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#### **Supplemental Information**

#### The Genomic Basis of Color Pattern

#### Polymorphism in the Harlequin Ladybird

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**Figure S1. Genome-wide association study focusing on the color pattern genomic region of the** *HaxR* **assembly. Related to Figure 1.** (A) Association with the proportion of Red-nSpots individuals. (B) Association with the proportion of Black-2Spots individuals. (C) Association with the proportion of Black-4Spots individuals. (D) Association with the proportion of Black-nSpots individuals.

Α



#### Figure S2. *pannier*, but not *GATAe*, is necessary for adult pigmentation patterns. Related to Figure 2; Figure S3.

(A-D) RNAi phenotypes after larval injection of dsRNA in Black-4Spots (left column) or Red-nSpots (right column). (A) dsRNA targeting *eGFP* (negative control). (B) dsRNA targeting *GATAe*.
(C) dsRNA targeting *pnr\_1* region. (D) dsRNA targeting *pnr\_2* region. *pnr\_1* and *pnr\_2* are non-overlapping regions of the *pannier* cDNA. Numbers in parentheses indicate the proportion of eclosed adults showing the pigmentation pattern of the representative individual. Scale bar; 1mm.





HaxB4(2.9 Mbp)



С

В

## Figure S3. Comparison of Pannier sequences and *utg676* and *HaxB4* sequences. Related to Figure 1; Figure 2; Figure S2.

(A) Alignment of Pannier sequences, resulting from conceptual translation of cDNA obtained by RT-PCR or by mapping two independent RNA-seq reads from Black-4Spots or Red-nSpots individuals. Asterisks indicate conserved amino acids. The positions of the two fragments amplified for dsRNAs (*ds\_pnr1* and *ds\_pnr2*) are shown in grey. (B) Dot-plot between genomic scaffolds from the Black-4Spots (assembly *HaxB4*) and Red-nSpots (assembly *HaxR*, contig *utg676*) forms. The most divergent sequence (white dashed box in B) is shown at a higher magnification in C). (C) Traces of a sequence inversion are visible in the divergent region (white dashed box).

Α

Pool of Red–nSpots Individuals only (HaxR assembly)

D



### Figure S4. Relative read mapping coverage for window of 10,000 positions. Related to STAR Methods.

(A-C) relative read mapping coverage over the extended Red-nSpots (*HaxR* assembly), and (D-F) relative read mapping coverage over the Black-4Spots allele sequence (HaxB4 assembly) for i) the four pools including Red-nSpots individuals only (A, D); ii) the three pools including Black-4Spots individuals only (B, E); and iii) the CH2-B2 and NOV-Bn pools including respectively Black-2Spots individuals only and Black-nSpots individuals only (C, F). The horizontal blue dotted line gives the value of 1 expected for the absence of departure in the relative coverage. The genomic region where the sequences of the Red-nSpots and Black-4Spots alleles diverge is represented as a shaded orange area. A purple line represents the position of the gene *pannier*. As expected, no obvious departure of the relative window coverage (from the expected value of 1) was observed over the entire Red-nSpots *utg676* contig for the four pools including Red-nSpots individuals only, although the BIO-R pools displayed several regions with high relative coverage values (A). In contrast, for the three pools including Black-4Spots individuals only, we found that the relative window coverage was almost halved in the ca. 170 kb region upstream *pannier*, with slight pattern differences likely explained by the pool frequency of heterozygous individuals carrying a Red-nSpots allele (B). When considering the extended Black-4Spots allele sequence as a reference (assembly *HaxB4*), the pattern was reverted: no obvious departure of the relative window coverage was observed for Black-4Spots pools (E) and the coverage was almost halved for Red-nSpots pools in the genomic region harbouring strong sequence divergence between the two color pattern form alleles (D). For the CH2-B2 pool including Black-2Spots individuals only, we observed a clear reduction in relative coverage over the entire genomic region where the sequences of the Red-nSpots and Black-4Spots alleles diverge on the HaxR assembly (C). Interestingly, the reduction in relative coverage concerned a more restricted region (located closer to *pannier*) on the *HaxB4* assembly (F). This suggests that the Black-2Spots allele might be more similar to the Black-4Spots allele, over at least a part of the upstream region of *pannier*, although it should be kept in mind that additional large scale variation (insertion or deletion) specific to the Black-2Spots allele are not identifiable with this approach. Finally, for the NOV-Bn pool including BlacknSpots individuals only, we observed a clear reduction in relative coverage over the second half of the region where the sequences of the Red-nSpots and Black-4Spots alleles diverge on the *HaxR* assembly (C). This pattern suggests a close similarity in sequence of the Black-nSpots allele with the Red-nSpots allele over the first half of its sequence. On the HaxB4 assembly we also observed a clear reduction of the NOV-Bn relative coverage over the first half of the region where the sequences of the Red-nSpots and Black-4Spots alleles diverge, whereas coverage reduction was less obvious in the second half of the region (F). This suggests some sequence similarity between the BlacknSpots and the Black-4Spots allele in the latter genomic region. It is worth noting, however, that for the Black-nSpots allele too, additional specific large-scale variations (insertion or deletion) are not identifiable with this approach.

