

Genetic Model for Color Polymorphism in *Jadera haematoloma* (Hemiptera: Rhopalidae)

LAURIE A. McLENNAN, FRANK K. LAKE, SCOTT P. CARROLL, HUGH DINGLE,
AND CHRIS BOYD¹

Center for Population Biology, University of California, Davis, CA 95616

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ABSTRACT Color markers in insects facilitate research in many areas, including developmental genetics and the study of sperm competition. Laboratory populations of the soapberry bug *Jadera haematoloma* contain two color variants, orange and lemon, in addition to the wild-type, red. Results of experimental crosses among these three types support a two-locus model with two alleles at each locus. The two loci interact epistatically such that individuals possessing two recessive genes at the second locus are lemon regardless of which genes are present at the first locus. The putative genotypes are red, $R/- B/-$; orange, $r/r B/-$; lemon, $-/- b/b$.

KEY WORDS *Jadera haematoloma*, soapberry bug, color polymorphism

Jadera haematoloma Herrich-Schaeffer, the "soapberry bug" (Carroll 1991), is a bright red and black insect that feeds on mature seeds of plants in the family Sapindaceae (Schaefer & Mitchell 1983, Carroll & Loye 1987). It is distributed from the southern United States to northern South America (Blatchley 1926). *J. haematoloma* is an important model system for studies of mating systems and host race evolution (e.g., Carroll & Boyd 1992, Carroll 1993). Here, we investigate visible genetic markers that may facilitate studies of developmental genetics and sperm competition in this species. The wild-type color of *J. haematoloma* is red. Two color mutants, orange and lemon, occur in laboratory populations. To determine the genetic basis of this color polymorphism, we experimentally crossed individuals of the three color types. We conducted the work in two stages. In stage 1 we performed exploratory crosses between red and orange individuals, as well as between their F_1 offspring. In stage 2, we constructed a two-locus genetic model based on the results from stage 1 and tested its predictions with lemon \times lemon, orange \times orange, and orange \times lemon crosses. For clarity, we first introduce the genetic model and then report the results of the two series of crosses.

Model

Based on extensive exploratory crosses (described below), we constructed a two-locus Men-

delian model with two alleles at each locus to explain the color polymorphism of *J. haematoloma*. The first locus contains two alleles; the allele for red (wild-type) (R) is dominant, and the allele for orange (r) is recessive. The second locus, which is unlinked to the first, also contains two alleles; wild-type (B) is dominant, and lemon (b) is recessive. The two loci interact epistatically such that individuals possessing two recessive alleles (b/b) at the second locus are lemon regardless of which alleles are present at the first locus. Thus, the putative genotypes are red, $R/- B/-$; orange, $r/r B/-$; lemon, $-/- b/b$.

Materials and Methods

Jadera haematoloma specimens were collected in Woodward, OK, and Leesburg, FL, and raised in large cultures at 32°C with a photoperiod of 14:10 (L:D) h. Orange- and lemon-color mutants were removed periodically from the main culture and raised in separate cultures; however, these lines were not pure breeding lines. Bugs were supplied with cotton-stoppered water vials and seeds from their respective host plants, the soapberry tree, *Sapindus saponaria* (L.) v. *drummondii* (Hooker & Arnott) Benson, and the goldenrain tree, *Koelreuteria paniculata* Laxman, for the Woodward population and a second species of goldenrain tree, *K. elegans* (Seemann) Smith, for Leesburg.

In each of the experiments described below, virgins were obtained by removing fifth instars from the main colonies and observing them daily for adult eclosion. Virgins were paired individually and placed in 90-mm petri dishes lined with

¹Department of Biology, University of Utah, Salt Lake City, UT 84112.

