Advances in Entomology, 2016, 4, 11-18
Published Online January 2016 in SciRes. http://www.scirp.org/journal/ae
http://dx.doi.org/10.4236/ae.2016.41002

Gold: A Unique Pigmentation Defective Laboratory Strain of the Lady Beetle

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Received 2 November 2015; accepted 8 January 2016; published 11 January 2016

Abstract

A laboratory colony of *Coleomegilla maculata* (De Geer) was selected for a novel phenotypic color trait. The phenotype was paler in color than the wild type, although not as pale as a previously described mutant strain, yellow (*ye*), and retained dark pigmentation in the eyes. This selected strain was named gold. Mendelian breeding experiments indicate a recessive biallelic inheritance. The strain has decreased fitness characteristics based on measurements of egg production and pupa size.

Keywords

Lady Beetle, Recessive Phenotype, Reciprocal Cross, Inbreeding Depression, Aposematism

1. Introduction

Aposematic coloration is an important evolutionary development for many insect species. Many species of lady beetles (Coleoptera: Coccinellidae) have bright aposematic coloration and patterning, and produce noxious chemical defenses. The species *Coleomegilla maculata* (De Geer) (Coleoptera: Coccinellidae) is commonly called the pink lady beetle or the twelve-spotted lady beetle. It is a beneficial omnivorous insect ubiquitous in North American agroecosystems. The name "pink lady beetle" describes the typical color hues attributed to *C. maculata*: dark spots on a lighter background that is pink to red or orange.

While numerous color and pattern variations in other species of coccinellids have been described, and the inheritance of those patterns have been analyzed [1], *C. maculata* does not exhibit dramatic polymorphism in wild populations, and therefore it has not been used as a model for phenotype inheritance. However, at the US Department of Agriculture National Biological Control Laboratory in Stoneville, MS, colonies of *C. maculata* were kept in continuous culture and inbred for over forty generations since 2009 to facilitate genetics and biological

control research. One result of inbreeding has been the discovery and selection of novel phenotypes unique to these inbred laboratory strains.

A stable homozygous strain of pale eyed beetles with pale yellow coloration in the cuticle, *ye*, was described [2], followed by description of a novel spotting pattern [3]. An additional phenotype appearing nearly as pale in the colored portion of the cuticle, but maintaining dark eye coloration similar to the wild type, was observed and selected for over 30 generations. The selected stable strain was named gold. Breeding the phenotype to stability took many years, because the inbred colonies exhibited apparent inbreeding depression characterized by decreased size, fertility, and fecundity. To evaluate the apparent decrease in fitness and reproductive characteristics, selected bionomic data were collected and compared to wild type and *ye* strains of *C. maculata*. Digital color signatures of the pigmentation phenotypes were analyzed. Classical Mendelian crossing experiments using the *ye* strain were performed to determine the heritability of the color trait.

2. Methods

During routine maintenance of laboratory colonies of *C. maculata*, individual specimens with pale cuticle colors were isolated and then selected over many generations (>40) until a strain that bred true for the pale yellow to brown colors in the adult cuticle was established. The characteristic color, primarily in the adult elytra, was yellow in teneral adults and darkened to a golden brown in mature adults. Eye color in the strain was dark like the wild type. The strain was named gold because the color of the mature adults was darker than those of the *ye* strain, but still of a yellow hue. The gold strain was analyzed using classical Mendelian breeding and documented by digital image collection. Insects were maintained as previously described [2] [4]. Individual insects were isolated for reciprocal strain crosses, and F1 crosses were pooled. Individual F1 specimens were bred back to individuals from the gold strain to verify the inheritance pattern of the phenotype. For female F1 insects, larger, and thus more likely to be female, teneral adults were isolated. The isolated adults were allowed to age to reproductive maturity, and those that laid eggs were placed overnight within the gold colony cage. Afterward the individuals were kept isolated in small dishes (100 mm D × 26 mm h) with fine mesh glued into the lid to permit ventilation. Individuals that produced viable eggs were maintained and eggs were collected daily. For male F1 backcrosses, teneral gold females were isolated and allowed to age and produce eggs to verify sex, then paired with F1 males, identified *in copula* from the pooled F1 mating cages. After mating was observed between F1 males and gold females, males were removed to prevent egg cannibalism.