|                      | Assembly HaxR            | Assembly <i>HaxB4</i>   |  |
|----------------------|--------------------------|-------------------------|--|
| Data                 | MinION long reads (65X)  | Illumina PE reads (65X) |  |
|                      | Illumina PE reads (100X) | Illumina MP reads (24X) |  |
| Assembler            | SMARTdenovo              | ALLPATH-LG              |  |
| Nb. of sequences     | 1,071 contigs            | 6,586 scaffolds         |  |
| Total length (Mbp)   | 429                      | 393.1                   |  |
| Average length (Kbp) | 400.9                    | 59.7                    |  |
| Max size (Kbp)       | 7,499                    | 5,635                   |  |
| Total Ns (bp)        | 22                       | 22,814,986              |  |
| N50 (Kbp)            | 1,434                    | 978.4                   |  |
| BUSCO (complete)     | 97.2 %                   | 86.0 %                  |  |
| BUSCO (fragmented)   | 1.3 %                    | 8.7 %                   |  |
| BUSCO (missing)      | 1.5 %                    | 5.3 %                   |  |

Table S1 Statistics characterizing the HaxR assembly obtained from Red-nSpots individuals andthe HaxB4 assembly obtained from Black-4Spots individuals. Related to STAR Methods.More details can be found in DataS1.

