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Congenic strains developed for alcohol- and drug-related phenotypes

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Abstract

Quantitative trait loci (QTLs) for many alcohol- and drug-related traits have been mapped using well-accepted mapping techniques. The ultimate goal of gene identification necessitates confirmation of the QTL and reduction of the interval surrounding the QTL; both can be accomplished in congenic strains. These strains carry a chromosomal region introgressed from a donor strain onto the genetic background of a second, recipient strain. Multiple generations of backcrossing reduce the unlinked donor genome to less than 0.1%. Then, phenotypic comparisons between mice congenic for the donor region and controls from the recipient strain allow confirmation of the QTL effect. Animals with recombinations in the donor region can be used to generate interval-specific congenic recombinant lines. Numerous congenic strains are currently being developed in which chromosomal regions carrying QTLs for alcohol- and drug-related traits have been transferred from one strain onto a second strain. The purpose of this review is to summarize the chromosomal regions, donor and recipient strains, and results obtained from these congenics. Most researchers developing such strains are willing to share these resources to facilitate localization of the genetic bases of other phenotypes.

Keywords: Congenic strain; Interval-specific congenic strain; Ethanol; Alcohol preference; Alcohol withdrawal; Alcohol sensitivity; Pentobarbitol withdrawal; Morphine preference; Morphine analgesia; QTL mapping

1. Introduction

Quantitative trait loci (QTLs) have been identified for an enormous number of phenotypes, many of them behavioral, with a large subset relating to alcohol and other drugs of abuse [4,9]. The initial mapping of a QTL is the first step in a long process whose desired outcome is identification of the gene(s) underlying the phenotype. Mapping algorithms typically localize a QTL to a fairly large chromosomal region (e.g., 20 cM). Subsequent stages involve confirmation of the effect and location of the QTL in independent populations, reduction of the size of the region in which the QTL is located, eventual cloning of the DNA sequence, and ideally, production of transgenics carrying the gene(s) on different backgrounds.

Congenic strains are increasingly being used in confirmation and fine mapping of QTLs. These strains are generated by transferring, or introgressing, a portion of the genome of one strain (the donor) to another strain, the recipient, or inbred partner (for review, see Ref. [12]); this transfer is accomplished through a series of backcrosses, thus, the process of breeding a congenic strain can take several years. Effect size and location of QTLs can, and should, be confirmed during the backcrossing (for discussion of why this is important, see Ref. [2]), and each QTL can be characterized in isolation from the others.

The process of congenic strain construction and the application of this strategy to QTLs underlying the action of alcohol and other drugs, are reviewed here. Specific details, such as the exact chromosomal location of the region transferred, and the identity of the donor and recipient strains, are provided with the hope that these strains, each of which represents years of work, will be used by other researchers. Candidate gene hypotheses, for traits other than the originally mapped phenotype, can be tested by obtaining these mice and comparing phenotypes of congenic animals with those of donor and/or recipient animals. For example, in our lab, we have generated congenic strains, each of which carries a QTL region influencing loss of righting response (LORR) to a sedative dose of ethanol. One of these introgressed regions is on distal chromosome 1, an area where many drug- and

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alcohol-related QTLs map. Mice congenic for this QTL show significantly less locomotor activation following a low dose of ethanol than the inbred, recipient strain (J. Owens, personal communication). Further, none of the other congenics for LORR QTLs show this differential activation, suggesting that the chromosome 1 QTL is involved in both LORR and low dose activation, or a linked gene(s) influences activation.

2. Congenic construction

The earliest congenic strains were made on the basis of phenotypic selection for single locus traits: each mating was preceded by selection for mice carrying the phenotype of the donor strain; these animals were then mated to mice of the inbred recipient strain [27]. Introgression of QTL regions is done by backcrossing mice heterozygous for the chromosomal segment where the QTL has mapped, defined by two or more microsatellite markers. It is possible to reduce the number of backcross generations by using genetic markers in the selection process to select for recipient genome in unlinked regions [11,15,31]. These so-called speed congenics have been widely used to introgress the genomic region containing a QTL onto another background. An alternative approach, when all major QTLs are known, is to select against the donor genome in the nonintrogressed QTL regions, while simultaneously selecting for the donor markers in the desired QTL region [2]. Marker-assisted selection has also been used to construct chromosome substitution strains, in which an entire chromosome from the donor strain is introduced into the recipient background [19].

With each generation of backcrossing, approximately one half of the donor genome is replaced by that of the recipient, except in the region linked to the chromosomal segment being introgressed, called the differential locus. Donor strain genomic material linked to the differential locus will be eliminated much more slowly. After 10 backcross generations, the recipient strain contributes 99.9% of the unlinked genome [11]. At this point, heterozygotes for the donor region are intercrossed to generate homozygotes at the differential locus. These homozygous animals are termed congenics (Fig. 1).

The congenic strain is named using the format: Recipient.Donor-Introgressed region$^{\text{differential allele}}$. For example, we [2] developed four sets of reciprocal congenics (i.e., each member of the pair of inbred strains is used as both donor and recipient) for QTLs termed *Lore1* (chromosome 1), *Lore2* (chromosome 2), *Lore4* (chromosome 11), and *Lore5* (chromosome 5), for *l*oss of *r*ighting due to *e*thanol. The congenic strain, in which $Lore1^L$, from ILS was moved onto an ISS background, is designated ISS.ILS-$Lore1^L$.