Table 1. Average color-morph ratios for F₂ offspring of red × orange crosses in families that produced no lemon offspring

Population	No. families	Total no. of offspring	Avg frequency red	Avg frequency orange	G _H	G _P	G _T
Woodward	16	2,002	0.765	0.235	12.96	0.35	13.32
Leesburg	14	1,075	0.747	0.253	20.24	0.02	20.25
Expected	—	—	0.750	0.250	—	—	—

$P > 0.05$ for all values of G .

filter paper and containing a water vial and seeds. Eggs, which were deposited loosely on the filter paper, were collected daily and stored in plastic vials; they were censused once a day for hatching. The color morph of each hatchling was recorded immediately. Color morph does not change during development.

Stage 1: Exploratory Crosses. For red × orange crosses, we established 23 pairs of Leesburg bugs and 23 pairs of Woodward bugs; pairs included both red males crossed with orange females and orange males crossed with red females. All F₁ offspring were red, except for siblings from a single Woodward family that were not used for further crosses. We crossed the resulting F₁ offspring (22 pairs from Leesburg and 24 from Woodward) and recorded the color of the F₂ offspring from each family. Families were divided into two groups based on the presence or absence of lemon offspring. For each group in each population we then computed a color-morph ratio as the mean of the family ratios; families producing <20 offspring were excluded from this analysis. We used a heterogeneity G test to compare the offspring color-morph ratios predicted by the model with those observed in the F₂ offspring. G_H (G for heterogeneity) tests whether the offspring color-morph ratios among families are homogeneous versus heterogeneous, G_P (G for pooled data) tests the goodness-of-fit of the pooled data to the expected color-morph ratios, and G_T ($= G_H + G_P$) is the total G .

Stage 2: Tests of the Model. In a second series of crosses, we tested the genetic model with predictions about lemon × lemon, orange × orange, and orange × lemon crosses. Lemon × lemon crosses should produce exclusively lemon offspring. To test this prediction, we paired 30 Woodward lemon males with 30 Woodward lemon females and recorded the color of the offspring.

Orange × orange crosses should produce either exclusively orange offspring or orange and lemon offspring in a ratio of 3:1. To test this, we paired nine Woodward orange males with nine Woodward orange females and recorded the color of their offspring.

Orange × lemon crosses should produce a mixture of offspring colors dependent upon the pa-

rental genotypes. Thus, to determine the expected offspring color-morph ratios, we first needed to determine the parental genotypes. We used the presence or absence of particular colors from the offspring of orange × lemon crosses to assign parental genotypes. Offspring color frequencies predicted from the parental genotypes were compared with observed frequencies to test the model.

Ten Woodward orange sires were paired serially with multiple (up to four) Woodward lemon females, and 12 Woodward lemon sires were paired serially with multiple orange females. Each male was kept with his mate for at least 1 d; he was then removed from the petri dish and paired with another female.

We assigned genotypes to each parent using the rules given below. The numbers in parentheses are the probabilities of erroneous genotype assignment, given that our two-locus model is correct. These error probabilities were calculated by computing the expected offspring color ratios of all six possible genetic combinations of orange and lemon parents and calculating the probability of not observing the least frequent offspring color, given that 20 offspring were produced.

I.) Orange parent

- A. If any offspring are lemon = $r/r B/b$ ($P = 9.54 \times 10^{-7}$)
- B. If no offspring are lemon = $r/r B/B$ ($P = 9.54 \times 10^{-7}$)

II.) Lemon parent

- A. If no offspring are orange = $R/R b/b$ ($9.54 \times 10^{-7} < P < 0.0032$)
- B. If no offspring are red = $r/r b/b$ ($0 < P < 0.0032$)
- C. If at least one offspring is orange and one is red = $R/r b/b$ ($0 < P < 9.54 \times 10^{-7}$)

After we had determined the parental genotypes, we calculated the predicted offspring color ratios using Punnett squares, and used chi-square tests to compare predicted and observed ratios. Then we looked for consistency of each male's assigned genotype across the multiple females he was paired with, because each male's genotype was calculated independently for each of the pairs.

