

Heldout, a Recessive Wing Mutation in *Drosophila melanogaster*

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During July 1935, a few flies holding their wings at right angles to their bodies were discovered in a stock bottle of *Drosophila melanogaster*. These were mated *inter se* and a pure strain was obtained. While the wings vary in extension from 45° to 90° from the body axis, they apparently never show the parallel or folded-together position of the wild-type fly. Because of this characteristic position of the wings, the mutation was named straight, symbol *str*, but it was renamed heldout, symbol *ho*, upon the suggestion of Dr. Bridges of the Carnegie Institution of Washington, to whom we are indebted for advice throughout the course of these experiments.

Allelomorphic tests were made with other wing characters with negative results. The results showed in addition that heldout is recessive and not sex-linked.

Chromosome Carrying Heldout

To determine whether the gene for heldout is in the third chromosome, a heldout female was crossed to a sepia male and three F₂ pair cultures raised (Table I).

TABLE I.—F₂ Distribution (P₁, heldout ♀ x sepia ♂; F₁ wild-type ♀ x F₁ wild-type ♂).

May 23, 1936	wild-type	heldout	sepia	heldout sepia
Total.....	187	53	34	18

The appearance of double recessives showed that heldout is not in the third chromosome. The failure of the phenotypes to appear in a ratio closer to the expected 9:3:3:1 was undoubtedly caused by not counting the offspring for more than two days because of confirmatory evidence from the simultaneous experiment described below.

A heldout female was crossed to a black male to find out if heldout is in the second chromosome. Three F₂ pair cultures were raised (Table II).

TABLE II.—F₂ Distribution (P₁, heldout ♀ x black ♂; F₁ wild-type ♀ x F₁ wild-type ♂).

May 23, 1936	wild-type	heldout	black	heldout black
Total.....	128	57	51	0

The results plainly show a 2:1:1:0 ratio, proving that heldout is in the second chromosome.

Preliminary Localization of Heldout

A heldout male was mated to a brown female, and several brown and heldout flies from the F_2 were mated *inter se*. From the F_3 , several heldout brown individuals were obtained, from which a stock was established. The ease with which the heldout brown combination was obtained gave preliminary indication of a high rate of crossing-over between heldout and brown. Several heldout brown males and females were mated to wild and heterozygous females from the F_1 backcrossed to heldout brown males (Table III).

TABLE III.—Result of Backcross (P_1 , heldout brown x wild; F_1 wild-type ♀ x heldout brown ♂).

Jan. 21, 1937	heldout brown	wild-type	held-out	brown	N	Recombinations (R)
Total.....	464	549	492	466	1971	48.6%

The very high percentage of recombination indicated that the locus of heldout is very distant from that of brown, which is at 104.5.

In the same way that the heldout brown stock was obtained, a stock containing heldout black and purple was made up. Males from the heldout black purple stock were mated to wild females, and heterozygous females from the F_1 were backcrossed to heldout black purple males (Table IV).

TABLE IV.—Results of Backcross (Feb. 18, 1937) (P_1 , heldout black purple ♂ x wild ♀; F_1 wild-type ♀ x heldout black purple ♂).

Parental Combinations			Recombinations								N	R ho-b	R b-pr		
ho	b	pr	ho	+	+	ho	b	+	ho	+				pr	
+	+	+	+	b	pr	+	+	pr	+	b				+	
heldout black purple	wild- type		heldout	black purple		heldout black	purple		heldout purple	black					
500	591		222	199		13	11		3	0		1539	27.6%		1.8%

The fairly large amount of recombination for heldout and black showed that the locus of heldout is probably more than 30 units to the left of the locus of black, which is at 48.5.

From the above experiment it seemed probable that the locus lies near 13, and, to determine whether it lies to the left or right of this locus, a cross was made with dumpy clot (*dp*, 13.0; *cl*, 16.5). A heldout male was mated to a dumpy clot female; all the F_1 were wild-type. Heterozygous females were backcrossed to dumpy clot males. Cross-overs from the F_2 , easily identified since they were dumpy only or clot

only, were mated to heldout females. There were three possibilities: if heldout should lie to the left of clot ($ho + +/+ dp cl$), then the not-dumpy clot cross-overs would also carry heldout while the dumpy not-clots would not; if heldout should lie to the right of dumpy ($dp cl +/+ + ho$), the dumpy not-clot cross-overs would carry heldout, whereas the not-dumpy clots would not; if, however, both kinds of cross-overs should carry heldout, then heldout would lie both to the right of dumpy and to the left of clot, that is, between them ($dp + cl/+ ho +$).

When males of the two kinds, dumpy and clot, were mated separately to heldout females, the clot flies produced both wild and heldout, whereas the dumpy produced only wild, showing that the locus of heldout actually lies to the left of clot.

A stock containing the recessives heldout and clot was obtained from the last experiment, and females were mated to males with the genotype $Mz/Inversion$ (Minute-z, $13.0 \pm$). F_1 females showing Minute were crossed to heldout clot males. The totals for this three point cross were as follows:

TABLE V.—Results of Backcross (May 30, 1937) (P_1 , $ho cl \text{ } \varnothing \times Mz/Inversion \text{ } \sigma$; F_1 $Mz \text{ } \varnothing \times ho cl \text{ } \sigma$).