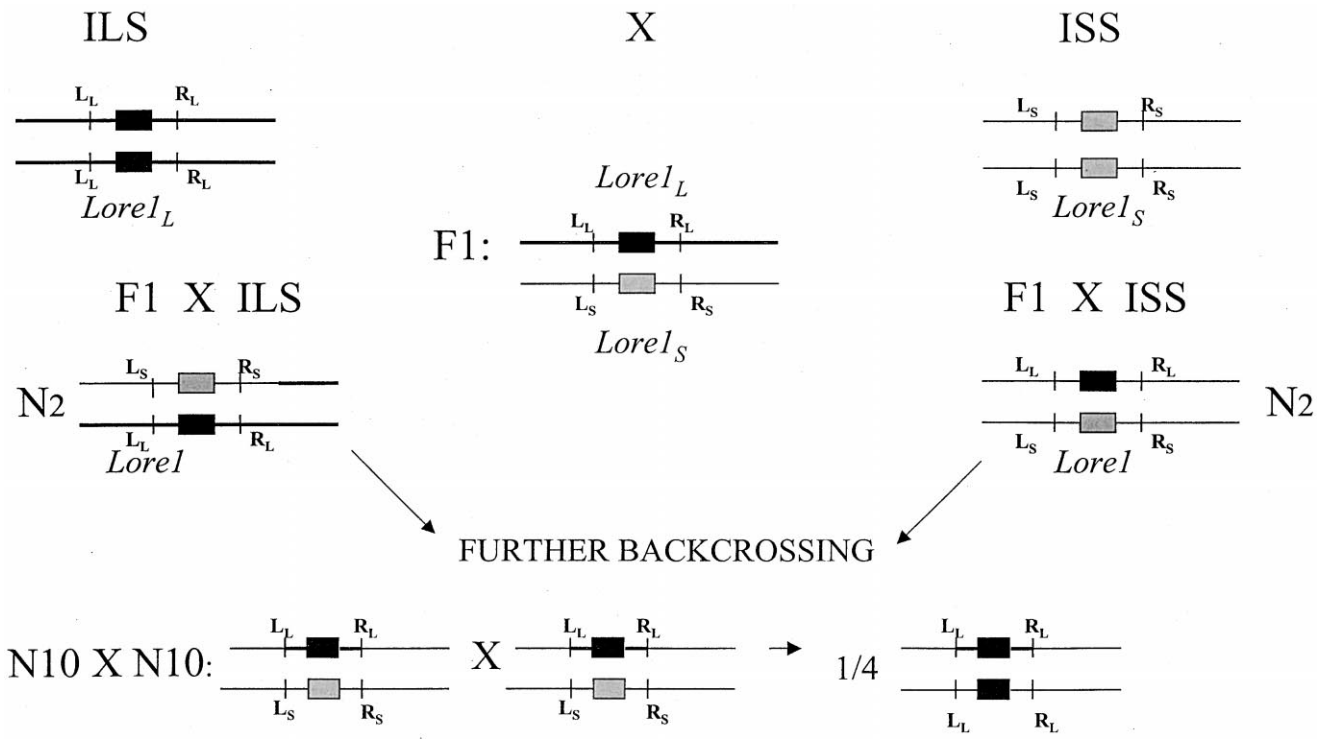

Fig. 1. Marker-assisted selection for congenic strain construction. An F_1 cross between two inbred strains, here shown as ILS (dark) and ISS (light), is the starting point for congenic production. The QTL to be introgressed, *Lore1*, is shown in a box. F_1 mice are backcrossed to the inbred parent that will provide the recipient genome. In subsequent generations, heterozygous, nonrecombinant parents are backcrossed to the recipient strain. Left (L) and right (R) flanking markers are subscripted in the diagram to indicate if they are $ILS_{(L)}$ or $ISS_{(S)}$ in origin. The derivation of the ISS.ILS-$Lore1^L$ congenics is shown at the bottom. After 10 generations of backcrossing, heterozygous, nonrecombinant parents are intercrossed; approximately 25% of the offspring should be homozygous for the $Lore1^L$ region.

2.1. Genetic chromosome dissection

During the backcrossing process, recombinations in the QTL interval will occur. Some of these recombinations will retain the donor QTL in a smaller donor fragment. Although these mice would be discarded for the purpose of congenic breeding, they are very useful for identifying a subset of the donor region where the QTL is located. The process of identifying a number of recombinant chromosomes that contain overlapping donor regions is termed