Table 2. Average color-morph ratios for F₂ offspring of red × orange crosses in families that produced at least one lemon offspring

Population	No. families	Total no. offspring	Avg frequency red	Avg frequency orange	Avg frequency lemon	G _H	G _P	G _T
Woodward	6	605	0.547	0.199	0.254	2.14	0.17	2.31
Leesburg	5	423	0.601	0.172	0.227	4.14	5.99	7.31
Expected	—	—	0.563	0.188	0.250	—	—	—

$P > 0.05$ for all values of G .

Results

Stage 1: Exploratory Crosses. Virtually all the F₁ offspring were wild-type (866 from Leesburg and 377 from Woodward); thus, the direction of the cross did not affect offspring color. The single exception was a Woodward family that produced 16 red, 9 orange, and 8 lemon offspring; this result is consistent with the model if the red parent was heterozygous at both loci. The F₁ crosses produced two types of families. Most pairs produced only red and orange offspring in a ratio of 3:1 (Table 1), whereas other families produced red, orange, and lemon offspring in a ratio of 9:3:4 (Table 2).

Stage 2: Tests of the Model. The lemon × lemon crosses produced 1,116 total offspring; 100% were lemon, as predicted by the model.

The results of the orange × orange crosses were also consistent with the model. Eight of nine crosses produced a total of 420 exclusively orange offspring; one cross produced both orange ($n = 41$) and lemon ($n = 5$) offspring.

For the crosses between orange males and lemon females (Table 3), all 17 offspring color ratios were consistent ($P > 0.05$) with our model,

and, for lemon male × orange female crosses (Table 4), 14 of 18 offspring ratios were consistent with the model. Three of the four inconsistent family ratios were the offspring of one male (Table 4, male no. 2); in those families, there were excess red individuals, too few orange siblings, and, in one case, too few lemon individuals ($P < 0.001$).

There are two cases in the lemon male × orange female crosses where the predicted male genotype is not consistent across dams (Table 4, males no. 1 and 4); however, in both of those cases, the family size is < 20 .

Discussion

The red/orange/lemon color polymorphism in *J. haematoloma* can be explained by our two-locus model. The first locus contains two alleles, R and r ; $R/-$ individuals are red, and r/r individuals are orange. A second independent locus, which also contains two alleles, B and b , interacts epistatically with the first locus such that the phenotype of $B/-$ individuals is determined by the first locus, and b/b individuals are always lemon.

Table 3. Offspring color-morph ratios resulting from orange male × lemon female crosses

Male no.	Female no.	No. eggs laid	No. eggs hatched	Observed color ratio of offspring L:O:R	Predicted male genotype	Predicted female genotype	Expected color ratio of offspring L:O:R	χ^2
2	2d	97	73	32:17:24	rrBb	Rrbb	2:1:1	2.45
4	4b	52	39	0:39:0	rrBB	rrbb	0:1:0	0.00
	4c	135	116	0:69:47	rrBB	Rrbb	0:1:1	4.17
5	4d	93	61	0:32:29	rrBB	Rrbb	0:1:1	0.15
	5c	85	71	35:18:18	rrBb	Rrbb	2:1:1	0.01
6	5d	68	57	32:0:25	rrBb	RRbb	1:0:1	0.86
	6a	137	108	0:62:46	rrBB	Rrbb	0:1:1	2.37
8	6b	26	11	0:5:6	rrBB	Rrbb	0:1:1	—
	6c	126	68	0:41:27	rrBB	Rrbb	0:1:1	2.88
	6d	105	92	0:40:52	rrBB	Rrbb	0:1:1	1.57
9	8a	115	77	41:12:24	rrBb	Rrbb	2:1:1	4.06
	8b	60	14	6:4:4	rrBb	Rrbb	2:1:1	—
	8c	189	7	6:1:0	rrBb	rrbb	1:1:0	—
	8d	195	146	69:77:0	rrBb	rrbb	1:1:0	0.44
10	9a	170	116	51:37:28	rrBb	Rrbb	2:1:1	3.09
	9b	189	120	61:59:0	rrBb	rrbb	1:1:0	0.03
	9c	313	159	82:77:0	rrBb	rrbb	1:1:0	0.16
	9d	113	83	37:0:46	rrBb	RRbb	1:0:1	0.98
10	10b	294	218	99:119:0	rrBb	rrbb	1:1:0	1.83
	10c	147	93	49:0:44	rrBb	RRbb	1:0:1	0.27