Digital images were collected using a Nikon digital camera, DMX 1200, with factory supplied ACT-1 software. The camera was mounted on a Nikon Stereomicroscope SMZ1500 (Nikon Corporation, Tokyo, Japan) with aperture fully closed to provide maximum depth of field.

Shutter speeds varied from 1/20 to 1/100 sec depending on magnification. Subjects were illuminated from two opposing sides to provide consistent color readings. Specimens were rotated to collect images that could be sampled for color analysis. Sections of cuticle that were neither in shadow nor reflecting from the light source were selected for color samples. 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 [5]. Four sections of each sampled individual whole insect were used for color analysis. Color samples were cropped from colored areas between the melanized spots on the elytra. Isolated right elytra were sampled from six mature (approximately 14 days post adult ecdysis) individuals of each strain and used for further color analysis. Color samples from individual elytra were sampled from two central colored areas between melanized spots.

To estimate strain fecundity, the number of eggs in egg masses were counted as harvested from mature strain oviposition cages. Live pupae were weighed individually using a Sartorius CP2P-F analytical balance. Data were analyzed by analysis of variance (ANOVA) using SigmaPlot, version 12 software (SSI, San Jose, CA, USA).

3. Results

Representative mature adult specimens of the yellow (*ye*), gold (gold), and wild type strains are shown in **Figure 1**. Based on the hypothesis that the gold phenotype was based on an allele or multiple alleles independent from the *ye* allele, the offspring of gold × *ye* and *ye* × gold parents would inherit wild type alleles from each parent and result in 100% pink offspring (wild type), assuming that the wild type allele was dominant to all paler phenotype alleles. These F1 offspring should carry alleles that would result in some proportion of phenotype recovery

Figure 1. Mature representative specimens of laboratory selected color strain phenotypes of *Coleomegilla maculata*.

in the F2 offspring when sibs of the F1 were mated. If a single recessive locus conferred the gold phenotype, 25% of the F2 offspring would have the recessive trait. If two recessive loci were responsible for the phenotype, 6.25% of the F2 offspring would segregate to display the trait. Additionally, if the F1 heterozygous offspring of the phenotype controlled by two recessive loci were mated to the homozygous recessive parental strain, 25% of the offspring would be expected to display the double recessive phenotype. As shown in **Table 1**, the F1 offspring of the gold × *ye* and *ye* × gold parents were consistently pink, conforming to the wild type phenotype. When these F1 offspring were crossed to one another, and to the parental gold strain (**Table 1(b)** and **Table 1(c)**), the expected ratios for a double recessive trait were recovered.

Empirical observations during the selection process of the gold strain indicated that the gold females laid smaller egg masses. To validate the observations, eggs of the egg masses collected from strain cages were routinely counted, and the count per mass data were analyzed by one way analysis of variance, and the means were significantly different (F (2254) = 39.615, $P < 0.001$). Egg masses collected from the ovipositing colony cages differed significantly in size (mean number of eggs/mass). Wild type egg masses contained 17.2 ± 5.7 eggs, *ye* egg masses contained 16.7 ± 4.8 eggs, and gold egg masses contained 11.5 ± 4.3 eggs. Means were compared using Holm-Sidak multiple all pairwise method, and while wild type and *ye* means did not differ (t = 0.613, P = 0.540), the gold mean eggs/mass were significantly different from both (t = 7.603, 7.322 respectively, $P < 0.001$ for both). These data are represented graphically in **Figure 2(b)**. The mass of pupae from the three strains, wild type, *ye*, and gold, and the offspring of the reciprocal crosses were compared (**Figure 2(a)**) and while the mean mass of the individuals from the gold strain were found to be significantly smaller than those of the wild type and *ye* strains (F (4114) = 4.023, P = 0.004), the mean mass of pupa of wild type and *ye* strains did not differ from each other (P = 0.521). These latter data contradict results from earlier work [3], but the pupae sampled for the present analysis were fewer in number and the means from the previous analysis resemble the data here: wild type average mass (mg) 17.0 vs. 16.4 and *ye* average mass 15.4 vs. 16.2 respectively. The pupae of the gold strain differed significantly from the parent strains at a mean of 14.6 mg; pupae of the offspring of gold and *ye* parents had means of pupa mass intermediate between the parents (**Figure 2(a)**).

