Genet. Res., Camb. (1972), **20**, *pp.* 263–268
With 2 text-figures
Printed in Great Britain

A subvital gene in *Rana pipiens* linked to the Burnsi locus*

By LEON W. BROWDER
University of Calgary

(*Received* 5 *June* 1972)

SUMMARY

A cross of a leopard frog heterozygous for the dominant Burnsi gene ($B/+$) with a wild-type ($+/+$) resulted in a large excess of $B/+$ progeny. Two descendent lines established by these $B/+$ progeny are characterized by excess $+/+$ progeny. These seemingly contradictory data are reconciled by postulating the existence of a dominant subvital gene (*Sbv*) linked to the $+$ allele of Burnsi in the initial cross. Progeny used to establish subsequent lines were recombinants with *Sbv* linked to *B*. This proposed linkage would constitute the first case of linkage between two mutant loci in anuran amphibians.

1. INTRODUCTION

The Burnsi phenotype in *Rana pipiens* is due to a simple dominant gene (Moore, 1942) and is characterized by a reduction in the number of black spots in the dorsal and lateral skin. Data from laboratory-reared crosses indicate that there is no demonstrable difference in viability between either the Burnsi heterozygote ($B/+$) or homozygote (B/B) as compared with wild-type, even under crowded conditions (Nace, Richards & Asher, 1970; Merrell, 1972).

In 1965, I crossed a Burnsi heterozygote with wild-type. One of the Burnsi heterozygotes resulting from this cross (designated no. 1) was crossed with a wild-type. The latter cross (no. 2) resulted in a large excess of $B/+$ progeny. These data have previously been published (Browder, 1968, Table 2). The data from cross 2 are also summarized in Table 1 of this report. No explanation for the aberrant $B/+ : +/+$ ratio was possible on the basis of the limited genetic data. However, subsequent crosses involving progeny of cross 2 have provided data that suggest a possible explanation. These data and their interpretation are presented below.

2. MATERIALS AND METHODS

Procedures used for induction of ovulation, artificial fertilization of eggs and rearing of progeny of crosses 1 and 2 were previously reported (Browder, 1968). Since that time, more efficient procedures have been developed. Details of these

* Supported by National Research Council Grant A6209; by United States National Institutes of Health Grants HD-02282 and HD-172; by funds from Institutional Grants from the American Cancer Society to the University of Colorado and the University of Minnesota. Portions of this research were conducted while the author was supported by United States Public Health Service Fellowships 1-F1-GM-32, 906-01; 1-F02-GM-32, 906-01; 1-F02-GM-32, 906-02. The helpful comments and advice made by Drs David J. Merrell and Sandra Smith-Gill during the preparation of the manuscript are gratefully acknowledged.

Table 1. *Results of crosses. See text for explanation*

Cross number	Description	Progeny			χ^2 Expected ratio indicated in parentheses	
		$B/-$	$+/+$	Intermediate		
2	$+/+ \times B/+$ (from cross 1)	99	59	–	10·17**	(1:1)
3	$+/+ \times B/+$ (from cross 2)	19	25	–	0·81	(1:1)
4	$+/+ \times B/+$ (from cross 3)	16	21	–	0·67	(1:1)
5	$+/+ \times B/+$ (from cross 3)	16	13	–	0·31	(1:1)
6	$+/+ \times B/+$ (from cross 2)	17	24	–	1·02	(1:1)
7	$+/+ \times B/+$ (from cross 6)	34	53	–	4·14*	(1:1)
8	$B/+ \times B/+$ (both from cross 2)	20	4	–	0·44	(3:1)
9	$+/+ \times B/+$ (from cross 8)	79	129	14	—	—
	Assign 'intermediate' to $B/+$	93	129	–	5·83*	(1:1)
	Assign 'intermediate' to $+/+$	79	143	–	18·44**	(1:1)
	Divide 'intermediate' equally	86	136	–	11·26**	(1:1)

* = Significant at the 5 % level. ** = Significant at the 1 % level.

methods will be published elsewhere. In all cases, temperature, water flow, availability of food, and density of animals have been adjusted for optimal survival and growth and to minimize environmentally determined variability in Burnsi phenotypes (Davison, 1964; Browder & Davison, 1967).