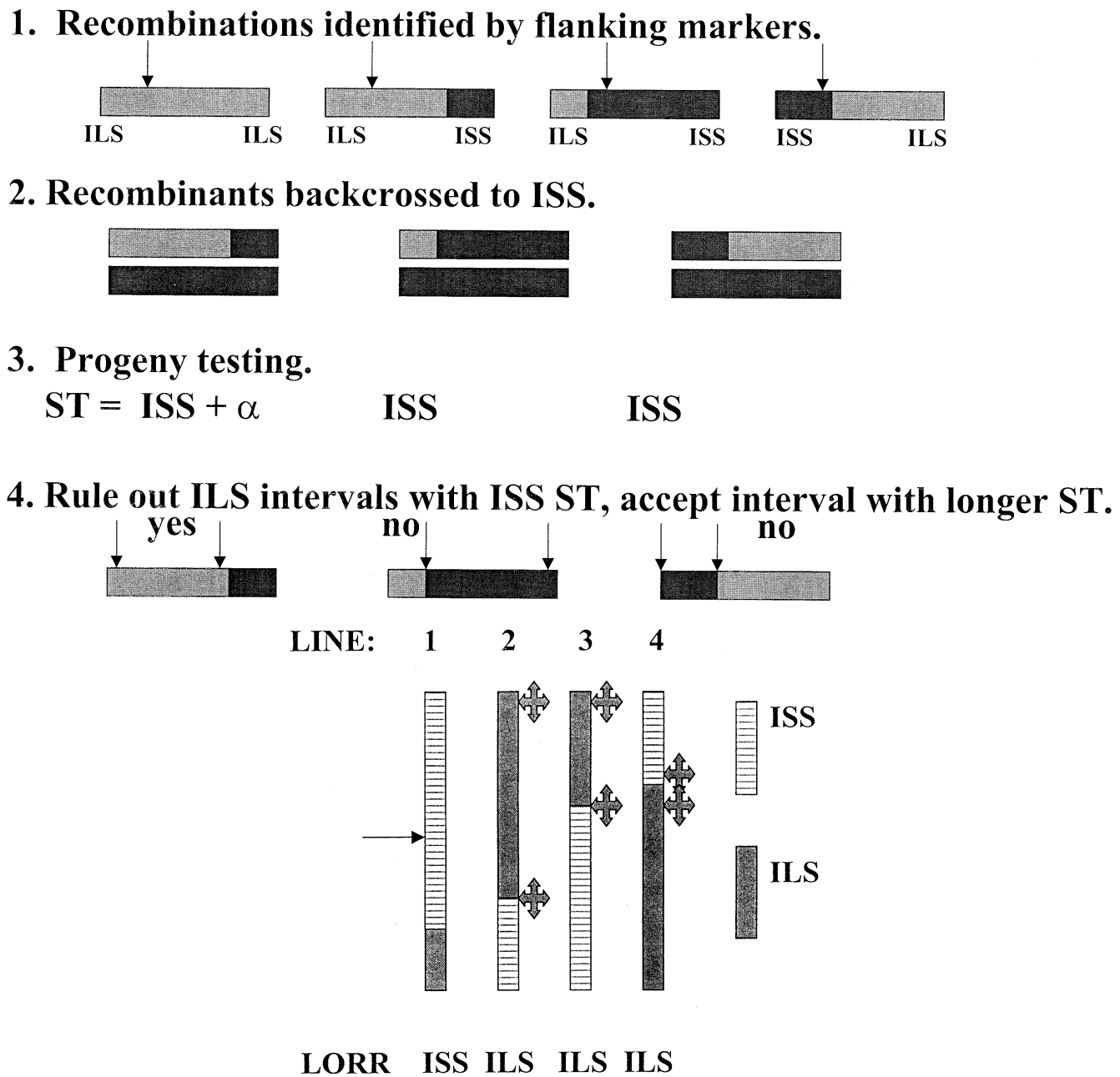

Fig. 2. Use of ISCR lines to localize a QTL (QTL position indicated by arrow). Donor (ILS) and recipient (ISS) genotypes are indicated in the diagrams. (a) Progeny testing. Mice with recombinations in the QTL-interval are identified by genotyping [1]; these animals are backcrossed to the inbred recipient strain [3], here, ISS [2]. All offspring from each cross are phenotyped [3]; mice with the recombinant chromosome are compared to homozygotes for the recipient genotype. If mice with the recombinant chromosome have a phenotype consistent with the effect from the donor strain (ILS), the QTL is localized to that smaller recombinant region [4]. (b) Pairwise comparisons. Phenotypic scores from mice carrying the recombinant chromosome are compared between lines; the observed phenotype (LORR) is indicated below the chromosome schematics. The donor region in line 1 can be excluded as mice carrying this recombination have the phenotype expected of the recipient strain (ISS). The donor region in line 2 increases the phenotype (now, ILS-like). Both lines 2 and 3 have the ILS phenotype, thus, the donor region carrying the ILS allele of the QTL can be narrowed to that of line 3. Finally, line 4 also has the ILS phenotype; by comparison with line 3, the interval can be narrowed substantially, as shown by the crosses.

genetic chromosome dissection [10]. These recombinant chromosomes can be tested as described below to localize the QTL to a smaller interval, or, to exclude the QTL from a region (Fig. 2).

Animals carrying a recombinant chromosome can be backcrossed to the recipient strain, and eventually intercrossed to generate new congenic strains. Strains developed in this fashion differ somewhat from interval-specific congenic strains [10], in that the former are discovered during the process of congenic construction and the latter are detected by screening an F_2 or backcross population for recombinations in the candidate region then backcrossed repeatedly to the recipient strain and finally intercrossed. I term the strains developed from recombinations discovered during congenic strain construction, interval-specific congenic recombinants (ISCR). The derivation of these strains takes less time than de novo congenics, as some backcrossing has already been done [12].

The phenotypic effect of the reduced donor interval can be assessed by progeny testing [10]: comparing the phenotype of mice with the recombinant chromosome to offspring from the same cross, which are homozygous for the recipient strain genotype (Fig. 2a). For QTLs with relatively large phenotypic effects, this comparison can be made, as shown in Fig. 2a, using mice heterozygous for the recombinant chromosome. As these mice are not homozygous for the donor region, hence, not true congenic strains, they are more properly termed ISCR lines. If progeny testing indicates that the QTL is located in the smaller region of donor genome, that is, mice with the recombinant chromosome have a phenotype significantly different, in the expected direction, from mice without the recombination, then the region containing the QTL has been narrowed.

Interval-specific congenic recombinant strains or lines can also be employed to test for the presence of QTLs influencing other phenotypes. For example, we are currently testing the activation phenotype in our chromosome 1 ISCR lines.

Alternatively, it is possible to compare the phenotypes of interval-specific lines (or strains) carrying different donor intervals to localize the QTL (Fig. 2b). By making pairwise comparisons between different recombinations, the location of a QTL can be progressively refined. A combination of the two strategies, progeny testing and pairwise comparisons, is useful for rapidly refining the location of a QTL. By starting with two nonoverlapping regions (Fig. 2b), one end can be eliminated, and then several lines carrying recombinant chromosomes whose donor regions overlap could be generated. Four large sets of these ISCRs are currently in development; each is discussed along with the original congenic from which they were originally derived.

