/Cub, SHR/Ola, and BN/Cub (57, 65, 66). As shown in Ref. 57, the metabolic traits examined were continuously distributed across the RI strain set, indicative of multigenic determination. Additionally, in several of the traits, evidence of transgressive variation was found, with the progenitors not constituting the extremes of the trait-strain distribution. We have documented a similar phenomenon for cardiovascular and behavioral traits (see below), whereas Zidek et al. (74) have shown it for several reproductive traits. Metabolic QTLs were mapped to chromosomes 3 and 17 for the insulin-to-glucose ratio and to chromosome 7 for the intraperitoneal glucose tolerance test (57). Suggestive loci were also identified and confirmed through congenic development to be

![Diagram of RI Strains](image-url)

**Fig. 2.** Illustration of continuous strain-dependent distribution of systolic arterial pressure (SAP) during active and rest periods. SAP averages are from a 3-wk period during baseline analysis (see text). Values reflect either pressures during the rest light period (○) or active dark period (●). Note the diurnal variation is present in some but not all strains. The BN-Lx has the lowest SAP, whereas the SHR/Ola has the highest SAP. RI, recombinant inbred.
present on chromosome 19 for HDL2 cholesterol (57) and chromosome 8 for serum triglycerides (66).

Aitman et al. (2, 3) reported on the utility of the SHR strain for studies of glucose and lipid metabolic traits. This was followed in 1999 (1) with the report that studies had led the authors to focus on a locus in chromosome 4 that they identified, through gene expression studies and congenic and radiation hybrid mapping, to be close to a candidate gene, CD36, a fatty acid translocase. They identified polymorphisms within the gene and proposed that altered CD36 may be involved in insulin resistance and dyslipidemia. In the past 3 yr, there has been a flurry of studies on CD36, which go beyond the content of this review. It is worth mentioning that the progenitor strains of the HXB/BXH set (SHR/Ola, BN/Cub, and PD/Cub), as well as CD36 targeted congenic and transgenic strains derived from them, have been successfully exploited in pharmacogenetic analysis of the antidiabetic drugs, thiazolidinediones, and studies of their effects on carbohydrate and lipid metabolism (60, 63, 64). The results from these studies highlight both the role of CD36 and the modifying effect of the genetic background on drug effect.

BEHAVIORAL, AIRPUFF STARTLE, AND STRESS-RELATED TRAITS WITHIN THE HXB/BXH PLATFORM

One of the most exciting new research areas for this RI strain set lies in studies of complex behavioral traits and endophenotypes. The HXB/BXH strain set was predicted to have traits for cardiovascular function (particularly arterial pressure) and for musculoskeletal abnormalities, and the finding of important metabolic traits was a bonus; however, it is now apparent that this RI strain platform is also of great value for behavioral traits. It is likely that, similar to findings for metabolic traits, the utility of the strains for behavioral traits stems from alleles present in all three progenitors and the interaction of the genetic backgrounds. Our behavioral studies with the RI strain set originated from studies of sensoriautonomic coupling that used the airpuff and acoustic startle response (19, 21, 61). In a repeated airpuff trial paradigm with normotensive Wistar-Kyoto (WKY/lj-cr), BN/hsd, and SHR (SHR/lj-cr) rats, the stress component (pressor and tachycardia response) was elicited on every trial, exhibited minimal habituation, and was strain dependent (21). The orienting response bradycardia was observed in adult WKY/lj-cr and BN/hsd animals, predominantly only on trials 1–3, and exhibited rapid habituation. In contrast, SHR animals, from both the La Jolla colony and commercially derived, generally failed to exhibit any bradycardia response, only tachycardia on the initial airpuff trials (21). In these and subsequent studies, evidence showed a genetic influence on both the stress (sympathetic) and the orienting response (parasympathetic) components of the airpuff startle reflex (20, 21, 30, 49, 59).

