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Genetics and Characteristics of a Pigmentation Defective Laboratory Strain of the Lady Beetle, *Coleomegilla maculata*

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Abstract

Beetles in the family Coccinellidae, commonly known as ladybugs, lady beetles, or ladybirds, are easily identifiable and popular beneficial insects. Current research aims to support conservation efforts of beneficial insects in agroecosystems by exploring genetic processes related to nutrition. As a part of this research, colonies of *Coleomegilla maculata* have been maintained in culture and inbred over many generations since 2009. One result of this inbreeding has been the discovery of novel morphological phenotypes unique to laboratory strains or present in wild populations at such low levels that they have not yet been described. One such phenotype is described here. The strain described here, *ye* (yellow elytra and eyes) was characterized with classical Mendelian breeding and digital image analysis. This phenotype differs from wild populations by possessing yellow pigment in the elytra and pale grey to white eyes. In contrast, wild populations of *C. maculata* possess pink or red pigmented elytra with black spots, and black eyes. *C. maculata* is not known to exhibit polymorphism in the field. Inheritance is autosomal and recessive. This species was not previously known to exhibit the dramatic variation of color described here. The strain is stable in the homozygous recessive form, and retains laboratory rearing characteristics similar to the wild type laboratory strain.

Keywords

Coccinellidae, Yellow, Autosomal, Recessive, Elytra, Eye Color, Inbreeding, Heritable Trait, Pigmentation, Mutant Phenotype, Cuticle

1. Introduction

The species complex *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) is commonly found in North

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American agroecosystems and widespread on the North American continent. It is a highly polyphagous species and is important in regulating pest insect populations by consuming many soft bodied insects including in its diet pollen, mites, aphids and moth eggs [1]. While performing serial isofemale selections with the goal of increasing homozygosity in a laboratory strain of *C. maculata*, a small number of insects that appeared to have yellow coloration rather than the normal pink to red were found.

2. Materials and Methods

2.1. Insect Cultures and Strain Establishment

The first three mutant beetles were observed among the progeny of the second generation of inbred beetles. Two males and one female were isolated and inbred, but only produced three viable offspring, all yellow males. These males were crossed to wild type virgin females. All surviving offspring (n = 30) exhibited the wild-type, pink/red, phenotype. These putative heterozygotes were crossed as a group, and produced yellow (n = 43) and pink (n = 125) progeny. Yellow insects were used to found a putatively homozygous colony of phenotypically stable yellow beetles. After the fourth generation of consistent phenotypic strain stability colony was judged sufficiently well established to be characterized with classical genetic crosses. The mutant strain was designated ye, for yellow elytra and eyes. Crosses were performed to test the hypothesis that the genetic basis of the phenotype was monofactorial.

2.2. Digital Images and Analysis

Images were collected using a Nikon Stereomicroscope SMZ1500 (Nikon Corporation, Tokyo, Japan) with the aperture fully closed for maximum depth of field (WD54 1x objective, C-W10xA/22 oculars, and 0.75 - 2 zoom). A Nikon NI-150 high intensity double gooseneck illuminator set at 75% intensity illuminated the subjects from opposing sides. A Nikon digital camera, DMX 1200, with factory supplied ACT-1 software was used to collect images (1/25 sec shutter speed). Images used to analyze cuticle color were cropped to include only the pink or yellow sections of the elytra (forewings) and converted to JPG files then analyzed using RGB software [2].

3. Results

Results of crosses are shown in **Table 1**. Phenotypic ratios did not differ from the 3 pink to 1 yellow expected from the crossing of heterozygous individuals with a single locus mutation [3]. No evidence of sex linkage was indicated by any of the crosses; both male and female individuals of each phenotype were produced in all offspring. Thus we conclude that the locus is autosomal. Digital images were collected and analyzed for future reference (**Figure 1**). At the time of the manuscript preparation, the yellow ye strain was stable and robust. Laboratory rearing characteristics of the strain do not obviously differ from the wild type laboratory strain (empirical observation). Egg hatch and pupation rates (**Table 1(c)**) are similar to those of the wild type strain, under laboratory conditions. Loss of pigmentation in eyes is visible in the advanced embryo stage an in hatching neonates, as shown in **Figure 2**.

