Characteristics of a Laboratory Strain of Coleomegilla maculata with a Novel Heritable Wing Spot Pattern Trait

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Abstract

The lady beetle Coleomegilla maculata is a common New World insect that is naturally colored pink to red or orange with black spots on the forewings of the adult stage. Previous laboratory inbreeding resulted in selection for a strain lacking red pigment in the cuticle and eyes. An additional strain selected for a novel spotting pattern is described here. The inheritance of the new trait, “ten spotted” (10sp), was determined by classical crossing experiments. Inheritance of the trait was autosomal and exhibited incomplete dominance. Bionomic strain measurements were compared to the parental strains and were similar overall. Two expressed sequences from C. maculata that may be related to the new phenotype were compared to model insect genes encoding a melanin biosynthesis enzyme and a patterning transcription factor.

Keywords

Coccinellidae, Mutant Phenotype, Melanin, Ebony, Wing Pattern

1. Introduction

Many beetles in the family Coccinellidae are identified by the number and pattern of spots appearing on the forewings, or elytra. Both the common and scientific names of many species describe the numbers of spots; for example, Adalia bipunctata (Linnaeus) is commonly called the two-spotted lady beetle, Coccinella septempunctata Linnaeus is the seven-spotted lady beetle, and Coccinella novemnotata Herbst is the nine-spotted lady beetle. The species Coleomegilla maculata (DeGeer) (Coleoptera: Coccinellidae) is commonly called the pink lady beetle or the twelve-spotted lady beetle. It is a beneficial omnivore found in US agroecosystems and occurs
throughout much of the North and South American continents. The name “twelve-spotted lady beetle” describes the typical spot pattern on *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* is not a species that exhibits dramatic polymorphism in wild populations, and therefore it has not been used as a model for phenotype inheritance.

To facilitate genetics and biological control research, colonies of *C. maculata* were kept in continuous culture and inbred for over 64 generations since 2009. One result of inbreeding was the discovery and selection of novel phenotypes unique to laboratory strains. A stable homozygous strain of pale eyed beetles with pale yellow coloration in the cuticle, ye, was described earlier [2]. After further laboratory inbreeding, an additional phenotypic trait was identified and selected and was described here. This trait involved the pattern of spots on the elytra of the adult beetles. The pattern was an expanded dark region of the two anterior spots on each elytron such that the spots merged. This trait was labeled “ten spotted” and the strain abbreviated 10sp. The pattern was observed in both the wild type strain and in the ye strain. Phenotypically distinct individuals exhibiting both the ye and 10sp traits combined were selected, bred to stability, and crossed back to the parental twelve spotted phenotype to determine the heritability of the trait. To evaluate the effects of the trait on overall fitness of the insects, selected biometric measurements of the ye/10sp strain were compared to those of the wild type and the ye laboratory strains. Two sequences that were predicted as potential candidate genes contributing to the phenotype were identified, and compared with genes from model insects.

2. Methods

The ye/10sp strain was analyzed using classical Mendelian breeding and documented by digital image collection. Insects were maintained as previously described [2] [3]. Individual insects were isolated for reciprocal strain crosses, and putative heterozygous first generation offspring (F1) crosses were pooled. Observed phenotype data were collected and entered into a spreadsheet, and phenotypic ratios were compared to expected Mendelian ratios for a single locus incomplete dominant allele using chi-squared distribution test (Excel®, Microsoft Corporation, Redmond, WA, USA). The expected phenotype of all offspring from a homozygous 10sp parent mated to an individual with wild type twelve spotted pattern was all 10sp, but with a heterozygous moderate expression pattern. Offspring from heterozygous parents were expected to result in 75% 10sp and 25% twelve spotted wild type phenotypes; offspring from a heterozygous parent mated to a wild type individual were expected to result in half 10sp and half wild type phenotypes. 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. Objective lens was WD54 1x, oculars C-W10xA/22. Zoom was set at 0.75, 1, and 2 for various images. Shutter speeds were 1/50 to 1/75 sec. A Nikon NI-150 high intensity double gooseneck illuminator set at 75% or higher intensity illuminated the subjects from two opposing sides.