Digital images of representative teneral adult specimens of the wild type, yellow (*ye*), and gold strains are shown in **Figure 3(a)** and **Figure 3(b)**. The young adults, within three days post imaginal ecdysis, had paler pigmentation in the cuticle which darkened with age. The wild type pigmentation, ranging from pale to deep pink or red, as shown in **Figure 1(a)** and **Figure 3(a)**, achieved full color clarity after a week or more of maturation. After death, and depending on the method of specimen preservation, these pigments darkened further so that the colors of the living insects could not be judged by preserved specimens. Thus specimens selected for phenotypic strain selection were monitored closely and repeatedly over time to segregate pale wild type insects

Table 1. (a)-(c) Crossing experiments to determine heritability of gold phenotype. Abbreviations: G = gold, Ye = ye (yellow), m = male, f = female, WT = wild type, F0 = parental generation, F1 = first filial generation. (a) Parental crosses of individuals from gold and ye colonies (virgin females). (b) Crosses of heterozygous sibling offspring (F1) from reciprocal crosses in **Table 1(a)**. (c) Crossing experiments using first generation (F1) individuals paired with gold individuals (virgin females).

(a)

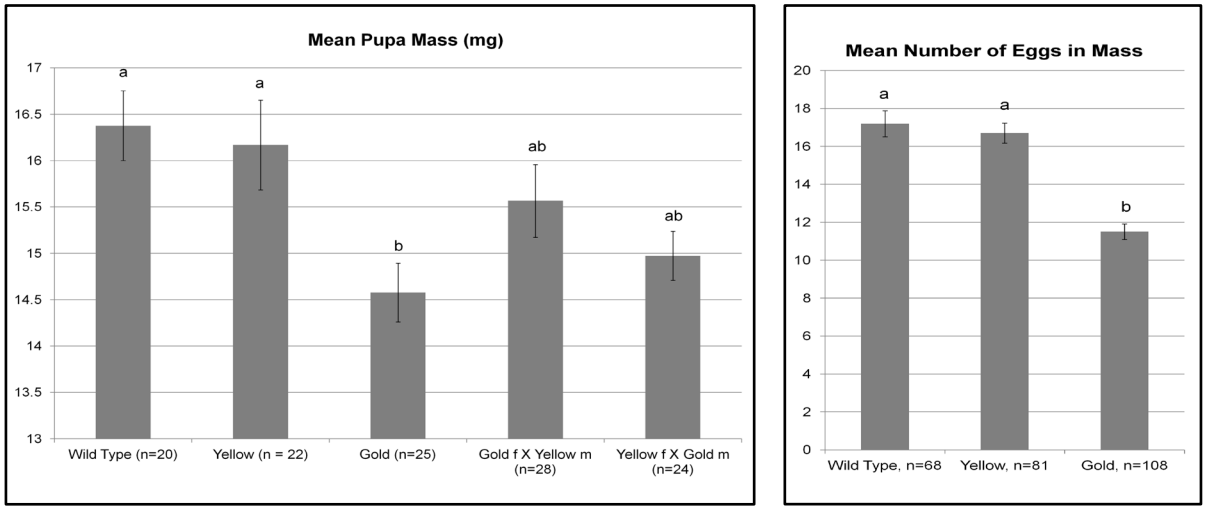
		Adult Phenotype (individuals)			Total	Expected Ratio Gold:Total	Actual Ratio Gold:Total	Chi Square
F0 Pairs	Specific ID	Ye	Gold	Pink (WT)	Adults			*P* value
G m X Ye f	1	0	0	24	24	0:All	0:24	n/a
G m X Ye f	2	0	0	19	19	0:All	0:19	n/a
G m X Ye f	3	0	0	21	21	0:All	0:21	n/a
G m X Ye f	4	0	0	27	27	0:All	0:27	n/a
Ye m X G f	1	0	0	17	17	0:All	0:17	n/a
Ye m X G f	2	0	0	18	18	0:All	0:18	n/a
Ye m X G f	3	0	0	13	13	0:All	0:13	n/a
Ye m X G f	4	0	0	11	11	0:All	0:11	n/a
Ye m X G f	5	0	0	14	14	0:All	0:14	n/a
							Combined Chi Square *P* value:	1.00