Because the SHR and BN (or WKY/lj-cr) strains exhibited marked trait differences in the airpuff cardiovascualr and behavioral components of the startle response, the RI strain set gave us an opportunity to seek stress-related QTLs for both the cardiovascular and behavioral (e.g., motor) traits associated with the airpuff startle reflex. Our laboratory (31) used our standard chronic catheterization procedure to examine a subset of the RI strains; with the new SDPs, QTLs were identified for both cardiovascular and behavioral traits (Fig. 3). We believe that this study is the first to report a QTL for the startle reflex and for loci controlling heart rate. Jaworski et al. (31) identified two significant QTLs for the early trial bradycardia associated with the orienting response: one on chromosome 2 (LOD 2.9, D2Rat62-D2Rat247) and a second on chromosome 3 (LOD 2.1, peak D3Rat20), together accounting for nearly 50% of the trait variance. Since the initial analyses, two additional loci have been confirmed with significant or suggestive LOD scores but smaller contributions to the variance; however, combining all four accounts for over 70% of the variance. For airpuff startle stress tachycardia responses, we identified two significant QTLs on chromosomes 1 (LOD 3.08, D1Rat287-D1Rat292) and 10 (LOD 2.4 D10Rat26-D10Rat267). A stress arterial pressure QTL (based on the trait, airpuff-elicited change in arterial pressure) was also identified on chromosome 6 (LOD 2.5, D6Rat80-D6Rat171), a chromosome not previously associated with genetic control of arterial pressure (31). The finding that QTLs for these divergent heart rate responses, elicited by an environmental startling stimulus, are located on different chromosomes likely reflects a multiplicity of complex control systems for

![Figure 3](https://example.com/f3.png)
both normal and stress-dependent trait expression. Important to recognize is that the loci for these heart rate responses are located on chromosomes that also contain arterial pressure loci. This raises the potential for linkage, thereby potentially predisposing to enhanced cardiovascular morbidity or mortality. To examine this question further, the HXB/BXH RI strain set, and associated congenics, provides a ready and available genetic and physiological platform.

ANXIETY-RELATED TRAITS AND ELEVATED PLUS MAZE

On the basis of our findings with airpuff startle, our laboratory also undertook an examination of the RI strains by using various behavioral traits. Using elevated plus maze, Conti et al. (22) characterized strains for several measures of anxiety and/or locomotion: percent time spent in the open arms, percent entries into the open arms, number of entries into both the open and closed arms, and number of entries only into the closed arms. SHR/Ola showed both greater percent time in the open arms and percent entries into the open arms than the BN-Lx progenitor, which was expected based on published studies of heightened measures of anxiety in this strain; however, it was found that there were no significant differences between progenitor strains on the other two measures. All four traits exhibited a continuous trait-strain distribution with significant differences among the RI strains on each of the four phenotypes, consistent with multigenic determination of the measures. With the use of the new SDPs, significant QTLs were identified for anxiety-like phenotypes on chromosomes 2, 5, 6, 7, and 17 and for a trait reflecting both locomotion and anxiety on chromosomes 2, 5, and 6. Finally, traits primarily associated with locomotion mapped to chromosomes 3, 8, and 18. For all but one strain examined, the presence of PLS did not appear to influence strain performance. In one strain, locomotion appeared to be a confound to performance, and this strain was not included in the linkage analyses. Interestingly, in an independent study (based on a cross between BN/hsd and WKY/lj-cr), a QTL for presiphon inhibition of the acoustic startle response, a measure of sensorimotor gating, was mapped by Palmer et al. (48) to a nearby locus on chromosome 2.

QTLS FOR STARTLE-ELICITED BEHAVIORAL TRAITS

As discussed above, both behavioral and cardiovascular traits are expressed in response to startling stimuli. Because the startle reflex is a stressor and may elicit measures related to fear, anxiety, and emotion, analyses of physiological and behavioral responses to a startling stimulus may provide insight into a number of human disorders, including anxiety, schizophrenia, and posttraumatic stress disorder as well as fear-mediated cardiovascular arrhythmia. Using the airpuff paradigm and only 23 RI strains, Jaworski et al. (Ref. 31 and unpublished observations) found significant strain effects on peak startle amplitude, startle latency, and percent startle habituation. Composite interval mapping (32) identified the first QTLs for several of these behavioral traits. Significant QTLs were found for peak startle amplitude on chromosomes 7, 17, and 20, whereas startle latency mapped to chromosomes 1, 4, and 9 and startle habituation mapped to chromosomes 1 and 9. Because several of these loci are “near” those found for plus maze anxiety traits (see above), the possibility exists that they represent anxiety-related alleles. Such QTLs and the RI strain platform should facilitate finding candidate genes important to these behavioral endophenotypes.