To estimate strain fecundity, the number of eggs in egg masses were counted as harvested from stable strain colony cages. A grouping of more than four eggs clustered together was considered a mass. To estimate fecundity and fertility on a finer scale, mature and apparently gravid individual females were isolated from each strain and eggs were collected and counted on a daily basis over ten consecutive days. Eggs were observed daily and the number of hatched neonates was counted. Pupae were weighed individually using a Sartorius CP2P-F analytical balance. Data were analyzed by one way analysis of variance using SigmaPlot, version 12 software (SSI, San Jose, CA USA).

As research on coccinellids advances, high-throughput sequencing is expected to play a greater role in both gene expression study and defining molecular markers [4]. A high-throughput sequencing project was undertaken utilizing total RNA extracted from highly inbred (six isofemale selection steps) specimens of *C. maculata*, resulting in two adult transcriptomes that assembled into over 33,000 sequences each [5]. Two predicted complete protein coding sequences identified from the *C. maculata* transcriptomes [5] were compared to sequences in NCBI GenBank using BLAST alignment [6]. Characterezed protein sequences from model insects were aligned with the predicted *C. maculata* sequences using DNASTARLasergene 8 MegAlign software (Madison, WI USA).

3. Results

Inheritance of the 10sp trait is autosomal and exhibits incomplete dominance. Reciprocal crosses of insects with
the trait and wild type or ye insects having the characteristic twelve spot phenotype produced offspring with expansion and merging of the anterior spots as heterozygotes, following expected Mendelian inheritance ratios (Table 1). All crosses resulted in ratios of offspring that did not differ from the expected ratios for an incomplete dominant autosomal allele (p > 0.05). The offspring of heterozygotes, when mated, produced offspring with a range of phenotypes including the normal spotting pattern, with twelve spots, and enlarged spots that were partially merged, and fully merged spots. Strain types are shown in Figure 1, with the homozygous characteristic pattern of the 10sp strain shown in Figure 1(B), and an example of a heterozygous ten spotted pattern shown in Figure 1(C). The differences between the homozygous and heterozygous patterns were subtle and for counts of the inherited trait, any variation of the trait was counted as ten spotted. Digital images of individual insects in homozygous and heterozygous form were compared by measuring the smallest region of the constriction between merged spots, the “waist”, and the widest region of the anterior spot, and using the measurements to estimate a ratio (Figure 2). The spot ratio of homozygous beetles was 0.886 ± 0.0347 (n = 13) while the ratio of heterozygous beetles was 0.542 ± 0.0819 (n = 8). These ratios were significantly different (student t-test, p < 0.001).

As represented in Figure 3, the strain ye/10sp was similar in fecundity and fertility to the laboratory wild type strain and the ye strain. Egg masses collected from the ovipositing colony cages did not differ significantly in size (F = 0.613, df = 2, p = 0.543). Because the egg masses collected from the colony cages might represent interrupted oviposition events, a second fecundity estimate was measured. Fecundity was measured by the number of eggs produced in ten days by individual gravid females from each of three strains. Individual gravid females produced quantities of eggs over a ten day period that were not significantly different (F = 3.052, df = 2, p = 0.067). However, wild type fertile egg masses had a higher hatch rate compared with the ye strain (F = 3.882, df = 2, p = 0.046), and the hatch rate was significant among the treatment groups (p = 0.039). However, the

### Table 1. Segregation of adult pattern in the progeny of crosses between 10sp and 12 spotted parents.

<table>
<thead>
<tr>
<th>Cross (female × male)</th>
<th>10 spot</th>
<th>12 spot</th>
<th>Total</th>
<th>Expected*</th>
<th>c²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong 10sp × 12 spot (Experiment 1)</td>
<td>74</td>
<td>-</td>
<td>74</td>
<td>74:0</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Strong 10sp × 12 spot (Experiment 1)</td>
<td>33</td>
<td>-</td>
<td>33</td>
<td>33:0</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Strong 10sp × 12 spot (Experiment 1)</td>
<td>63</td>
<td>-</td>
<td>63</td>
<td>63:0</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>12 spot × Strong 10sp (Experiment 1)</td>
<td>77</td>
<td>-</td>
<td>77</td>
<td>77:0</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>12 spot × Strong 10sp (Experiment 2)</td>
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<td>-</td>
<td>46</td>
<td>46:0</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>12 spot × Strong 10sp (Experiment 3)</td>
<td>54</td>
<td>-</td>
<td>54</td>
<td>54:0</td>
<td>n/a</td>
<td></td>
</tr>
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*For dominant phenotype, 100% 10sp is expected