Chi-square tests were performed only on families with at least 20 offspring. $P > 0.05$ for all values of χ^2 .

Table 4. Offspring color-morph ratios resulting from lemon male \times orange female crosses

Male no.	Female no.	No. of eggs laid	No. of eggs hatched	Observed color ratio of offspring L:O:R	Predicted male genotype	Predicted female genotype	Expected color ratio of offspring L:O:R	χ^2
1	1b	15	13	4:8:1	Rrbb	rrBb	2:1:1	—
	1c	48	35	0:35:0	rrbb	rrBB	0:1:0	0.00
	1d	99	92	46:46:0	rrbb	rrBb	1:1:0	0.00
2	2a	195	96	46:10:40	Rrbb	rrBb	2:1:1	18.90***
	2b	98	49	3:7:39	Rrbb	rrBb	2:1:1	67.75***
	2c	98	37	0:1:36	Rrbb	rrBB	0:1:1	33.11***
3	3a	11	8	2:4:2	Rrbb	rrBb	2:1:1	—
	3b	69	34	14:9:11	Rrbb	rrBb	2:1:1	1.29
	3c	64	51	0:28:23	Rrbb	rrBB	0:1:1	0.49
	3d	30	26	11:5:10	Rrbb	rrBb	2:1:1	2.54
4	4b	33	14	10:4:0	rrbb	rrBb	1:1:0	—
	4d	60	15	0:8:7	Rrbb	rrBB	0:1:1	—
	5a	220	158	0:74:84	Rrbb	rrBB	0:1:1	0.63
5	5b	95	50	0:25:25	Rrbb	rrBB	0:1:1	0.00
	5c	25	5	0:1:4	Rrbb	rrBB	0:1:1	—
	7a	26	12	2:5:5	Rrbb	rrBb	2:1:1	—
7	7d	70	45	19:12:14	Rrbb	rrBb	2:1:1	1.27
	8b	162	93	34:32:27	Rrbb	rrBb	2:1:1	7.26*
	8c	115	35	17:10:8	Rrbb	rrBb	2:1:1	0.26
	8d	212	77	31:21:25	Rrbb	rrBb	2:1:1	3.34
10	10a	166	64	33:31:0	rrbb	rrBb	1:1:0	0.06
	10b	259	112	49:63:0	rrbb	rrBb	1:1:0	1.75
	10d	23	15	6:9:0	rrbb	rrBb	1:1:0	—
11	11a	25	6	3:0:3	Rrbb	rrBb	2:1:1	—
	11b	20	10	8:1:1	Rrbb	rrBb	2:1:1	—
	11c	84	54	22:21:11	Rrbb	rrBb	2:1:1	5.56
	11d	124	51	29:11:11	Rrbb	rrBb	2:1:1	0.96
12	12a	107	4	2:1:1	Rrbb	rrBb	2:1:1	—
	12c	36	10	0:8:2	Rrbb	rrBB	0:1:1	—
	12d	22	17	0:7:10	Rrbb	rrBB	0:1:1	—

Chi-square tests were performed only on families with at least 20 offspring. *, $P < 0.05$; ***, $P < 0.001$.