Parental Combinations			Recombinations						N	R. ho-Mz	R. Mz-cl
ho	+	cl	ho	Mz	+	ho	+	+			
+	Mz	+	+	+	cl	+	Mz	cl			
heldout clot	Minute-z		heldout Minute-z	clot		heldout	Minute-z clot				
638	661		62	158		16	23		1558	14.1%	2.5%

From the above table it is evident that the locus of heldout is considerably to the left of Minute-z. A cross was made therefore with Star (S , 1.3), by mating Star/Curly females to heldout males, and crossing the F_1 Star females to heldout males.

TABLE VI.—Results of Backcross (P_1 , $S/Cy \text{ } \varnothing \times ho \text{ } \sigma$; F_1 $S \text{ } \varnothing \times ho \text{ } \sigma$).

July 2, 1937	Star	heldout	Star heldout	wild-type	N	R.
Totals.....	1786	1787	49	52	3674	2.7%

The locus is therefore about 2.7 units from Star. To find out whether it is to the right or left of Star, females having the genotype $S ho +/+ + Mz$ or else $ho S +/+ + Mz$ were mated to heldout males. The cross-overs examined included Star Minute flies and heldout flies, showing that heldout lies to the right of Star. Therefore the locus was placed at approximately 4.

Final Localization of Heldout

The final step in the localization of heldout was a three-point back-cross involving aristaless (*al*, 0.0) Star and heldout (*al S ho/+ + +*). To get the *al S ho* combination, *al S/Pm*, *ds^{32k}* males were mated to heldout females and F₁ Star females were backcrossed to heldout males (Table VII).

TABLE VII.—Results of Backcross (P₁, *al S/Pm*, *ds^{32k}* ♂♂ x *ho* ♀♀; F₁ *al S +/+ + ho* ♀ x *ho* ♂).

Nov. 2, 1937	Star	heldout	Star heldout	wild-type	N	R. S-ho
Totals.....	520	493	13	13	1039	2.5%

Cross-overs showing both Star and heldout were then mated to Star/Curly and the Star Curly offspring inbred to give a balanced lethal stock with the genotype *al S ho/Cy*. Similar cross-overs were mated to wild to get the heterozygous females necessary for the three-point experiment.

In order to obtain an aristaless heldout stock, an aristaless male was mated to a *S ho/Cy* female (Table VIII). F₁ Star females then were mated, not to heldout, as would ordinarily be done, but to *S ho/Cy* males. The Curly offspring were discarded without counting, and advantage was taken of the lethality of homozygous Star in eliminating half of the remaining offspring. This type of "backcross" is advantageous over the ordinary backcross in the following respects: first, there are only two (non-Curly) phenotypes to be counted instead of the usual four; second, all the *S ho* flies carry the desired *al ho* genotype; and third, all the cross-overs can be identified quite easily. The objections to this procedure are: first, the number of flies counted is one-fourth normal; and second, there is no balancing of differential mortality. In this particular case these objections do not hold because the purpose of the experiment was to obtain an *al ho* strain, counts for the percentage of recombinations being incidental, and both *ho* and *S* are practically as viable as wild.

TABLE VIII.—Results of "Backcross" (Sept. 29, 1937) (P₁, *S ho/Cy* ♀ x *al* ♂; F₁ *S* ♀ x *S ho/Cy* ♂—*Cy* flies discarded).

Zygotes	<i>al + +</i>	<i>+ S ho</i>	<i>al + ho</i>	<i>+ S +</i>	N	R. S-ho
	<i>+ S ho</i>	<i>+ S ho</i>	<i>+ S ho</i>	<i>+ S ho</i>		
Phenotypes	Star	hom. Star (dies)	Star heldout	hom. Star (dies)		
Total.....	375	0	7	0	382	1.8%

Females having the genotype *al S ho/+ + +* obtained from mating the cross-overs from Table VII were backcrossed to *al ho* males procured by selfing the cross-overs from Table VIII. The results of this cross (Table IX) confirm previous localizations of heldout.

TABLE IX.—Results of Backcross (Nov. 26, 1937) (*al S ho/+ + +* ♀
x *al ho* ♂).

Parental Combinations			Recombinations						N	R. al-S	R. S-ho
al	S	ho	al	+	+	al	S	+			
+	+	+	+	S	ho	+	+	ho			
aristaless Star heldout	wild- type		aristaless		Star heldout	aristaless Star		heldout			
3593	3821		16		17	123		94	7664	0.4%	2.8%

Although the 0.4% of crossing-over between aristaless and Star is much smaller than the generally accepted value of 1.3%, the agreement of the percentage between Star and heldout with that obtained in other experiments indicated that the experiment is reliable.

A total of 12,759 flies were counted in experiments giving 351 cross-overs. This is a cross-over percentage of 2.7, which, when added to the locus of Star, 1.3, places the locus of heldout at 4.0 in the second chromosome.

The accurate localization of heldout at 4.0, its high viability, strategic position, and easily distinguishable characteristic make it a mutant of the first rank (RK1). Its characteristics are summarized in the manner customarily used in describing *Drosophila* mutants, as follows: *ho*—heldout; Novitski, 35g (2-4.0) Spontaneous. Wings extended at 45° to 90° from body axis. RK1.

References

For an account of the previously known mutants in this region, as well as the various mutants used in the course of this problem, consult the following:

1. Stern, C. and C. B. Bridges, 1926. The mutants at the extreme left end of the second chromosome of *Drosophila melanogaster* *Genetics* 11:503-530.
2. *Drosophila* Information Service, 1935. Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, N. Y.