<table>
<thead>
<tr>
<th>Cross (female × male)</th>
<th>10 spot</th>
<th>12 spot</th>
<th>Total</th>
<th>Expected*</th>
<th>c²</th>
<th>p value</th>
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<tbody>
<tr>
<td>Moderate 10sp group (Experiment 1)</td>
<td>31</td>
<td>18</td>
<td>49</td>
<td>36.75:12.25</td>
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<tr>
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<td>39.75:13.25</td>
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<tr>
<td>Moderate 10sp group (Experiment 3)</td>
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<td>0.21232</td>
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*For dominant phenotype, 100% 10sp is expected

<table>
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<th>Cross (female × male)</th>
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<th>12 spot</th>
<th>Total</th>
<th>Expected*</th>
<th>c²</th>
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<tbody>
<tr>
<td>Moderate 10sp × 12 spot (Experiment 1)</td>
<td>34</td>
<td>33</td>
<td>67</td>
<td>33.5:33.5</td>
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<td>Moderate 10sp × 12 spot (Experiment 3)</td>
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<td>27</td>
<td>55</td>
<td>27.5:27.5</td>
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*For heterozygous cross, a 3:1 ratio is expected

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<th>Total</th>
<th>Expected*</th>
<th>c²</th>
<th>p value</th>
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<tr>
<td>12 spot × Moderate 10sp (Experiment 1)</td>
<td>39</td>
<td>38</td>
<td>77</td>
<td>38.5:38.5</td>
<td>0.84727</td>
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</tr>
<tr>
<td>12 spot × Moderate 10sp (Experiment 2)</td>
<td>29</td>
<td>27</td>
<td>56</td>
<td>28:28</td>
<td>0.73160</td>
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</tr>
<tr>
<td>12 spot × Moderate 10sp (Experiment 3)</td>
<td>27</td>
<td>30</td>
<td>57</td>
<td>28.5:28.5</td>
<td>0.66618</td>
<td></td>
</tr>
</tbody>
</table>

*For heterozygous to wild type cross, a 1:1 ratio is expected

<table>
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<th>Cross (female × male)</th>
<th>10 spot</th>
<th>12 spot</th>
<th>Total</th>
<th>Expected*</th>
<th>c²</th>
<th>p value</th>
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<td>30</td>
<td>57</td>
<td>28.5:28.5</td>
<td>0.66618</td>
<td></td>
</tr>
</tbody>
</table>

*For heterozygous to wild type cross, a 1:1 ratio is expected
difference between the ye/10sp and ye rate was not statistically significant. The mass of pupae from the three strains were compared and while the mean mass of the individuals from the ye strain were found to be significantly smaller than those of the wild type strain ($F = 10.172, df = 2, p < 0.001$), the difference was small (<10%) and not visually obvious.

Sequences involved in melanin biosynthesis (predicted N-β alanlyldopamine synthase, ebony) and patterning (predicted transcription factor bric a brac) were identified from C. maculata transcriptomes [5]. Predicted translations of the sequences were very similar to those from the red flour beetle, Tribolium castaneum, and somewhat similar to genes from the fly Drosophila melanogaster, both genetically robust model organisms. Table 2 summarizes the similarities by alignment of the C. maculata sequences to genetics model insects. Translated sequences and ClustalW alignments are included as Supplementary Figures S1-S4.

4. Discussion

The dark colors of insects are often based on pigments in the cuticle. One of the best known dark pigment
Figure 3. Bionomic measurements of colony strain fitness. Columns depict means with ± standard error bars. Means labeled with the same letter, or unlabeled columns are not significantly different. Upper left: mean number of eggs per egg mass collected from colonies of a stable homozygous strain of 10 spotted yellow phenotype of *Coleomegilla maculata* (*ye/10sp*), a stable homozygous strain of twelve (wild type) spotted yellow phenotype of *C. maculata* (*ye*), and a wild type strain (pink with twelve spots) of *C. maculata* (*wt*). Upper right, mean number of eggs per individual isolated gravid female from a stable homozygous strain of 10 spotted yellow phenotype of *C. maculata* (*ye/10sp*), a stable homozygous strain of twelve (wild type) spotted yellow phenotype of *C. maculata* (*ye*), and a wild type strain (pink with twelve spots) of *C. maculata* (*wt*). Lower left: mean ratio of hatched eggs to unhatched eggs from masses collected from the three strains: *ye/10sp*, *ye*, and *wt*. Lower right: mean mass of individual pupae (mg) collected from the three strains: *ye/10sp*, *ye*, and *wt*.