(b)

	F1 Pool	Adult Phenotype (individuals)			Total	Expected Ratio Gold:Total	Actual Ratio Gold:Total	Chi Square
F0 Parents	Specific ID	Ye	Gold	Pink (WT)	Adults			*P* value
G m X Ye f	m1	84	23	234	341	1:16 (0.0625)	0.0674	0.2861
G m X Ye f	m2	65	8	220	293	1:16 (0.0625)	0.0273	0.7850
G m X Ye f	m3	22	3	67	92	1:16 (0.0625)	0.0326	0.7587
G m X Ye f	m4	28	2	80	110	1:16 (0.0625)	0.0182	0.5627
Ye m X G f	f1	14	5	50	69	1:16 (0.0625)	0.0725	0.4215
Ye m X G f	f2	41	7	104	152	1:16 (0.0625)	0.0461	0.4288
Ye m X G f	f3	15	3	32	50	1:16 (0.0625)	0.0600	0.6249
Ye m X G f	f4	13	4	34	51	1:16 (0.0625)	0.0784	0.6003
Ye m X G f	f5	14	4	58	76	1:16 (0.0625)	0.0526	0.6994
							Combined Chi Square *P* value:	0.1598

(c)

Individual		Adult Phenotype (individuals)			Total	Expected Ratio Gold:Total	Actual Ratio Gold:Total	Chi Square
Back Cross	Specific ID	Ye	Gold	Pink (WT)	Adults			*P* value
F1f2 m X G f	HB3F	5	9	25	39	1:4 (0.25)	0.2308	0.6003
G m X F1m1 f	HB1	0	37	115	152	1:4 (0.25)	0.2434	0.9359
G m X F1m2 f	HB2	0	33	98	131	1:4 (0.25)	0.2519	0.8334
G m X F1f2 f	HB3A	28	16	62	106	1:4 (0.25)	0.1509	0.4272
G m X F1f2 f	HB3B	30	17	47	94	1:4 (0.25)	0.1809	0.2359
G m X F1f2 f	HB3C	14	13	33	60	1:4 (0.25)	0.2167	0.4685
							Combined Chi Square *P* value:	0.2771



(a) (b)

Figure 2. Comparison of fitness bionomics associated with strains of laboratory selected *Coleomegilla maculata*. Columns depict means with ±standard error bars. Means labeled with the same letter are not significantly different. (a) Mean mass of pupae sampled from established strains yellow (*ye*), gold (g), wild type, and from reciprocal cross offspring. The x-axis indicates the strain or cross, and the number of specimens sampled and analyzed; the y-axis is the mean mass in mg. (b) Mean number of eggs per egg mass collected from colony cages. The x-axis indicates the strain or cross, and the number of masses sampled and analyzed; the y-axis is the mean number of eggs per mass.