Four sections of each sampled individual were used for color analysis (see **Figure 1(b)**, **Figure 1(c)**). Color samples were cropped from colored areas between the melanized spots on the elytra. Averaged trichromatic color percentages are shown in **Table 2** as Red (% R), Green (% G), or Blue (% B) as detected by the digital camera. A composite of pixel components are also shown. The mutant strain, while appearing yellow visually, differed from wild-type primarily in the values for green and blue pixels. Mature specimens, with completed pigmentation deposition in the wings had higher green (Student t-test, $p < 0.0001$) and blue (Student t-test, $p < 0.0001$) pixel values when measured as live whole specimens. Chemical characterization of pigments in mutant and wild-type strains is in progress using chemical and physical extraction and isolation methods.

4. Discussion

This trait is of interest to insect dieticians because some Coccinellids possess carotenoid pigments [4] [5]; however no biochemical studies have focused on *C. maculata*. Light coloration and the ability to produce eggs when reared on an artificial diet without prey supplementation are correlated in some Coccinellids (*i.e. Harmonia axiridis* and *C. maculata*, but not *Hippodamia* or *Coccinella sp.*) [6] indicating that carotenoids may be essential for

Table 1. Crossing experiments between two strains of lady beetles, *Coleomegilla maculata*. (a) Expected results of crosses betweeen wild type pink (+) and variant yellow (*ye*), based on the null hypothesis that a single autosomal allele is responsible for the phenotype. (b) Results from paired matings. Analyses indicating a "do not reject the null hypotheses" supporting a single autosomal allele controlling the phenotype are shown as ns (not significant). wt = wild type, f = female, m = male, G = generation, R = reciprocal, y = yellow, F = filial, avg = average. (c) Hatch rate and pupation rate of crossing experiments. No significant differences were identified (Student t-test, $p > 0.05$) between the homozygous recessive cross nor reciprocal crosses in either hatch or pupation rates as compared with the wild type strain.

(a)

Phenotype+ = pink *ye* = yellow			
Male Parent	Female Parent	Offspring Ratio	Phenotype
++	++	++ 100%	pink
yeye	*yeye*	*yeye* 100%	yellow
+*ye*	*yeye*	1:1*	pink: yellow
yeye	+*ye*	1:1*	pink: yellow
+*ye*	+*ye*	3:1	pink: yellow
++	*yeye*	+*ye* 100%	pink
yeye	++	+*ye* 100%	pink

*cross not performed.

(b)

Cross number	Female name	Male name	Assumed female genotype	Assumed male genotype	Number of eggs laid	Number of eggs hatched	Number of adults reaching maturity	Expected color ratio based on model (pink:yellow)	Observed color ratio in F1 offspring (pink:yellow)	Uncorrected chi-square	F1 hybrid family chosen for F2
Parental Cross 0	P0 f1	P0 m1, P0 m2	*yeye*	*yeye*	20	10	3	0:1	0:3	ns	all (all male)
Out Cross 1	wt f1, wt f2	P1 m1	++	*yeye*	nc	nc					
Out Cross 1	wt f3, wt f4	P1 m2	++	*yeye*	nc	nc	69	1:0	69:0	ns	all (pooled)
Out Cross 1	wt f5, wt f6	P1 m3	++	*yeye*	nc	nc					
Filial Cross 1	Yellow F1f	Yellow F1m	+ye	+ye	nc	nc	168	3:1	125:43	ns	yellow only
Cross R1A	wt fA	G5 mA	++	*yeye*	244	157	93	1:0	93:0	ns	
Cross R1B	wt fB	G5 mB	++	*yeye*	186	162	58	1:0	58:0	ns	F2 pool ABC
Cross R1C	wt fC	G5 mC	++	*yeye*	284	233	90	1:0	90:0	ns	
Cross R1D	wt fD	G5 mD	++	*yeye*	12	0	infertile				
Cross R1E	G5 fE	wt mE	*yeye*	++	242	160	100	1:0	100:0	ns	
Cross R1F	G5 fF	wt mF	*yeye*	++	180	131	81	1:0	81:0	ns	F2 pool EFG
Cross R1G	G5 fG	wt mG	*yeye*	++	157	106	53	1:0	53:0	ns	
Cross R1H	G5 fH	wt mH	*yeye*	++	67	55	nc				
Cross Y1	G5 f1	G5 m1	*yeye*	*yeye*	146	84	46	0:1	0:46	ns	
Cross Y2	G5 f2	G5 m2	*yeye*	*yeye*	118	88	17	0:1	0:17	ns	
Cross Y3	G5 f3	G5 m3	*yeye*	*yeye*	64	30	14	0:1	0:14	ns	
Cross wt1	wt f1	wt m1	++	++	89	60	20	1:0	20:0	ns	
Cross wt2	wt f2	wt m2	++	++	119	100	27	1:0	27:0	ns	
Cross wt3	wt f3	wt m3	++	++	139	66	37	1:0	37:0	ns	
Cross wt5	wt f5	wt m5	++	++	28	17	nc				
Cross wt6	wt f6	wt m6	++	++	46	40	nc				
Cross F2ABC	pooled	pooled	+ye	+ye	181	118	88	3:1	67:21	ns	
Cross F2EFG	pooled	pooled	+ye	+ye	195	89	60	3:1	44:16	ns	