compounds is melanin, a derivative of the amino acid tyrosine. Melanization in insects is influenced by multiple genes at multiple loci. In *Drosophila melanogaster* pigment metabolism involves tyrosine hydroxylase, encoded by the gene *pale*; phenol oxidases and dopa decarboxylase; genes in the *Yellow* family; the genes *black* and *tan*; and N-β-alanyldopamine synthase, encoded by the gene *ebony*. In *Bicyclus anynana* butterflies, different mutations implicating an entirely different enzyme, cysteine sulfenic acid decarboxylase, produce melanin mutants in the larva and adult forms of the insect [7]. The gene *ebony* has been described in the beetle *Tribolium castaneum*, and when it is disrupted the resulting phenotype is uniformly dark [8]. Melanic forms of insects, or “dark morphs” may be adapted to resist diseases. For example, a dark form of the wax moth, *Galleria mellonella*, had a thicker cuticle and was resistant to infection by the entomopathogen *Beauveria bassiana*, a fungus used commercially for biological control of insect pests [9]. Melanin in insects is also associated with temperature modulation, as demonstrated in the lady beetle *Adalia bipunctata*. Forms with dark elytra may be better adapted for climate tolerance, particularly in cold conditions, and an associated increase in activity may influences mate choice in some populations [10]. Recent research on the model organism *Drosophila melanogaster* indicates that dark pigmentation patterning is associated with resistance to ultraviolet radiation exposure, and melanin forms are most closely correlated with high levels of exposure [11]. Toxic defensive compounds and elytra coloration are correlated in *Harmonia axyridis* [16] and *Coccinella septempunctata* [17]; whether this is also true for *C. maculata* is a topic for further research. Both the *ye* and *ye/10sp* insects produce reflex hemolymph leakage accompanied by a distinctively unpleasant odor when disturbed (empirical observation), but variation between the
phenotypic strains has not yet been examined or analyzed.

Patterning in insects may be regulated by transcription factors such as *optomotor-blind* (*omb*) or *bric a brac* (*bab*) [11]. Color patterns in wings of butterflies are complex, and may be the product of the co-option of developmental pathways, as exemplified by the eye development gene *optix* which is correlated with wing patterns of *Heliconius* butterflies [12]. The genes involved in color patterning in beetles have not yet been discovered.

The ladybird beetle *C. maculata* has appealing characteristics for use as a genetic model organism. It is relatively easy to find and maintain in culture, it is visually appealing and unthreatening, and 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 [5]. The species exhibits high genetic polymorphism across its wide geographic distribution [13], and laboratory cultures kept for gene sequencing are more suitable for sample submission when inbred through multiple isofemale selections to increase homozygosity. Naturally occurring mutant strains of other insect species, and of course in domesticated vertebrates, have proven extremely valuable in genetic discovery. The ye and 10sp strains of *C. maculata* do not appear to arise in natural populations, but arose spontaneously during laboratory inbreeding. Recently, a visible pigmentation marker was constructed based on the *ebony* gene from the silk moth *Bombyx mori* for use in insect transgenesis [14]. The gene sequences presented herein could be used for similar biotechnology applications research. Coloration in lady beetles is indicative of a wide range physiological and ecological traits of interest. The combination of distinctive phenotypic laboratory strains and 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.