LOCI FOR CONDITIONED TASTE AVERSION

Bielavská et al. (6) used the HXB/BXH RI strain set to examine conditioned taste aversion by using lithium chloride as the aversive substance. They observed a continuous distribution of values across the RI strain set, with the BN-Lx progenitor exhibiting a stronger aversion than the SHR progenitor, whereas seven strains exhibited even stronger taste aversion values than BN-Lx. The SHR value was on the opposite extreme. An association of conditioned taste aversion (CTA) was found with two loci, one was mapped on chromosome 2, around D2Cebr11s4 and D2Arb24, and one on chromosome 4, D4Cebrp149s8. The likelihood distribution on chromosome 2 was broad; therefore, the relationship, if any, between this locus and that found for other behavioral or stress loci (discussed above) cannot as yet be determined. However, it is unlikely that these dissimilar traits identify the same locus. As reported by the authors, the chromosome 2 CTA locus was supported by examining an SHR congenic carrying an introgressed segment of BN-Lx chromosome 2.

It is of interest that a small number of chromosomes appear to contain a collection of loci for different traits all related to behavioral performance or behavior-autonomic coupling. This may be coincidental or a reflection of a clustering of alleles involved in a common goal. The HXB/BXH RI strain set provides a platform for examining this issue at several levels of investigation.

DEVELOPMENTAL TRAITS WITHIN THE HXB/BXH AND PXO PLATFORMS

There is a substantial difference in the genetic determination between the original two model diseases integrated into the experimental model of RI strains. Spontaneous hypertension from the SHR strain is genetically determined by many factors that contribute in varying degrees to the resulting blood pressure phenotype, thereby SHR is multigenic and multifactorial. On the contrary, the PLS traits are genetically determined by one major gene, Lx, the inheritance of which is basically Mendelian but modified in its expressivity and penetrance by polygenic influences of the genetic background (7, 34, 35, 38). Because the morphometric traits exhibit continuous distribution and are determined by allelic contributions from the genetic background, these loci are QTLs. The extreme variability of
the PLS fixed in individual strains of both HXB/BXH and PXO sets of RI strains, as well as congenic strains, is therefore due to the interaction of the major gene, \( Lx \), with specific combinations of polymorphic morphogenetic genes of BN or SHR origin.

The \( Lx \) mutation was randomly distributed through the HXB/BXH set of inbred strains, permitting studies of the genetic determinants of limb development. The vertebrate limb has been generally recognized as a model system for the study of the mechanisms that control pattern formation during development (18, 25). Limb malformation mutants were also proposed to be a useful tool for studying mechanisms of normal development by Etelson et al. (26). Tabin (71) proposed a molecular genetic model of limb development involving complex interactions among retinooids, homeoboxes, and growth factors. It is now generally accepted that genetic determinants of human limb development find considerable homology of mechanism with animal model systems. The HXB/BXH RI strains, along with the new PXO RI set (37) and congenic strains that carry leg paw malformations as a model of genetically determined disease, facilitate genomic studies for understanding the complex interactive processes underlying genetic control of development and also genetic origins of similar human pathologies.

The original malformation phenotype of PLS in outbred Wistar rats (34) consisted of preaxial polydactyly and zeugopod affliction of the hind feet, which imparted a “luxate” appearance to the carriers. During introgression of PLS onto the genetic background of inbred SHR and BN strains, it was found that the disease phenotype was coded for by a major gene designated \( Lx \), the phenotypic performance of which was a function of the genetic background. On the SHR genetic background, the \( Lx \) gene performance was completely recessive and the only manifestation of PLS in homozygous SHR.Lx congenic animals was preaxial polydactyly of the hind feet without significant zeugopod affliction. On the contrary, on the BN genetic background, the \( Lx \) gene inheritance was semidominant, with 60% penetrance in +/\( Lx \) heterozygotes that carry preaxial polydactyly of the hind feet. Homozygous \( Lx/Lx \) BN-Lx congenic animals exhibit polyphalangy up to polydactyly of the front feet and preaxial polydactyly of the hind feet with prominent affliction of the zeugopod. Such a substantial difference in the phenotypic expression of the \( Lx \) mutant gene between SHR and BN genetic backgrounds strongly indicates polymorphisms in many modifying genes involved in morphogenetic processes. The PLS phenotype was randomly fixed in 21 of 36 originally derived RI strains and currently resides in 19 of the surviving 32 strains. Morphometric analysis has demonstrated that any individual RI strain with PLS represented a unique malformation phenotype, provided that all the morphometric traits were taken together (36).