(c)

Cross number	Female name	Male name	Assumed female genotype	Assumed male genotype	Number of eggs laid	Number of eggs hatched	% Hatch	avg	Number of adults reaching maturity	% pupation	avg
Cross R1A	wt fA	G5 mA	++	*yeye*	244	157	64.34%		93	59.24%	
Cross R1B	wt fB	G5 mB	++	*yeye*	186	162	87.10%	77.83%	58	35.80%	44.55%
Cross R1C	wt fC	G5 mC	++	*yeye*	284	233	82.04%		90	38.63%	
Cross R1D	wt fD	G5 mD	++	*yeye*	12	0	0.00%		infertile		
Cross R1E	G5 fE	wt mE	*yeye*	++	242	160	66.12%		100	62.50%	
Cross R1F	G5 fF	wt mF	*yeye*	++	180	131	72.78%	72.12%	81	61.83%	58.11%
Cross R1G	G5 fG	wt mG	*yeye*	++	157	106	67.52%		53	50.00%	
Cross R1H	G5 fH	wt mH	*yeye*	++	67	55	82.09%		nc		
Cross Y1	G5 f1	G5 m1	*yeye*	*yeye*	146	84	57.53%		46	54.76%	
Cross Y2	G5 f2	G5 m2	*yeye*	*yeye*	118	88	74.58%	59.66%	17	19.32%	40.25%
Cross Y3	G5 f3	G5 m3	*yeye*	*yeye*	64	30	46.88%		14	46.67%	
Cross wt1	wt f1	wt m1	++	++	89	60	67.42%		20	33.33%	
Cross wt2	wt f2	wt m2	++	++	119	100	84.03%		27	27.00%	38.80%
Cross wt3	wt f3	wt m3	++	++	139	66	47.48%	69.32%	37	56.06%	
Cross wt5	wt f5	wt m5	++	++	28	17	60.71%		nc		
Cross wt6	wt f6	wt m6	++	++	46	40	86.96%		nc		
Cross F2ABC	pooled	pooled	*+ye*	*+ye*	181	118	65.19%		88	74.58%	
Cross F2EFG	pooled	pooled	*+ye*	*+ye*	195	89	45.64%		60	67.42%	

Table 2. Average percentages of RGB values of specimens of yellow or wild type *Coleomegilla maculata*. Individual specimens are those shown in Figure 1. Specimens 2 and 3 of each strain are newly eclosed adults (<24 hours post adult eclosion), while specimens 4 and 5 are mature adults (2 weeks post adult eclosion). Specimen 1 is an "ideal" representative of the phenotype. Computer generated composites are shown.

	sex	% R	% G	% B	composite
Yellow Specimen 1	unknown	41.62	34.79	23.59	
Yellow Specimen 2	female	37.08	35.88	27.03	
Yellow Specimen 3	male	38.05	36.08	25.85	
Yellow Specimen 4	female	40.48	35.83	23.65	
Yellow Specimen 5	male	39.50	36.08	24.40	
WT Specimen 1	unknown	59.11	26.28	14.62	
WT Specimen 2	female	45.68	33.88	20.48	
WT Specimen 3	male	49.00	32.25	18.80	
WT Specimen 4	female	53.65	28.90	17.45	
WT Specimen 5	male	56.85	27.78	15.40	

egg production. Extensive molecular genetic and biochemical characterization of the yellow family of genes found in the red flour beetle, *Tribolium castaneum*, identified genes and proteins associated with the elytra, but not with eye color. One phenotype of *T. castaneum* appears visually similar to the mutation described here, *glossy*, but has not been characterized [7]. Correlation of elytra coloration with levels of toxic defensive compounds has been demonstrated in *H. axiridis* [8] [9] and *Coccinella septempunctata* [10]. Defensive chemistry of