3. Congenic strains currently in production

A comprehensive, though certainly not exhaustive, listing of strains derived to confirm alcohol- or drug-related QTLs, that are completely or partially congenic, is given in Table 1. Interval-specific congenic recombinant lines are summarized in Table 2. These congenic strains carry (or will carry, upon completion of backcrossing) a homozygous region from the donor strain on a recipient inbred strain background. The map positions of the introgressed region, and, in some cases, the markers defining this region, are given in Tables 1 and 2, although some groups designate their congenic strain by a candidate gene. Some background information on the congenic strains summarized in Tables 1 and 2 is given below; however, the salient information is presented in Tables 1 and 2 and much of it is not recapitulated in the text. With few exceptions [2,29,33] these data are largely unpublished, and few of the originally mapped phenotypes have been confirmed in the incipient congenic strains, because backcrossing is incomplete at this time for many of the strains. These strains should provide a valuable resource for the rodent research community, as genetic hypotheses relating to other phenotypes can easily be tested [2,24].

3.1. Congenic strains for alcohol-related traits

3.1.1. Sensitivity to ethanol-induced sedation

Sensitivity to the sedative effect of ethanol is most often assessed by duration of LORR. QTLs for this trait have been mapped in an F_2 cross between Inbred Long Sleep (ILS) and Inbred Short Sleep (ISS) mice. Four major QTLs, (*Lore1*, *2*, *4*, *5*) accounting for 60% of the genetic variance in LORR, confirmed in the ISS.ILS congenic strains; a fifth QTL, on chromosome 8, failed this confirmatory test [2]. Confirmation of the effect of the ISS allele on the ILS background is underway. Four sets of reciprocal congenics have been completed.

Four other alcohol-related phenotypes have been tested in ISS.ILS-$Lore1^L$,2^L,5^L congenic strains that were intercrossed after eight generations of backcrossing: Low-dose ethanol-induced locomotor activation was significantly lower in congenics carrying the $Lore1^L$ region than in ISS controls. Hypothermia in response to 4.1 g/kg was significantly different between every congenic and ISS at time of injection, but not at any other time point up to 180 min. Blood ethanol concentration (BEC) at regaining the righting response, following a dose of 4.1 g/kg, did not differ between any congenic strain and ISS, nor did ataxia, measured by rotorod performance after 2.0 g/kg (J. Owens, personal communication).

A small number (approximately three per chromosome) of ISCR lines are in production for the ISS.ILS-$Lore^L$ congenics. Progeny testing in a larger set of these lines has been successful in narrowing the QTL interval to that shown for the extant recombinant lines (unpublished data).

3.1.2. Alcohol preference and consumption

Their marked differences in voluntary ethanol consumption made the C57BL/6 (B6) and DBA2 (D2) inbred

strains the obvious choice for mapping this trait. QTLs for consumption have been identified on chromosomes 1 (*Ap1q*) [28], 2 (*Alcp1*), [16], 4 (*Ap3q*) [28], and 9 (*Pref1*) [22]. Phillips et al. [22] confirmed these QTLs using data from F_2, RI, and selected lines. The putative QTL on chromosome 9 is of interest because two likely gene candidates (the dopamine D2 receptor, and the 5-HT_{1B} receptor) lie on chromosome 9 [9]. Two congenic strains carrying B6 regions from chromosome 9 have been introgressed onto the D2 background, and one reciprocal strain has also been constructed.

A phenotypic selection method was used to move D2 alleles onto a B6 background using males with low drinking scores as sires [33]. A genome scan of N_7 mice identified a D2 region on chromosome 2, likely the same QTL identified by others (*Alcp1*), which was confirmed on the basis of phenotypic testing (i.e., mice with one D2 allele had lower drinking scores than mice with only B6 alleles). Introgression of *Alcp1* from D2 onto the B6 background is complete and the D2 phenotype persists (Table 1, Line 2.2). A denser mapping effort at the N_9 generation revealed D2 regions on chromosomes 1, 3, 6, and 9; however, none of these lines show the D2 phenotype. Approximately 10 recombination events have been identified from the B6.D2-*Alcp1*^{D} congenic strain. These ISCR lines are being backcrossed, although no progeny testing has been done.

The BALB/cJ strain, which also differs significantly from the B6 in ethanol preference drinking, was used as the source of donor genetic material in the construction of quasicongenic recombinant QTL introgression (RQI) strains on a C57BL/6By background [29]. These strains are similar to interval-specific congenics, but derived by phenotypic selection, in this case for extremes of a quantitative dopamine system measure. These RQI have been used to analyze the effect of various passenger regions of the introgressed genome (BALB) on ethanol preference. It is important to realize that, unlike a true congenic, these RQI carry multiple passenger regions (given in Table 2) from the donor strain. Two of these strains (α10, 11; Table 2) are similar to BALB in their preference scores, while two others (β13, 14; Table 2) surpass the B6 strain in preference drinking scores.

A different model for voluntary ethanol consumption exists in the selected preferring (P) and nonpreferring (NP) rat lines. These lines were inbred following selection. An F_2 cross between the inbred strains was the basis for QTL mapping, turning up a significant QTL on rat chromosome 4, syntenic to a region on mouse chromosome 6 [8]. A pair of reciprocal congenic strains is in development for this region.

3.1.3. Alcohol withdrawal

Differences between D2 and B6 are also observed during acute withdrawal from alcohol: D2 respond with severe handling-induced convulsions whereas B6 have a much less intense response. Three QTLs accounting for 68% of the genetic variance in this phenotype have been mapped and confirmed [5]. These three B6 regions, on chromosomes 1 (*Alcw1*), 4 (*Alcw2*), and 11 (*Alcw3*), are being introgressed onto a D2 background.