(a) (b)

Strain		Whole Adults	Whole Insect color	Saturated Whole Insect color (Red = 255)	Isolated Wings	Isolated Wing color	Saturated Isolated Wing color (Red = 255)
Wild Type	r	56.196±2.399			58.417±1.278		
	g	28.300±0.159			29.250±0.429		
	b	15.513±1.622			12.333±0.955		
Yellow	r	38.270±0.333			39.842±0.159		
	g	35.320±0.085			35.725±.0783		
	b	26.405±0.365			24.433±0.171		
Gold	r	46.575±0.465*			41.642±0.273*		
	g	35.220±0.368			33.992±0.109*		
	b	18.195±0.506*†			24.333±0.260		

*Gold mean is significantly different from Yellow as well as Wild Type; †Gold mean is not significantly different from Wild Type ($P = 0.251$). All other means of color percent readings of Gold and Yellow strains differ significantly from Wild Type. r = red, g = green, b = blue

(c)

Figure 3. Laboratory selected color strain phenotypes of *Coleomegilla maculata*. (a) Offspring from mating parental gold male with F1 female from gold male × yellow (*ye*) female cross. Adults are aged less than 48 hours post adult ecdysis, and have not achieved final colors. Clockwise from upper left: yellow (*ye* strain phenotype), note pale eyes and elytra; wild type pink or red; gold (gold strain phenotype), note dark eyes with pale elytra; pale wild type, note dark eyes and pink hued elytra. (b) Wing samples from mature adults used for color analysis: top, wild type; center, yellow (*ye*); lower, gold. (c) Mean color percentages of mature samples, and digital representations. Saturated indicates that the red (dominant) color value in the digital representation was set to full saturation (255) and other colors were adjusted proportionally based on the measured mean percents.

from the gold phenotype.

The representative strain color samples and mean percent color components are shown in **Figure 3(c)**. The empirically visible characteristics were supported by color analysis; all color components of gold pigmentation differed significantly from wild type (Holmes-Sidak, $P < 0.050$), with the exception of the blue value in the whole insect samples, which only accounted for the minority of the color, less than 20%.

4. Discussion

Aposematism in lady beetles is often characterized by red to yellow pigments in the adult cuticle, and the production of unpleasant warning odors and taste, and toxic alkaloid defensive compounds. These aposematic signals: color, odor, and toxicity, have been studied and shown to be correlated. In one species of lady beetle, *Hippodamia convergens*, with a natural range of elytra cuticle coloration from saturated red to translucent yellow-orange, insects of both sexes with redder coloration possessed significantly higher concentrations of defensive alkaloid toxins.

Red females of this species contained double the concentration of alkaloids compared to males. However, the warning odor compound concentrations were highest in red males and lowest in red females, with an overall negative correlation between toxin and odor compounds [6]. A study of multiple species of lady beetles demonstrated that, in general, red pigmentation was more likely to correlate with high toxicity compared with more yellow-hued colors [7]. Bright, conspicuous colors and patterns deter predation; this is true not only for vertebrate insectivores but for predatory invertebrates such as spiders [8]. Studies of aposematism have not previously had genetic models with which to evaluate signal honesty, and the availability of selected and genetically stable strains with clear variations in color provides a new avenue for future research.

Laboratory inbreeding of organisms frequently leads to decreases in fitness characteristics. The gold strain was difficult to rise to consistent phenotypic stability. Not only were the egg masses and adults (as measured by pupa mass) of the gold strain significantly smaller as reported herein, but the hatch rate of the strain appeared very low, the lifespan appeared shorter, and the individuals of all stages seemed more fragile than other strains. These empirical observations could not be effectively measured because of the fragility of the gold insects; handling them resulted in unacceptable mortality. The pupae of the gold strain were noticeably as well as significantly smaller than the parent strains at a mean of 14.6 mg; pupae of the offspring of gold and *ye* parents had mean pupa masses intermediate between the parents, indicating that the size difference was inherited along with the color trait. Interestingly, other selected *C. maculata* laboratory strains with variations in pigmentation [2] and pattern (Allen, accepted) suffered little from inbreeding. Variation in fitness characteristics in mutant phenotypes is expected; for example, melanistic mutants of the Colorado potato beetle, *Leptinotarsa decimlineata*, showed decreased growth and survival while a white mutant retained fitness [9].