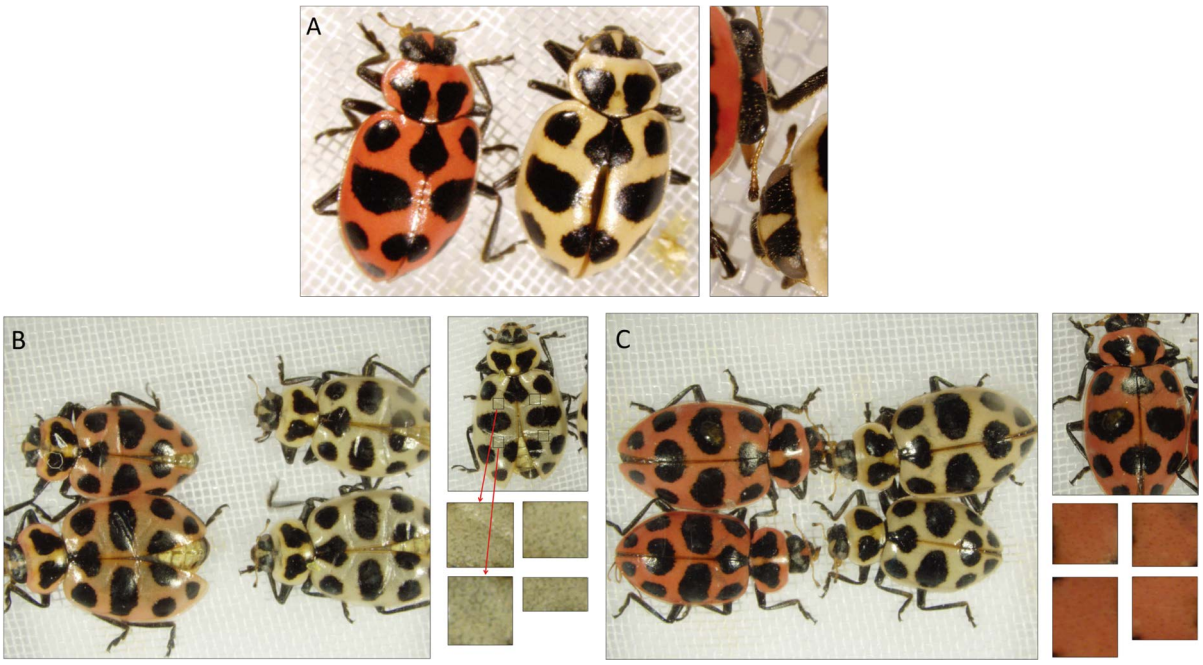

Figure 1. Phenotypes of adult wild type and mutant (*ye*) specimens of *Coleomegilla maculata*. (A) Random representative specimens, dorsal view. Left: whole insects; right: close up of heads, showing pale eyes of mutant adult. (B) Newly eclosed (teneral, <24 hours post ecdysis) adults with incomplete pigment deposition and sclerotization (hardening) of elytra. Note bending of soft elytra under the weight of a glass cover slip. (C) Mature adults. (B) and (C) show male (smaller) and female specimens. To the right, subsections of the pigmented elytra sampled for comparative image analysis are shown. Boxes and arrows (B) represent the selections for color samples.

Figure 2. Mature embryos and neonates of wild type and mutant (*ye*) specimens. Upper images are wild type, lower are mutants. Arrows indicated stemmata, or developing eye-spots.

coccinellids is of interest to organic chemists and evolutionary biologists.

Elytra color may be involved with the modulation of body temperature. Color forms of *Adalia bipunctata* with dark elytra are partially responsible for climate tolerance and associated increase in activity influences mate choice in some populations [11]. Color patterns in insect wings are often the product of the co-option of developmental pathways, as exemplified by *Heliconius* butterflies [12]. Coloration in lady beetles is associated with a range of physiological and ecological traits of interest to biocontrol workers. Transcriptome analyses will shed more light on the relationship between the genetic processes responsible for elytra coloration, and the aforementioned traits in lady beetles. The *ye* strain will be useful for molecular genetic and biochemical studies of pigments, defensive mechanisms, and evolution of gene regulatory networks. Many studies of *C. maculata* and related Coccinellids will be facilitated by this unique strain of beetles. Better understanding of this important beneficial insect will help farmers and land managers conserve beneficial insects and reduce the need for pesticide applications.

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