3.1.4. Alcohol-conditioned taste aversion

Risinger and Cunningham [25] mapped a QTL for taste-aversion conditioned by an alcohol injection, to distal chromosome 1. The B6 allele for this region is being introgressed onto the D2 background. Three other groups have mapped putative QTLs to this region, based on fear-conditioning paradigms [7,20,33], suggesting that gene(s) in this region may influence learning and memory, or, alternatively, aversive behavior. A significant positive correlation has been found between acute functional tolerance to ethanol and fear conditioning in lines selected for high and low tolerance to ethanol [23]. This finding emphasizes the possibility of overlap between genes influencing ethanol-related behavior and fear conditioning. The D2 QTL region identified by Wehner [23] is being introgressed onto a B6 background. Backcrossing is almost complete in 13 ISCR lines, which break up the large D2 interval into smaller, overlapping pieces. Progeny testing of these lines has just begun.

3.2. Congenic strains based on phenotypes related to other drugs

3.2.1. Morphine preference and analgesia

A major QTL (*Mop2*) for morphine preference drinking has been mapped to chromosome 10, using B6 and D2 crosses [3]. This QTL is important as it maps to a region with a very suggestive candidate gene: *Oprm*, which encodes the μ-opioid receptor. A QTL for morphine analgesia (*Moan2*) also maps to this region [9]; a second one to chromosome 9 (*Moan1*). Congenics for this region are currently in development; additionally, congenics for a smaller region, which more tightly brackets *Oprm* are being developed. Morphine preference did confirm at N_4, but no further phenotypic data are available for this strain (Ferraro, personal communication).

3.2.2. Methamphetamine

The BXD RI series was used to map QTLs for activity and thermal responses to different doses of methamphetamine [13]. QTLs for activity mapped to chromosomes 6, 7, 9, and several suggestive ones to 19; for thermal effects to 1, 9, 10, and 19. The genetic correlation between these two phenotypes was high, indicating some of the same genes influence both. Congenic strains for several subsets of the chromosome 9 region are nearly complete; most of these strains carry the B6 region on a D2 background, as does the congenic strain carrying the chromosome 19 interval.

3.2.3. Pentobarbitol withdrawal

Pentobarbitol is similar to ethanol in its pharmacology [17]. Not surprisingly, a QTL for acute pentobarbitol withdrawal (*Pbw1*), mapped to the same region of chromosome 1 where alcohol withdrawal mapped [6]. Suggestive QTLs

Table 1
Congenic strain summary

PI or group[a] developing	Original phenotypic selection	Strain designation[b]	Chromosome: introgressed region (cM)	Recipient strain	Donor strain	Current state of development	Confirmed[c]	Other PT tested[d]	References
Berrettini	morphine preference	D2.B6-*Mop2*^{B}	**10**:2–40	D2	B6	completed	at N4		[23]
	morphine preference	D2.B6-*Mop2*^{B}	**10**:2–7	D2	B6	completed	at N4		[23]
	morphine preference	D2.B6-*Mop2*^{B}	**10**:2–40	B6	D2	N6	at N4		[23]
	morphine preference	D2.B6-*Mop2*^{B}	**10**:2–7	B6	D2	N6	at N4		[23]
Carr	alcohol preference	P.NP-D4Mgh16,D4Rat55^{NP}	**4**:57–90	P	NP	N5			[22]
	alcohol preference	NP.P-D4Mgh16,D4Rat55^{P}	**4**:57–90	NP	P	N5			[22]
Hitzemann	haloperidol-induced catalepsy	D2.B6-D9Mit90,74^{B}	**9**:10–40	D2	B6	completed	yes	activity,	[6,14]
	haloperidol-induced catalepsy	B6-D2-D9Mit90,74^{B}	**9**:10–40	B6	D2	completed	yes	preference	[6,14]
Johnson	alcohol sensitivity	ISS.ILS-*Lore1*^{L}	**1**:37–59	ISS	ILS	completed	N4–N10	BEC, ataxia,	[15]
	alcohol sensitivity	ISS.ILS-*Lore2*^{L}	**2**:80–92	ISS	ILS	completed	N4–N10	hypothermia,	[15]
	alcohol sensitivity	ISS.ILS-*Lore4*^{L}	**11**:37–56	ISS	ILS	completed	N4–N10	ethanol-	[15]
	alcohol sensitivity	ISS.ILS-*Lore5*^{L}	**15**:38–55	ISS	ILS	completed	N4–N10	activation	[15]
	alcohol sensitivity	ILS.ISS-*Lore1*^{S}	**1**:37–59	ILS	ISS	completed			[15]
	alcohol sensitivity	ILS.ISS-*Lore2*^{S}	**2**:80–92	ILS	ISS	completed			[15]
	alcohol sensitivity	ILS.ISS-*Lore4*^{S}	**11**:37–56	ILS	ISS	completed			[15]
	alcohol sensitivity	ILS.ISS-*Lore5*^{S}	**15**:38–55	ILS	ISS	completed			[15]
PARC	alcohol withdrawal	D2.B6-D1Mit200,150^{B}	**1**:73–102	D2	B6	N7			[8]
	pentobarbital withdrawal	D2.B6-D1Mit200,150^{B}	**1**:73–102	D2	B6	N7			[26]
	taste aversion	D2.B6-D1Mit200,150^{B}	**1**:73–102	D2	B6	N7			[9]
	alcohol withdrawal	D2.B6$^{-Tyrp1\ or\ b}$	**4**:28–40	D2	B6	completed			[16]
	pentobarbital withdrawal	D2.B6$^{-Tyrp1\ or\ b}$	**4**:28–40	D2	B6	completed			[3]
	morphine analgesia	D2.B6$^{-Myo5a\ or\ d}$	**9**:9–55	D2	B6	completed			[9]
	alcohol preference	D2.B6$^{-Myo5a\ or\ d}$	**9**:9–55	D2	B6	completed			[16]
	methamphetamine activity	D2.B6$^{-Myo5a\ or\ d}$	**9**:9–55	D2	B6	completed			[3]
	cocaine seizures	D2.B6$^{-Myo5a\ or\ d}$	**9**:9–55	D2	B6	completed			[9]
	morphine analgesia	D2.B6-D9Mit90,18^{B}	**9**:9–71	D2	B6	completed			[9]
	alcohol preference	D2.B6-D9Mit90,18^{B}	**9**:9–71	D2	B6	completed			[16]
	methamphetamine activity	D2.B6-D9Mit90,18^{B}	**9**:9–71	D2	B6	completed			[3]
	cocaine seizures	D2.B6-D9Mit90,18^{B}	**9**:9–71	D2	B6	completed			[9]