| sequencing<br>sample codeCountryRegion or cityyearthe poolsequenced<br>individualsCH1-RChinaJilin2013Red-nSpots100CH1-BJilin201330 Black-4Spots58CH2-RRed-nSpots100CH2-B4ChinaChangchun2015Black-2SpotsCH2-B4ChinaChangchun2015Black-4Spots67OH2-B2Black-2Spots73onlyJP-RJapanKyoto2009onlyJP-B4JapanKyoto2009onlyNOV-BnRussiaNovosibirsk2007Black-nSpots58NOV-BnFranceBourgogne2013Red-nSpots50SRG-RFranceBourgogne2013Red-nSpots50MA-RUSAGeorgia2007Red-nSpots50onlyWAS-RUSAWashington2007Red-nSpots44onlyWashington2007Red-nSpots40onlyMAS-RUSAWashington2007Red-nSpots40   | Pooled-     | Populati | on sampling site | Sampling | Colour form in  | No. of      |
|--|-------------|----------|------------------|----------|-----------------|-------------|
| sample codeindividualsCH1-RChinaJilin2013Red-nSpots<br>only100CH1-B30 Black-4Spots5828 Black-2Spots58CH2-RRed-nSpots10001ly100CH2-B4ChinaChangchun2015Black-4Spots67CH2-B2Red-nSpots10001ly100CH2-B2Red-nSpots7301ly100JP-RJapanKyoto2009Red-nSpots57JP-B4and other cities01lyBlack-4Spots58NOV-BnRussiaNovosibirsk2007Black-nSpots58BRG-RFranceBourgogne201301ly100BRG-RFranceBourgogne201301ly100BRA-RUSAGeorgia2007Red-nSpots50OnlyWAS-RUSAWashington2007Red-nSpots44OnlyOnlyOnly01ly01lyBrack-4Spots5001ly01ly01ly01lyBrack-ROnlyOnly01ly01ly01lyBrack-ROnlyOnly01ly01ly01lyBrack-ROnlyOnly01ly01ly01lyBrack-ROnlyOnly01ly01ly01lyOnlyOnlyOnly01ly01ly01lyOnlyOnlyOnly01ly01ly01lyOnlyOnlyOnly01ly01ly<  | sequencing  | Country  | Region or city   | year     | the pool        | sequenced   |
| CH1-R<br>CH1-BChinaJilin2013Red-nSpots<br>only100<br>onlyCH1-B30 Black-4Spots58<br>28 Black-2Spots58<br>28 Black-2Spots58<br>28 Black-2SpotsCH2-R<br>CH2-B4ChinaChangchun2015Red-nSpots<br>only100<br>onlyCH2-B2CH2-B2Black-4Spots<br>only67<br>only67<br>onlyJP-R<br>JP-R<br>Black-2SpotsJapanKyoto<br>and other cities2009<br>only73<br>onlyJP-R<br>BRG-R<br>BRG-B4FranceBourgogne2007<br>onlyBlack-nSpots<br>only58<br>onlyBRG-R<br>BRG-B4FranceBourgogne2013Red-nSpots<br>only50<br>onlyWAS-RUSAGeorgia2007Red-nSpots<br>only45<br>only  | sample code |          |                  |          |                 | individuals |
| ChinaJilin2013onlyCH1-B30 Black-4Spots5828 Black-2Spots28 Black-2SpotsCH2-RRed-nSpots100CH2-B4ChinaChangchun2015CH2-B2Black-4Spots67JP-RJapanKyoto2009JP-B4and other citiesBlack-4Spots57MOV-BnRussiaNovosibirsk2007Black-nSpots58BRG-RFranceBourgogne2013Red-nSpots50BRG-B4USAGeorgia2007Red-nSpots50WAS-RUSAWashington2007Red-nSpots44onlyMarket and the comparison of the compariso | CH1-R       |          |                  |          | Red-nSpots      | 100         |
| CH1-B30 Black-4Spots58<br>28 Black-2SpotsCH2-RRed-nSpots100<br>onlyCH2-B4ChinaChangchun2015Black-4Spots67<br>onlyCH2-B2Black-2Spots73<br>only73<br>only73<br>onlyJP-RJapanKyoto<br>and other cities2009onlyJP-B4and other citiesBlack-4Spots57<br>onlyNOV-BnRussiaNovosibirsk2007Black-nSpots58<br>onlyBRG-RFranceBourgogne2013OnlyBRG-B4FranceBourgogne2013OnlyWAS-RUSAGeorgia2007Red-nSpots50<br>onlyWAS-RUSAWashington2007Red-nSpots40<br>only  |             | China    | Jilin            | 2013     | only            |             |
| 28 Black-2SpotsCH2-RRed-nSpots100CH2-B4ChinaChangchun2015Black-4Spots67OnlyBlack-2Spots730000CH2-B2Black-2Spots730000JP-RJapanKyoto20090000JP-B4and other citiesBlack-4Spots57ONV-BnRussiaNovosibirsk2007Black-4Spots58ONV-BnRussiaNovosibirsk2007Black-nSpots44ONUBRG-RRed-nSpots5000BRG-B4FranceBourgogne20130001ENA-RUSAGeorgia2007Red-nSpots45ONLONLONL000000WAS-RUSAWashington2007Red-nSpots40ONLONLONL000000   | CH1-B       |          |                  |          | 30 Black-4Spots | 58          |