All crosses represent single-pair matings. All male parents were Burnsi heterozygotes. With the exception of one cross (see below), female parents were wild-type and were obtained from J. R. Schettle Biologicals, Stillwater, Minnesota. Progeny were scored at the time of metamorphosis.

3. RESULTS

Data are summarized in Table 1. Cross 2 was between a $B/+$ derived from cross 1 and $+/+$. Burnsi progeny were in excess.

The various lines of descent from cross 2 (Fig. 1) differ in the kinds of Burnsi: wild-type ratios obtained. Line A (crosses 3, 4 and 5) is characterized by normal segregation ratios. Cross 3 was between a $B/+$ from cross 2 and $+/+$. $B/+$ progeny from cross 3 were crossed with wild-types (crosses 4 and 5).

Line B (crosses 6 and 7) is characterized by excesses of $+/+$ progeny. Cross 6 was between a $B/+$ from cross 2 and $+/+$. The excess of $+/+$ progeny of cross 6 is not significant at the 5 % level. A $B/+$ from cross 6 was mated with $+/+$ (cross 7). The excess $B/+$ progeny of cross 7 is significant at the 5 % level.

Cross 8 (line *C*) was between sibling $B/+$ from cross 2. No significant difference in the expected 3:1 ratio of $B/- : +/+$ was found. A $B/+$ from cross 8 was crossed with $+/+$ (cross 9). Cross 9 resulted in a large excess of $+/+$ progeny.

Most of the Burnsi parents and progeny discussed in this paper possessed a small number of dorsal and lateral spots. Such Burnsi are frequently encountered by investigators and can result from either genetic or environmental factors or a combination of both Merrell (1972). Merrell (personal communication) has applied the following criteria to distinguish between these Burnsi and their wild-type siblings:

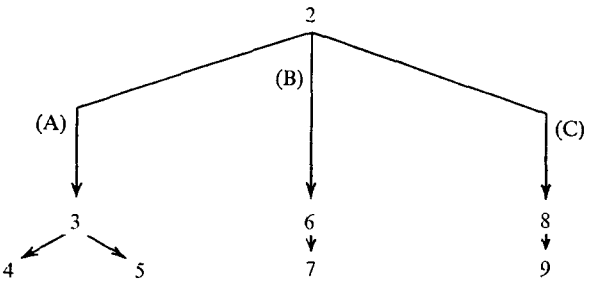

Fig. 1. Descent from cross 2.

1. Spot number of progeny has a bimodal distribution. Wild-types are clustered around the high spot number mean, while Burnsi are clustered around the low spot number mean.

2. Wild-type dorsal spots are more or less evenly spaced from one another between the dorsolateral lines. Burnsi dorsal spots tend to lie along the mid-dorsal line.

3. Wild-type lateral spots are more or less evenly distributed along the flanks. Lateral spots are rare in Burnsi and, when found, are localized just below the dorsolateral lines and near the front or hind legs.

I use one additional criterion. Burnsi dorsal spots are unevenly distributed from front to rear; they are seldom found as far forward as the level of the pectoral region. Spots on Burnsi frogs produced in this laboratory are never present over the eyes. Wild-type frogs on the other hand, nearly always have a spot over at least one eye with the remaining spots being present in both anterior and posterior skin.

The latter criterion is vital for scoring frogs produced in this laboratory since wild-type frogs are frequently produced with low spot number and whose spots are not evenly spaced from one another. The validity of the criterion of eye spotting has been experimentally demonstrated (Browder, unpublished).

The above criteria normally allow positive identification of progeny. However, in cross 9, a few frogs were recovered whose phenotypes were still intermediate between Burnsi and wild-type using all the above criteria. They had higher spot numbers than those positively identified as Burnsi, and the spots were unevenly distributed. The ultimate criterion (eye spots) could not be applied with certainty since all of them had spots very near, but slightly posterior to, the eyes. Wild-type frogs of this phenotype have previously been recovered, and, in my judgement, all of the intermediate progeny are wild-type. However, the data are analysed in alternative ways to demonstrate that the excess of wild-type progeny is a real phenomenon and not the result of scoring bias. The intermediate progeny were either all assigned to the $B/+$ class, all assigned to the $+/+$ class, or divided equally between the two classes. No matter how these progeny are assigned, the excess of $+/+$ is statistically significant.