Our experimental crosses strongly support our model. The one exception is that the offspring color-morph ratios of male no. 2 in the lemon male \times orange female crosses (Table 4) have an excess of red individuals, too few orange siblings, and, in family no. 2b, too few lemon siblings. Although the offspring color-morph ratios of families no. 2a and 2c could be explained by a back mutation in the male's spermatogonium from $R/r\ b/b$ to $R/R\ b/b$, the offspring color-morph ratio of family no. 2b is not consistent with this explanation. The inheritance of color morph may involve a third locus.

The two-locus/two-allele mode of inheritance of color morph found in *J. haematoloma* is also found in other species (Johnson 1984, Strickberger 1986, Farnsworth 1988). Natural populations of the Jersey tiger moth *Callimorpha quadripunctaria* Poda have individuals with red, orange, or yellow hindwings, and the mode of inheritance is analogous to the inheritance of red, orange, and lemon color morphs in *J. haematoloma* (Liebert & Brakefield 1990). In *C. quadripunctaria*, the second locus (analogous to the "B" locus in *J. haematoloma*) may control metabolism of a basic yellow pigment to an intermediate orange pigment, and the first locus may control the metabolism of the orange pig-

ment to a red pigment (Liebert & Brakefield 1990).

The color mutants we studied have not been observed in nature. However, a population in the state of Puebla, Mexico, contained "color variants" on the red wild-type that included reddish individuals with pink, orange, golden, and green elements in their base color (S.P.C., personal observation). Pink bugs also occur in the laboratory and appear to be related genetically to lemon-colored bugs (L.A.M., unpublished data). Although the Puebla population has not been studied, it is possible that the genetic elements that contribute to the color variation are related to those controlling the color mutants observed in the laboratory.

The mode of color inheritance we have found should be a useful tool for genetic studies. Lemon-colored individuals are true-breeding, and both color mutants are recessive to the wild-type. By sequentially mating a mutant female with same-color and wild-type males, we can obtain information on sperm competition. In addition, if such males differ in other phenotypic traits, such as wing length, this model provides a means of distinguishing genetic control versus maternal effects in determining the phenotypes of the resulting offspring.

Wild-type and mutant individuals derived from several United States populations are maintained in our laboratory. Interested parties may gain access to them by contacting S.P.C.

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References Cited

- Blatchley, W. S. 1926. Heteroptera or true bugs of eastern North America, with special reference to the faunas of Indiana and Florida. *Nature*, Indianapolis.
- Carroll, S. P. 1991. The adaptive significance of mate guarding in the soapberry bug, *Jadera haematoloma* (Hemiptera: Rhopalidae). *J. Insect Behav.* 4: 509-530.
1993. Divergence in male mating tactics between two populations of the soapberry bug: I. Guarding versus nonguarding. *Behav. Ecol.* 4: 156-164.
- Carroll, S. P. & C. Boyd. 1992. Host race radiation in the soapberry bug: natural history, with the history. *Evolution* 46: 1052-1069.
- Carroll, S. P. & J. L. Loye. 1987. Specialization of *Jadera* species (Hemiptera: Rhopalidae) on the seeds of Sapindaceae (Sapindales) and coevolutionary responses of defense and attack. *Ann. Entomol. Soc. Am.* 80: 373-378.
- Farnsworth, M. W. 1988. *Genetics*, 2nd ed. Harper & Row, New York.
- Johnson, C. 1984. Color-morph genetics in the terrestrial isopods *Armadillidium nasatum* and *Porcellionides virga*. *Can. J. Genet. Cytol.* 26: 167-173.
- Liebert, T. G. & P. M. Brakefield. 1990. The genetics of colour polymorphism in the aposematic Jersey Tiger Moth *Callimorpha quadripunctaria*. *Hereditas* 64: 87-92.
- Schaefer, C. W. & P. L. Mitchell. 1983. Food plants of the Coreoidea (Hemiptera: Heteroptera). *Ann. Entomol. Soc. Am.* 76: 591-615.
- Strickberger, M. 1986. *Genetics*, 3rd ed. Macmillan, New York.

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