To expand the utility of the HXB/BXH RI strain set for developmental analyses of limb malformation genetics, an additional set of strains was developed. Progenitors for the new set of strains were two strains exhibiting the most extreme malformation phenotypes: the SHR.Lx congenic and the BXH2 RI strain. From these progenitors, 10 complementary strains were derived (39) and designated PXO to refer to hind feet polydactyly in SHR.Lx and oligodactyly in BXH2. These strains were inbred through brother-sister mating and are currently over 30 generations. A genetic map of the PXO set has been derived consisting of more than 200 polymorphic framework markers arranged according to the new HXB/BXH map (32) with residual heterozygosity being <5% (Kemlink D, Jeaabkova M, Janku D, Krenova D, and Kren V, unpublished observations). The malformation phenotypes exhibit similar variability of malformation expression as the original HXB/BXH sets and, together with the original set of strains, should prove valuable in future studies to identify genes responsible for phenotype variability.

A detailed morphometric digital image analysis of both the HXB/BXH and the PXO strain sets was performed by measuring 12 selected parameters and 2 indexes of the zeugopod (36) of 4-mo-old animals (Fig. 4). It was found that most of the traits examined exhibited continuous distributions across affected RI strains and progenitors, as illustrated for tibia length (Fig. 5). Interstrain statistical comparisons with the 14 trait measures supported the finding that genetic background strikingly modified expression of the \( Lx \) mutation (Table 2). There were no significant differences between SHR.Lx and SHR, reflecting the moderating effect of the SHR genetic background on \( Lx \) expression.

Association analysis was conducted and indicated several significant and suggestive associations between morphometric traits and gene markers. With the use of the new framework-based map and SDPs for HXB/BXH (32), several QTLs were identified, some found to be closely linked with genes involved in morphogenetic pathways, for example, D10Rat116, which mapped to 45.38 cM, closely linked (according to UNIGENE) to Aldh3a2. To test these associations, double
congenic strains have been initiated by introgressing segments of SHR chromosomes 2 and 4 onto the BN-Lx strain background. Each double congenic strain should thus carry a differential segment of RNO8, with the major PLS gene Lx and a second differential segment of SHR origin with putative loci modifying the PLS malformation phenotype.

USE OF THE HXB/BXH STRAIN SET FOR STUDIES OF TERATOGEN GENETICS

Multigenic control of the physiological effect of teratogens and a combined interaction with malformation mutant genes in mice were reported as early as the 1960s (23, 24). Bila and Kren (8, 9) developed a test for teratogenicity by using PLS congenic strains on varying genetic backgrounds. This test exploited the interactions of the teratogen with the Lx mutation, as well as the influences of the genetic background on gene-teratogen interaction. When pregnant females carrying heterozygous +/Lx fetuses were administered a teratogen, such as bromodeoxyuridine or cyclophosphamide, on fetal days 11–13, the critical period for limb development, newborns exhibited paw affliction with polydactyly up to oligodactyly and variably afflicted zeugopod. The same dose of teratogen given to females with +/+ fetuses was largely without effect, i.e., these females develop normodactylous progeny. Thus the presence of the Lx mutant allele sensitized the carrier to the malformation effects of the administered teratogenic substance. However, because the effect of the mutant allele and the entire genome interacted in phenotypic expression, background alleles either suppressed or enhanced the teratogen influence. In the case of the SHR genome, the effect is suppression, whereas with the BN genetic background there is enhanced effects; thus the results argued for the presence of both positive and negative modifying loci. Further application of the test yielded positive results with drugs with known teratogenic, mutagenic, or immunosuppressive potential (8–11, 15).