Any differential mortality must have occurred prior to the tadpole stage, since

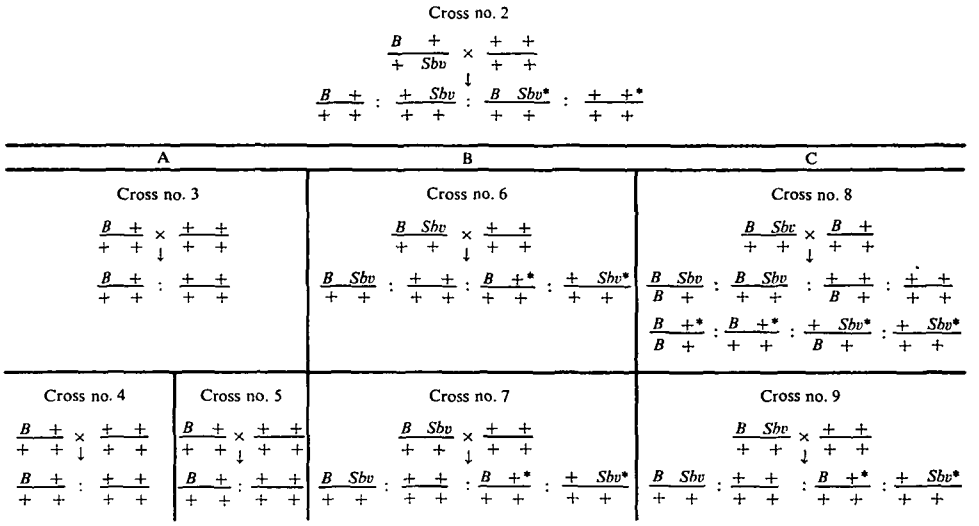

Fig. 2. Behaviour of the *B* and *Sbv* genes. Asterisks indicate cross-overs.

the death of tadpoles was insufficient to account for the observed ratios. For example, 160 tadpoles were reared from cross 2; 158 of them survived until they were scored at metamorphosis.

4. DISCUSSION

The pattern of aberrant $B/+ : +/+$ ratios reported in this paper could not be the result of viability differences between the Burnsi gene and its wild-type allele. One cross (no. 2) produced an excess of $B/+$ progeny, while various subsequent crosses had either normal $B/+ : +/+$ ratios or excess $+/+$ progeny. The simplest interpretation of the data is that a subvital gene (*Sbv*) linked to the Burnsi locus is segregating in the various crosses. *Sbv* could produce its effect either during embryogenesis, at fertilization, or even earlier, during gametogenesis. In each case where aberrant ratios were obtained, the male parent was a Burnsi heterozygote. It is possible, therefore, that the effect of *Sbv* was exerted during spermatogenesis, resulting in unequal numbers of + and *B*-bearing sperm. However, in the absence of reciprocal crosses, this question is unresolved.

The behaviour of *Sbv* in the various crosses is outlined in Fig. 2. The *Sbv* gene was initially linked to the wild-type allele of the Burnsi gene in the Burnsi parent of cross 2. The cross resulted in non cross-over Burnsi that lacked *Sbv* and recombinants with *B-Sbv* linkage. A non cross-over was used to establish line A, while recombinants were used to establish lines B and C. Cross 8 (line C), which was between two Burnsi sibling heterozygotes, produced a straight 3:1 ratio. However, a male Burnsi derived from cross 8 was mated with a wild-type female (cross 9), producing excess $+/+$ progeny. Thus, cross 8 showed no ratio distortion but transmitted the gene. Two possible explanations for this are proposed. If the effect of *Sbv* is on spermatogenesis, the male parent of cross 8 would have had the geno-

type $B+/++$, while the female was $BSbv/++$, producing a normal 3:1 ratio. The effects of *Sbv* are undoubtedly influenced by the genetic background. This is obvious if one compares the various ratios obtained. Thus, the genetic background in cross 8 may have ameliorated the effects of *Sbv*.