	morphine analgesia	B6.D2-D9Mit90,274^{D}	**9**:9–50	B6	D2	N8			[9]
	alcohol preference	B6.D2-D9Mit90,274^{D}	**9**:9–50	B6	D2	N8			[16]
	methamphetamine activity	B6.D2-D9Mit90,274^{D}	**9**:9–50	B6	D2	N8			[3]
	cocaine seizures	B6.D2-D9Mit90,274^{D}	**9**:9–50	B6	D2	N8			[9]
	morphine analgesia	D2.B6-D10Mit28,3^{B}	**10**:4–21	D2	B6	completed			[9]
	morphine activity	D2.B6-D10Mit28,3^{B}	**10**:4–21	D2	B6	completed			Belknap (personal communication)
	morphine preference	D2.B6-D10Mit28,3^{B}	**10**:4–21	D2	B6	completed			[9]
	methamphetamine thermal responses	D2.B6-D10Mit28,3^{B}	**10**:4–21	D2	B6	completed			[3]
	alcohol withdrawal	D2.B6-D11Mit78,179^{B}	**11**:2–52	D2	B6	N6			[8]
	pentobarbital withdrawal	D2.B6-D11Mit78,179^{B}	**11**:2–52	D2	B6	N6			[26]
	methamphetamine activity	D2.B6-D19Mit109,89^{B}	**19**:4–41	D2	B6	N3			[3]
	methamphetamine thermal responses	D2.B6-D19Mit109,89^{B}	**19**:4–41	D2	B6	N3			[3]
	dopamine transporter B_{max}	D2.B6-D19Mit109,89^{B}	**19**:4–41	D2	B6	N3			Belknap (personal communication)
Rikke	propofol sensitivity	ISS.RI6-D7Mit347, Tyr^{LS}	**7**:38–47	ISS	ILS	completed	no		[17]
	propofol sensitivity	ISS.RI32-D7Mit184,262^{LS}	**7**:46–50	ISS	ILS	N5			[17]
	propofol sensitivity	ILS.ISS-D7Mit347,184^{S}	**7**:38–47	ILS	ISS	N4			[17]
	propofol sensitivity	ILS.ISS-D7Mit184,262^{S}	**7**:43–50	ILS	ISS	N4			[17]
Wehner	fear conditioning (Line 1)	B6.D2-D1Mit490,113^{D}	**1**:59.5–92.3	B6	D2	N7	N4	LORR	[20]
Whatley	alcohol preference (Line 2.2)	B6.D2-Alcp1^{D}	**2**:18–53	B6	D2	completed	yes		[10]
	alcohol preference (Line 3.42)	B6.D2-D9Mit129,166^{D}	**9**:26–41	B6	D2	completed	no		[10]

[a] PI given except for Portland Alcohol Research Center (PARC), where numerous PIs collaborated in congenic development.

[b] Recipient.Donor-Introgressed region^{Allele} (Alleles: B = B6; D = D2; L = ILS; S = ISS; LS = Long Sleep from LSXSS RI).

[c] Confirmation of original phenotype (either partway through strain construction, or after intercrossing following N_{10}). Yes indicates that the phenotype of the donor strain is retained in the congenic strain; No, the donor strain phenotype is not seen in the congenic; a blank means that confirmatory testing has not been done. Interim phenotyping at generation$_x$ before intercrossing is indicated by N_x.

[d] BEC: Blood ethanol concentration at awakening. LORR: Loss of righting response.