Comparison of two sequences that are similar to the genes *ebony* and *bric-a-brac* from the *C. maculata* transcriptomes (Supplementary Figures S1-S4) with sequences from the model insects *D. melanogaster*, *B. mori*, and *T. castaneum* (Table 2) indicate that similar genes involved in melanization and patterning are present in *C.*

<table>
<thead>
<tr>
<th>Table 2. Comparisons of predicted ebony and bric-a-brac sequences from transcriptomes to model insects in GenBank.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Comparable to Coleomegilla maculata predicted ebony</strong></td>
</tr>
<tr>
<td><strong>Nucleotide sequence</strong></td>
</tr>
<tr>
<td>Predicted: XM_008199683.1 ebony</td>
</tr>
<tr>
<td>NM_079707.4 ebony</td>
</tr>
<tr>
<td>NM_001145321.1 Ebony</td>
</tr>
<tr>
<td><strong>Similarity Scores</strong></td>
</tr>
<tr>
<td>Predicted: XP_008197905.1 a-aec 19</td>
</tr>
<tr>
<td>NP_524431.2 Ebony</td>
</tr>
<tr>
<td>NP_001138793.1 Ebony</td>
</tr>
</tbody>
</table>

| **B. Comparable to Coleomegilla maculata predicted bric-a-brac** | **Similarity Scores** |
| **Nucleotide sequence** | **Length** | **Species** | **Bit score** | **Expect** | **Identities** | **% identities** | **Gaps** |
| Predicted: XM_008200884.1 bric-a-brac | 1352 | *Tribolium castaneum* | 457 | 1.00E−76 | 243/304 | 80% | 0/304 |
| NM_163881.2 mdg4 | 1839 | *Drosophila melanogaster* | 44.6 | 0.006 | 71/102 | 70% | 0/102 |
| NM_001112758.1 mdg4 | 1331 | *Bombyx mori* | n/a | n/a | none | n/a | n/a |
| **Similarity Scores** | **Translated amino acid sequence** | **Length** | **Species** | **Bit score** | **Expect** | **Identities** | **% identities** | **Gaps** |
| Predicted: XP_008199106.1 | 350 | *Tribolium castaneum* | 434 | 8.00E−151 | 230/387 | 59% | 51/387 |
| NP_732741.1 CG34376 | 681 | *Drosophila melanogaster* | 158 | 2.00E−42 | 66/114 | 58% | 0/114 |
| NP_001106229.1 mdg4 | 344 | *Bombyx mori* | 173 | 4.00E−50 | 127/369 | 34% | 55/369 |
While these particular genes may not play a role in the phenotypes described here, they provide an initiation point for further studies on pigmentation biochemistry and pattern formation in a novel and important beneficial lady beetle. Predictably, the *C. maculata* sequences are more similar to *T. castaneum* sequences than to those of *D. melanogaster* or *B. mori*, because the genes of two beetles, albeit distantly related, should logically be more similar to each other than to those from other insect orders, either Diptera or Lepidoptera (respectively). As more genes from non-model organisms are annotated and curated, the conservation and divergence of gene evolution and function will be better understood.

The cost of producing genetic strains is inbreeding depression. The fitness assessments presented here, indicating relatively robust fecundity, fertility, and size, bode well for continuing availability of the strains described here, and other strains. Laboratory inbreeding of biological control agents may change predation characteristics [15], therefore it is important to maintain and improve awareness and understanding of those traits that could lead to changes in the effectiveness of beneficial insects.

Additional studies of coccinellid pigments will require chemical or physical extraction and isolation methods and molecular biological methods such as gene disruption or over expression.

Further studies of *C. maculata* and related coccinellids and the genetic and biochemical processes responsible for elytra coloration will be facilitated by this unique strain of beetles. Molecular genetic markers will facilitate mark and recapture studies to evaluate immigration and emigration to and from natural and managed ecosystems [4], and even from plant type or species may be possible. This strain is stable in the homozygous form. The strain will be useful for molecular genetic and biochemical studies of insect pigments, immunity, and evolution of gene regulatory networks.

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Competing Interests

The author claims no competing interests.