The teratogenicity test was also used for the characterization of a class of antiviral acyclic nucleotide analogs, specifically 9-(2-phosphonomethoxyethyl)adename (PMEA), which interacted with the mutant allele to produce preaxial polydactyly of the hind feet, whereas the 1-(3-hydroxy-2-phosphonomethoxethyl)cytosine (HPMPC) exhibited embryolethal effects (15). The teratogenic effect of thalidomide (12) was established after the administration of the drug to females carrying +/Lx heterozygous fetuses, whereas no malformations were ascertained in fetuses homozygous.
gous in the standard allele. In heterozygous fetuses, the Lx allele penetrance was increased up to 87% (10, 12). The genotype dependence of thalidomide teratogenicity indicated the usefulness of a genetically defined system of RI and congenic strains (8, 12).

The HXB/BXH strains have also been used to explore the teratogenic effects of retinoic acid (RA) (13, 14). Vitamin A is known to have a key role in embryonal development, and its metabolite, RA, has been implicated in anterior-posterior patterning in vertebrate embryos (62). RA, acting directly or indirectly, affects a complex network of receptors, transcription factors, and cytokines, and this has been reinforced by recent gene expression studies (5). From 532 genes evaluated, 27 genes were identified as being unquestionably direct targets of RA, whereas hundreds of genes were influenced indirectly. To examine alleles involved in the action of RA, studies were conducted in PLS carrying HXB/BXH RI strains and congenics by administration of the RA to pregnant females (14). It was found that RA administration resulted in a significantly decreased body weight of all fetuses irrespective of their genotype. The embryo-lethal effect of RA administration was most pronounced in fetuses with the homozygous BXH2 genome (Fig. 6), where more than 90% of fetuses died (14). Further studies have established that the SHR genome not only ameliorated the Lx gene phenotypic manifestation but also buffered the teratogenic effect of RA. In this regard, the SHR.Lx congenic strain and BXH2 RI strain appear to be the two extremes in the sensitivity to RA teratogenic action, which parallels their sensitivity to PLS expression. These findings would argue that both the Lx mutation and varying genetic background within the HXB/BXH and PXO RI strains are important determinants of the complex interplay of genetic factors in RA teratogenesis.

**SUMMARY AND CONCLUSION**

The rat has been the primary model organism for comparative physiological and pharmacological studies, and a wealth of information has accumulated over the years in the literature. Only a few years ago, a genetic map for the rat to facilitate genomic studies was not available (67); however, all that has changed. Although the rat has been the organism of choice for physiologists and pharmacologists and the mouse has been the organism of choice for geneticists (67), integration of the sciences in this era of genomics has blurred the distinction between these species. Popularity of murine strains among physiologists and pharmacologists over the past 5 yr is largely attributable to the advent of gene targeting afforded by the unique L129 strain. Without question, for studies seeking to model or study a single gene product on a single genetic background, genetically engineered mouse models are today the logical choice; nevertheless, this may need to be reexamined in the near future because of advances in nuclear transfer. However, if the goal is to define and thoroughly understand human and comparative genomics, it can be argued that the rat remains a more logical model organism for study. This position is based not only on the vast literature but also on the availability of a large number of well-defined inbred strains, unique genetically informative model constructs, including a rapidly growing number of congenic substra and the subject of this review, the HXB/BXH RI strain set. In addition, the rat genetic map rivals the mouse and the recently announced rat genomic sequence makes the rat an ideal comparative model for human disorders.

The HXB/BXH RI strain set along with congenics and the new PXO RI set has been shown to have utility in five major research areas: cardiovascular, metabolic, behavioral, developmental, and toxicological. Additionally, the strains can be starting points for new constructions designed to address specific questions and gene targets. Lastly, with today’s technology and the accumulated knowledge of inbred rat strains facilitating selection of future progenitors, the construction of new rat RI strain sets can be accomplished expeditiously. As pointed out by Blizard (16) through genetic correlation analysis, the use of RI strains provides an alternative method to physiological or pharmacological methods to explore relationships between different domains in the behavioral sciences. Recombinant inbred methodology should enhance studies of the relationships between complex processes, and this application should be considered separate from its use in gene mapping (16).

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