Coleomegilla maculata has several features that endorse its use as a genetic model organism. It is common in its native habitat, and is therefore relatively easy to locate and collect. Rearing of *C. maculata* is possible on the laboratory scale [4] and interest in expanding to commercial scales is increasing, especially since this lady beetle has been demonstrated as an effective biological control agent [10]. Its bright contrasting color pattern is visually appealing, its reputation as a beneficial makes the species unthreatening to humans, and it has a relatively rapid reproductive rate. For molecular genetics, it has a small genome, and a pair of transcriptomes of the adult life stage have been sequenced [11]. The *ye*, 10 *sp*, and gold strains of *C. maculata* do not appear to arise in natural populations, but arose spontaneously during laboratory inbreeding. The combination of multiple, complementary phenotypic laboratory strains plus increasing genetic sequence availability provide valuable scientific resources for studying complex and fascinating interactive relationships of organisms with the environment, with each other, and in trophic relationships with other organisms.

Genes involved in metabolism of carotenes are not well studied in insects, especially outside of the visual function. Recently, a gene encoding carotene synthesis was identified in the pea aphid [12], and using sequence comparison to that gene a similar gene was discovered in two-spotted spider mite, *Tetranychus urticae* [13]. The genes encoding the biosynthetic or transport pathway or pathways for coccinellid pigments are completely unknown. Coccinellid pigments are reportedly carotenoids, are present in multiple forms, and may be derived from dietary compounds obtained from non-plant sources, possibly associated with fungal diet or symbionts [14]. Future studies of coccinellid pigments should include chemical or physical extraction and isolation methods and molecular biological methods such as gene disruption by RNAi to simulate mutant phenotypes, similar to the

experiments performed with *Tribolium castaneum* [15] and *Oncopeltus fasciatus* [16].

Acknowledgements

An earlier version of this manuscript was reviewed by William Rodney Cooper, Louis S. Hesler, and Michael Grodowitz, and the comments and suggestions provided helpful improvement. The author thanks Mary Elizabeth Huddleston, Joseph Grey Ballenger, and Morgan Holmes for technical support and assistance with insect maintenance. The United States Government has the right to retain a non-exclusive, royalty-free license in and to any copyright of this article. This article reports the results of research only. Mention of a commercial or proprietary product does not constitute an endorsement of the product by the United States Department of Agriculture. USDA is an equal opportunity provider and employer.

Competing Interests

The author claims no competing interests.

Authors' Contributions

Margaret L. Allen designed and analyzed all experiments and data.

References

[1] Sloggett, J.J. and Honek, A. (2012) Genetic Studies. In: Hodek, I., van Emden, H.F. and Honek, A., Eds., *Ecology and Behaviour of the Ladybird Beetles* (*Coccinellidae*), Wiley-Blackwell, Oxford, 561 p. http://dx.doi.org/10.1002/9781118223208.ch2

[2] Allen, M.L. and Ballenger, J.G. (2014) Genetics and Characteristics of a Pigmentation Defective Laboratory Strain of the Lady Beetle, *Coleomegilla maculata*. *Advances in Entomology*, **2**, 161-166. http://dx.doi.org/10.4236/ae.2014.24024

[3] Allen, M.L. (2015) Characteristics of a Laboratory Strain of *Coleomegilla maculata* with a Novel Heritable Wing Spot Pattern Trait. *Advances in Entomology*.