Table 2
Interval-specific congenic recombinant lines in development

PI or group	Original phenotypic selection basis	Strain designation	Chromosome: introgressed	Background strain	Donor strain	Current state of development	Confirmed?
Johnson	alcohol sensitivity (Line 10)[a]	ISS.ILS-D1Mit161,215	**1**:26–47	ISS	ILS	N10	yes
	alcohol sensitivity (Line 6)[a]	ISS.ILS-D1Mit161,46	**1**:26–44	ISS	ILS	N10	yes
	alcohol sensitivity (Line 10.9)[a]	ISS.ILS-D1Mit180,215	**1**:42–47	ISS	ILS	N10	yes
	alcohol sensitivity (Line 1)[a]	ISS.ILS-D2Nds3,Mit280	**2**:73–81.7	ISS	ILS	N10	yes
	alcohol sensitivity (Line 3.9)[a]	ISS.ILS-D2Mit107,194	**2**:75.6–81.4	ISS	ILS	N10	yes
	alcohol sensitivity (Line 12)[a]	ISS.ILS-D2Mit21,280	**2**:80–81.7	ISS	ILS	N10	yes
	alcohol sensitivity (Line 10)[a]	ISS.ILS-D11Mit30,54	**11**:40–56	ISS	ILS	N10	yes
	alcohol sensitivity (Line 4)[a]	ISS.ILS-D11Mit5,36	**11**:37–49	ISS	ILS	N10	yes
	alcohol sensitivity (Line 5)[a]	ISS.ILS-D11Mit5,90	**11**:37–42	ISS	ILS	N10	yes
	alcohol sensitivity (Line 9)[a]	ISS.ILS-D11Mit5	**11**:37	ISS	ILS	N10	yes
	alcohol sensitivity (Line 5)[a]	ISS.ILS-D15Mit185,171	**15**:41–55	ISS	ILS	N10	yes
Wehner	fear conditioning (Line 1-AA)[a]	B6.D2-D1Mit490,420^{D}	**1**:59.5–63.8	B6	D2	N9	
	fear conditioning (Line 1-BB)[a]	B6.D2-D1Mit490,192^{D}	**1**:59.5–64	B6	D2	N9	
	fear conditioning (Line 1-CC)[a]	B6.D2-D1Mit490,312^{D}	**1**:59.5–70	B6	D2	N9	
	fear conditioning (Line 1-GG)[a]	B6.D2-D1Mit490,500^{D}	**1**:59.5–79	B6	D2	N9	
	fear conditioning (Line 1-HH)[a]	B6.D2-D1Mit490,33^{D}	**1**:59.5–81.6	B6	D2	N9	
	fear conditioning (Line 1-II)[a]	B6.D2-D1Mit490,314^{D}	**1**:59.5–86.6	B6	D2	N9	
	fear conditioning (Line 1-KK)[a]	B6.D2-D1Mit490,145^{D}	**1**:59.5–89	B6	D2	N9	
	fear conditioning (Line 1-A)[a]	B6.D2-D1Mit192,113^{D}	**1**:64–92.3	B6	D2	N9	
	fear conditioning (Line 1-B)[a]	B6.D2-D1Mit218,113^{D}	**1**:67–92.3	B6	D2	N9	
	fear conditioning (Line 1-C)[a]	B6.D2-D1Mit312,113^{D}	**1**:70–92.3	B6	D2	N9	
	fear conditioning (Line 1-F)[a]	B6.D2-D1Mit42,113^{D}	**1**:78–92.3	B6	D2	N9	
	fear conditioning (Line 1-G)[a]	B6.D2-D1Mit105,113^{D}	**1**:80–92.3	B6	D2	N9	
	fear conditioning (Line 1-I)[a]	B6.D2-D1Mit110,113^{D}	**1**:87.9–92.3	B6	D2	N9	
	fear conditioning (Line 2-A)[a]	B6.D2-D2Mit495,266^{D}	**2**:73.2–109	B6	D2	N5	
	fear conditioning (Line 2-AA)[a]	B6.D2-D2Mit79,52^{D}	**2**:10–99	B6	D2	N5	
	fear conditioning (Line 3-AA)[a]	B6.D2-D3Mit227,159^{D}	**3**:22–61.8	B6	D2	N5	
	fear conditioning (Line 3-HH)[a]	B6.D2-D3Mit149,342^{D}	**3**:2.4–49.7	B6	D2	N5	
	fear conditioning (Line 10-CC)[a]	B6.D2-D10Mit75,158^{D}	**10**:2–40.7	B6	D2	N5	
	fear conditioning (Line 16-A)[a]	B6.D2-D16Mit160,D16128^{D}	**16**:21.5–66.3	B6	D2	N5	
	fear conditioning (Line 16-B)[a]	B6.D2-D16Mit160,151^{D}	**16**:4.6–45.5	B6	D2	N5	
Whatley	alcohol preference (Line 1)[a]	B6.D2-D2Mit83,351^{D}	**2**:22–50	B6	D2	N12	
	alcohol preference (Line 4)[a]	B6.D2-D2Mit61,351^{D}	**2**:34–50	B6	D2	N12	
	alcohol preference (Line 6)[a]	B6.D2-D2Mit7,56^{D}	**2**:28–38	B6	D2	N12	

	alcohol preference (Line 8)[a]	B6.D2-D2Mit83,9^{D}	**2**:28–37	B6	D2	N12	
	alcohol preference (Line 12)[a]	B6.D2-D2Mit7,298^{D}	**2**:28–31	B6	D2	N12	
	alcohol preference (Line 14)[a]	B6.D2-D2Mit9^{D}	**2**:37	B6	D2	N12	
	alcohol preference (Line 16)[a]	B6.D2-D2Mit433,9^{D}	**2**:31–37	B6	D2	N12	
	alcohol preference (Line 19)[a]	B6.D2-D2Mit61,9^{D}	**2**:34–37	B6	D2	N12	
	alcohol preference (Line 17)[a]	B6.D2-D2Mit433,61^{D}	**2**:31–34	B6	D2	N12	
	alcohol preference (Line 26)[a]	B6.D2-D2Mit37^{D}	**2**:45	B6	D2	N12	
Vadasz	mesencephalic TH activity[b]	B6.Cb4i5-a10/Vad	**2**:21.9–27.3[c]	BALB	B6	inbred RQI (30+bxs matings)	yes
			2:71	BALB	B6		yes
			6:0.5	BALB	B6		yes
			6:33.5–37	BALB	B6		yes
			7:53.5–65.2	BALB	B6		yes
	mesencephalic TH activity[b]	B6.Cb4i5-a11/Vad	**2**:27.3[c]	BALB	B6	inbred RQI (30+bxs matings)	yes
			5:59	BALB	B6		yes
			9:29–48	BALB	B6		yes
			13:16–21	BALB	B6		yes
			13:62–71	BALB	B6		yes
			17:45.3	BALB	B6		yes
	mesencephalic TH activity[b]	B6.Cb4i5-b13C/Vad	**1**:59.7–63.1[c]	BALB	B6	inbred RQI J16 (30+bxs matings)	yes
			7:59.7–63.1	BALB	B6		yes
			15:51–55.6[c]	BALB	B6		yes
			17:55.7	BALB	B6		yes
			18:57	BALB	B6		yes
			19:24	BALB	B6		yes
			19:26	BALB	B6		yes
			19:51	BALB	B6		yes
			19:53	BALB	B6		yes
	mesencephalic TH activity[b]	B6.Cb4i5-b14/Vad	**1**:59.7–63.1[c]	BALB	B6	inbred RQI (30+bxs matings)	yes
			7:49.9–72.4	BALB	B6		yes
			8:31–37	BALB	B6		yes
			10:62	BALB	B6		yes
			12:58	BALB	B6		yes
			15:51–55.6[c]	BALB	B6		yes

[a] Interval specific congenic recombinant strain, for smaller portion of originally introgressed region.
[b] Recombinant QTL introgression strains. Note that these carry multiple passenger regions.
[c] Candidate region for the alcohol preference phenotype.

for pentobarbitol withdrawal were also found on chromosomes 4 and 11, mirroring regions implicated in alcohol withdrawal. Three congenic strains, in which the B6 alleles from chromosomes 1, 4, and 11 are being introgressed onto a D2 background, are in production.