Authors’ Contributions

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

References


List of Abbreviations Used

10sp = Ten spotted pattern (strain)
wt = wild type
f = female
m = male
G = generation
R = reciprocal
ye = yellow eyes and elytra (strain)
F = filial
avg = average
Supplementary

ggtgaatgtgtcactcttgtgaagagctcgtcgacggtggatattcaaacggaatccatacaccaggaattggatacttcctccttcctcttttctctatatttagatgat
  metgslpqfsil

AAA GGG CCG ACC CCA AGA TTC AAC CCT GAA TAT ATA AAT GAT
  kgptrrfnpeyind

GTC ATT GAA TCT ACT CTA TCA GAT TCG AAC ACT GCT GAT AAA
  viesltstdntsbdka

ATT GCT CTA ATA TAC GAG GAT GAG GAA ACA TGC GTC AAG CAC
  iailiedyedectcvkh

ACG TAC GCT GAA CTG AAC ATC ATC ACC AAT AAA CTT GCG AGG
  tyaelniiitnklar

GTT ATC AAG AAT AAA ATA ACA CAA GAA AAG ACT CCA AGA AGC
  viknkitqenlqnr

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M. L. Allen

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C S G E T L V K S A A V D F

TAC AAG TAT TTC CCT GAA AAC GAA CAT CGT CTT TGT AAT TTT
Y K Y F P E N E H R L C N F

TAC GGA AGC ACT GAA ATT ATG GCC GAC GTA ACT TAC TAC GTC
Y G S T E I M G D V T Y V

ATA AAA GGA TTA GAT CAA CTG GCT ACT ATT GAA AAA ATT CCT
I K G L D Q L A T I E K I P

ATA GGC GTC CCA GTC GAC AAC ACC ATC ATC TAT CTT CTG GAC
I G V P V D N T I I Y L L D

CCG GAG TTC CGC CCT GTA AAA GCA GGA GAA ATT GGT GAA TTA
P E F R P V K A G E I G E L

TAC GTT TCC GTT TTA AAT CTT GCA GCG GGA TAC ATA AAT GGT
Y V S G L N L A A G Y I N G

AGA GAT CCT GAC AAA TTC CTC GAT AAT CCC TTA GCC ATA GAT
R D P D K F L D N P L A I D

CCA ACA TAT GCT AAA ATT TAT AGA ACA GCC GAT TTC GCT AGG
P T Y A K I Y R T G D F A R

TTG GAG AAA GGA GTT CTC TTA TAC GAA GGA AGA ACC GAT TCA
L E K G V L L Y E G R T D S

CAG GFA AAA ATT AGG GGA CAT CGT GTA GAT CTG AGC GAG GTA
Q V K I R G H R V D L T E V

GAA AAG GCA GTT TCT TCA ATA GAA GAG ATA GAA AAG GCC GTT
E K A V S S I E E I E K A V

GTC CTC TGC TAT AAA CCT GGT GAA ATG AGT CAG GCA CTT TTA
V L C Y K P G E M S Q A L L

GCT TTC GTA ACA ACC AAA CAA TTA GTA AGC GAA AGT TGG ATT
A F V T T K Q L V S E S W I

GAA GCT TAT TTG AGA AAA AAA CTA ACT CCA TAC ATG ATT CCA
E A Y L R K K L T P Y M I P

CAA GTG ATT CTT GTA GAA TTC ATA CCG CTC TTG GTA AAC GGA
Q V I L V E S I P L L V N G

AAA ATT GAC CGG CAG AGC TTG CTC AAG ATG TAC GAA AAC ACT
K I D R Q S L L K M Y E N T

AAC AAT AAC AAT GAT GAT CAA TAC CAA GTC GAT ATA GAT TAC
N N N N D D Q Y Q V D I D Y
Figure S1. Nucleotide sequence and translated amino acid sequence for predicted *ebony* gene transcript from transcriptomes of two individual adult *C. maculata* specimens. Illumina sequences were assembled individually and combined, and all assemblies resulted in the same predicted sequence. Lower case letters indicate untranslated sequence.

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**Figure S2.** ClustalW alignment (Gonnet method) of predicted translated *ebony* sequence with predicted sequence from *Tribolium castaneum* genome project, and validated sequence from *Drosophila melanogaster*.
Figure S3. Nucleotide sequence and translated amino acid sequence for predicted *bric-a-brac* gene transcript from transcriptomes of two individual adult *C. maculata* specimens. Illumina sequences were assembled individually and combined, and all assemblies resulted in the same predicted sequence. Lower case letters indicate untranslated sequence.
Figure S4. ClustalW alignment (Gonnet method) of predicted translated bric-a-brac sequence with predicted sequence from Tribolium castaneum genome project, and validated sequence from Drosophila melanogaster.