Gill (1970) reported significant excesses of Burnsi progeny from several reciprocal $B/+ \times +/+$ crosses. However, $B/+ \times B/+$ crosses involving the same parents produced 3:1 ratios (Gill, personal communication). Thus, it is unlikely that a subvital gene was responsible for her aberrant ratios. No follow-up data resulting from crosses involving progeny are available. Thus, it is uncertain whether her aberrant ratios have a genetic or environmental explanation.

The proposed linkage between *B* and *Sbv* would constitute the first case of linkage between two mutant loci in anuran amphibians. Previous linkage studies in *Rana pipiens* have involved the four well-characterized pigment mutants of *Rana pipiens:* Burnsi, Kandiyohi (*K*), Speckle (*Sp*) and melanoid (*m*). Crosses of Burnsi–Kandiyohi double heterozygotes with the recessive wild-type have resulted in four classes of progeny in equal numbers: double mutant, Burnsi, Kandiyohi, and wild-type (Volpe, 1960; Merrell, 1972). Thus, these genes are on different chromosomal pairs. Likewise, Browder (1968) demonstrated independent assortment of *Sp* and *B* by crossing the double heterozygote with wild-type.

Nace *et al.* (1970) investigated linkage relationships of *m* with *B* and *K* respectively by producing gynogenetic progeny from double heterozygotes ($B/+$; $m/+$ and $K/+$; $m/+$). Gynogenesis involves activation of eggs without fertilization and subsequent inhibition of the second meiotic division to reconstitute diploidy. *B* and *K* segregated independently of *m*. Nace *et al.* were also able to map these genes in relation to the centromeres on their respective chromosomes. The gene-centromere distance for *B* is 41·4 map units. Volpe (1970) estimated the Burnsi-centromere distance to be at least 32 map units. Nace *et al.* (1970) recalculated Volpe's data to 37·7 map units. Gynogenesis is an extremely useful tool for mapping and determining linkage relationships in the amphibian. As more genes are identified and become available for laboratory experimentation, detailed linkage maps of *Rana pipiens* can be prepared utilizing this procedure.

Frogs derived from crosses in which *Sbv* has been postulated are under culture in the Amphibian Facility, University of Michigan. Inquiries concerning the availability of *Sbv* frogs should be sent to the Director, Dr George Nace.

REFERENCES

Browder, L. W. (1968). Pigmentation in *Rana pipiens*. I. Inheritance of the speckle mutation. *Journal of Heredity* **59**, 162–166.

Browder, L. W. & Davison, J. (1964). Spotting variations in the leopard frog. A test for the genetic basis in the *Rana pipiens* 'burnsi' variant. *Journal of Heredity* **55**, 234–241.

Davison, J. (1964). A study of spotting patterns in the leopard frog. III. Environmental control of genic expression. *Journal of Heredity* **55**, 47–56.

Gill, S. J. (1970). Differential viability associated with the burnsi and kandiyohi heterozygotes in the leopard frog, *Rana pipiens*. *Genetics Supplement* **64**, S 23.

Merrell, D. J. (1972). Laboratory studies bearing on pigment pattern polymorphisms in wild populations of *Rana pipiens*. *Genetics* **70**, 141–161.

MOORE, J. A. (1942). An embryological and genetical study of *Rana burnsi* Weed. *Genetics* **27**, 408–416.

NACE, G. W., RICHARDS, C. M. & ASHER, JR., J. H. (1970). Parthenogenesis and genetic variability. I. Linkage and inbreeding estimation in the frog, *Rana pipiens*. *Genetics* **66**, 349–368.

VOLPE, E. P. (1960). Interaction of mutant genes in the leopard frog. *Journal of Heredity* **51**, 151–155.

VOLPE, E. P. (1970). Chromosome mapping in the leopard frog. *Genetics* **64**, 11–21.