3.2.4. Cocaine seizures

QTLs for cocaine-induced seizures have been mapped to chromosomes 9 (*Cosz1*), 14 (*Cosz2*), and 15 (*Cosz3*) [9]. Reciprocal congenic strains for the *Cosz1* region have been produced, but the phenotype has yet to be confirmed in these strains [9].

3.2.5. Propofol sensitivity

Long Sleep and Short Sleep and ILS and ISS mice are differentially sensitive to the general anesthetic propofol [26]. A major QTL, *Lorp1*, accounting for 80% of the genetic variance for Propofol-induced LORR, was mapped to chromosome 7 in the LSXSS recombinant inbred (RI) panel and confirmed in ILSXISS F_2 [26]. This is an interesting QTL, in terms of possible cloning, as the LOD score from mapping is so large [26] and the 2-LOD support interval (2.5 cM) is very tight. Three congenic strains carrying this QTL are in production; one is complete. Two of these strains take advantage of the increase in recombination characteristic of RI panels by introgressing regions known to be derived from LS and containing $Lorp1^L$, from the LSXSS RI, onto an ISS background. A second pair carries the same region, from ILS, being moved onto the ISS background.

The completed congenic for *Lorp1*, which carries a 9-cM region containing the tyrosinase locus from LSXSS RI32, is interesting because the LS phenotype was lost at some point in the backcrossing. Mice carrying the introgressed LS region retained differential LORR after propofol injection at N_6; when tested at N_{10}, there was no phenotypic difference between congenic mice and ISS controls (Rikke, personal communication). This finding emphasizes the importance of testing for the phenotype through the backcrossing process. It is possible that small effect modifier loci with a significant impact on the phenotype, but not identified in a mapping study, may be lost during backcrossing.

3.2.6. Haloperidol-induced catalepsy

Haloperidol, a dopamine D2 agonist, used in the treatment of schizophrenia, induces a reversible Parkinsonism in human patients. A similar phenotype can be seen in mice; two QTLs, on chromosomes 4 and 9 for this trait have been mapped and confirmed [14,21]. The QTL on chromosome 9 is of particular interest because it is linked to *Drd2*, the dopamine receptor gene. In DXB F_2 mice, the D2 allele was associated with higher receptor binding in most brain areas [14], supporting a dopaminergic mechanism for the catalepsy. A pair of reciprocal congenic strains for this chromosome 9 region has been completed. The D2 receptor binding phenotype has confirmed and decreased ethanol preference is seen in B6.D2 congenics.

4. Discussion

The utility of congenic strains, in confirming an alcohol- or drug-related phenotype putatively associated with a QTL-region, has been demonstrated in several published studies [2,33]. Clearly, other strategies, such as the use of independent segregating populations to map the same trait, provide a quicker and cheaper way to confirm, but most researchers develop congenics as a stepping stone to eventual cloning of the genes underlying the QTL. The success of the congenic approach, in conjunction with gene expression data, has been demonstrated in the identification of the gene causing defective fatty acid and glucose metabolism in rats [1]. A discussion of suitable methodology for utilizing congenic strains for these goals is beyond the scope of this review, but Darvasi [10] has developed theoretical approaches, and others have published empirical considerations [2,15,31].

The large set of congenic strains currently in production (Tables 1 and 2) indicates that the number of confirmation studies will soon increase dramatically. Obviously, confirmation of the phenotype for which the strain was developed will take place first; eventually, these strains will be used to test candidate regions and genes for other traits. Initially, these other phenotypes will most likely be drug- and alcohol-related phenotypes. As knowledge of, and interest in, the congenic methodology spreads, a variety of other phenotypes can be tested in these strains, which will, for the most part, be made available to the research community, for linkage to the introgressed regions.

Development or testing of congenic strains in which the same chromosomal region has been introgressed onto different backgrounds will allow assessment of strain background effects upon individual regions. This type of testing will be especially interesting when applied in ISCR strains. These strains, which are homozygous for the reduced interval, are not available for any of the drug- or alcohol-related phenotypes reported in this review, except for the morphine preference strain, D2.B6-$Mop2^B$. Preliminary results from the ISS.ILS-$Lore^L$ and fear conditioning ISCR lines are positive in terms of narrowing the interval containing the QTL.

Alcohol and drug researchers could also utilize some of the many congenic strains that have been developed to study other phenotypes. For instance, the Jackson Lab website (jaxmice.jax.org/html) lists 88 H2 (major histocompatibility locus) and 48 H (minor histocompatibility locus) congenic strains, developed for immunogenetic studies. Approximately half of these congenics are on a B6 background, but C3H, B10, BALB, A, AKR, and NOD are also utilized. A variety of other congenic strains, many for spontaneous or induced mutations, are also maintained by the Jackson Lab. Clicking on the strain name and gene symbol gives the chromosomal location of the introgressed gene. These strains have been constructed to carry a known allele; thus, the extent of the introgressed segment is unknown in most cases. Numerous other congenic strains have been developed on the basis of QTLs for phenotypes such as systemic lupus

erythematosus [18], obesity [34], insulin-dependent diabetes [35], and dopamine system activity in midbrain [30].

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