[4] Allen, M.L. and Riddick, E.W. (2012) A System for Harvesting Eggs of the Pink-Spotted Lady Beetle. *Psyche*, **2012**, 1-6. http://dx.doi.org/10.1155/2012/923653

[5] Byers, J.A. (2006) Analysis of Insect and Plant Colors in Digital Images Using Java Software on the Internet. *Annals of the Entomological Society of America*, **99**, 865-874. http://dx.doi.org/10.1603/0013-8746(2006)99[865:AOIAPC]2.0.CO;2

[6] Wheeler, C., Millar, J. and Cardé, R. (2015) Multimodal Signal Interactions in the Ladybeetle, *Hippodamia convergens*, Aposematic System. *Chemoecology*, **25**, 123-133. http://dx.doi.org/10.1007/s00049-014-0181-2

[7] Arenas, L.M., Walter, D. and Stevens, M. (2015) Signal Honesty and Predation Risk among a Closely Related Group of Aposematic Species. *Scientific Reports*, **5**.

[8] Taylor, L.A., Maier, E.B., Byrne, K.J., Amin, Z. and Morehouse, N.I. (2014) Colour Use by Tiny Predators: Jumping Spiders Show Colour Biases during Foraging. *Animal Behaviour*, **90**, 149-157. http://dx.doi.org/10.1016/j.anbehav.2014.01.025

[9] Boiteau, G. (1985) Bionomics and Genetics of a Black Mutant Colorado Potato Beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Annals of the Entomological Society of America*, **78**, 663-666. http://dx.doi.org/10.1093/aesa/78.5.663

[10] Allen, M.L. (2015) Greenhouse Evaluation of Neonate and Adult Applications of *Coleomegilla maculata* (Coleoptera: Coccinellidae) to Control Two Spotted Spider Mite Infestations. *Florida Entomologist*, **98**, 714-720. http://dx.doi.org/10.1653/024.098.0247

[11] Allen, M.L. (2015) Characterization of Adult Transcriptomes from the Omnivorous Lady Beetle *Coleomegilla maculata* Fed Pollen or Insect Egg Diet. *Journal of Genomics*, **3**, 20-28. http://dx.doi.org/10.7150/jgen.10385

[12] Moran, N.A. and Jarvik, T. (2010) Lateral Transfer of Genes from Fungi Underlies Carotenoid Production in Aphids. *Science*, **328**, 624-627. http://dx.doi.org/10.1126/science.1187113

[13] Altincicek, B., Kovacs, J.L. and Gerardo, N.M. (2012) Horizontally Transferred Fungal Carotenoid Genes in the Two-Spotted Spider Mite *Tetranychus urticae*. *Biology Letters*, **8**, 253-257. http://dx.doi.org/10.1098/rsbl.2011.0704

[14] Britton, G., Lockley, W.J.S., Harriman, G.A. and Goodwin, T.W. (1977) Pigmentation of the Ladybird Beetle *Cocci-*

nella septempunctata by Carotenoids Not of Plant Origin. *Nature*, **266**, 49-50. http://dx.doi.org/10.1038/266049a0

[15] Arakane, Y., Dittmer, N.T., Tomoyasu, Y., Kramer, K.J., Muthukrishnan, S., Beeman, R.W. and Kanost, M.R. (2010) Identification, mRNA Expression and Functional Analysis of Several Yellow Family Genes in *Tribolium castaneum*. *Insect Biochemistry and Molecular Biology*, **40**, 259-266. http://dx.doi.org/10.1016/j.ibmb.2010.01.012

[16] Liu, J., Lemonds, T.R. and Popadić, A. (2014) The Genetic Control of Aposematic Black Pigmentation in Hemimetabolous Insects: Insights from *Oncopeltus fasciatus*. *Evolution & Development*, **16**, 270-277. http://dx.doi.org/10.1111/ede.12090

List of Abbreviations

g = gold (strain);
wt = wild type;
f = female;
m = male;
ye = yellow eyes and elytra (strain);
F (0,1,2) = filial generation;
avg = average.