| CH2-RRed-nSpots100CH2-B4ChinaChangchun2015Black-4Spots67CH2-B2Black-2Spots730nly0nly0nlyJP-RJapanKyoto20090nly0nly0nlyJP-B4and other citiesBlack-4Spots580nly0nlyNOV-BnRussiaNovosibirsk2007Black-nSpots58MGG-RFranceBourgogne2013Red-nSpots50BRG-B4USAGeorgia2007Red-nSpots50WAS-RUSAWashington2007Red-nSpots45ONLOnlyOnlyOnly0nly0nlyWAS-RUSAWashington2007Red-nSpots40OnlyOnlyOnlyOnly0nly0nlyOnlyOnlyOnlyOnlyOnly0nlyOnly <t< td=""><td></td><td></td><td></td><td></td><td>28 Black-2Spots</td><td></td></t<>   |             |          |                  |          | 28 Black-2Spots |             |
| CH2-B4<br>CH2-B2ChinaChangchun2015Only<br>Black-4Spots67<br>onlyCH2-B2Black-2Spots73<br>onlyJP-RJapanKyoto<br>and other cities2009NolyJP-B4JapanKyoto<br>and other cities2009OnlyNOV-BnRussiaNovosibirsk2007Black-4Spots<br>only58<br>onlyBRG-RFranceBourgogne2013OnlyBRG-B4USAGeorgia2007Red-nSpots<br>only50<br>onlyWAS-RUSAWashington2007Red-nSpots<br>only40<br>only   | CH2-R       |          |                  |          | Red-nSpots      | 100         |
| CH2-B4ChinaChangchun2015Black-4Spots67CH2-B2Black-2Spots73JP-RJapanKyoto2009JP-B4and other citiesBlack-4Spots57ONV-BnRussiaNovosibirsk2007Black-nSpots58OnlyBRG-RRed-nSpots5000BRG-RFranceBourgogne20130000BRG-B4VSAGeorgia2007Red-nSpots50OnlyUSAWashington2007Red-nSpots45OnlyOnlyOnlyOnly00Brack-RVSAWashington2007Red-nSpots40OnlyOnlyOnlyOnly00OnlyOnlyOnlyOnly00OnlyOnlyOnlyOnly00OnlyOnlyOnlyOnly00OnlyOnlyOnlyOnly00OnlyOnlyOnlyOnly00OnlyOnlyOnlyOnly00OnlyOnlyOnlyOnly00OnlyOnlyOnlyOnly00OnlyOnlyOnlyOnly00OnlyOnlyOnlyOnly00OnlyOnlyOnlyOnly00OnlyOnlyOnlyOnly00OnlyOnlyOnlyOnly00OnlyOnlyOnlyOnly00OnlyOnl  |             | _        |                  |          | only            |             |
| CH2-B2onlyJP-RJapanKyoto<br>and other cities2009Red-nSpots<br>only57JP-B4and other citiesBlack-4Spots<br>only58NOV-BnRussiaNovosibirsk2007Black-nSpots<br>only44NOV-BnFranceBourgogne2013OnlyBRG-RFranceBourgogne2013OnlyBRG-B4VSAGeorgia2007Red-nSpots<br>only50WAS-RUSAWashington2007Red-nSpots<br>only40  | CH2-B4      | China    | Changchun        | 2015     | Black-4Spots    | 67          |
| CH2-B2Black-2Spots73JP-RJapanKyoto<br>and other cities2009Red-nSpots57JP-B4and other cities2009onlyBlack-4Spots58NOV-BnRussiaNovosibirsk2007Black-nSpots44BRG-RFranceBourgogne2013only50BRG-B4FranceBourgogne2013Black-4Spots50WAS-RUSAGeorgia2007Red-nSpots45WAS-RUSAWashington2007Red-nSpots40onlySASa Shington2007Red-nSpots40  |             |          |                  |          | only            |             |
| JP-RRed-nSpots57JapanKyoto2009onlyJP-B4and other citiesBlack-4Spots58NOV-BnRussiaNovosibirsk2007Black-nSpots44NOV-BnRussiaNovosibirsk2007Black-nSpots44BRG-RFranceBourgogne2013onlyBlack-4Spots50BRG-B4FranceBourgogne2007Red-nSpots50USAGeorgia2007Red-nSpots50WAS-RUSAWashington2007Red-nSpots45OnlyUSAWashington2007Red-nSpots40OnlyUSAWashington2007Red-nSpots40   | CH2-B2      | -        |                  |          | Black-2Spots    | 73          |
| JP-R<br>   |             |          |                  |          | only            |             |
| JapanKyoto<br>and other cities2009<br>and other citiesonlyJP-B4and other citiesBlack-4Spots<br>only58<br>onlyNOV-BnRussiaNovosibirsk2007Black-nSpots<br>only44<br>onlyBRG-RFranceBourgogne2013OnlyBRG-B4FranceBourgogne2007Red-nSpots<br>only50<br>onlyENA-RUSAGeorgia2007Red-nSpots<br>only45<br>onlyWAS-RUSAWashington2007Red-nSpots<br>only40<br>only   | JP-R        |          |                  |          | Red-nSpots      | 57          |
| JP-B4and other citiesBlack-4Spots<br>only58NOV-BnRussiaNovosibirsk2007Black-nSpots<br>only44BRG-RFranceBourgogne2013000000000000000000000000000000000  |             | Japan    | Kyoto            | 2009     | only            |             |
| NOV-BnRussiaNovosibirsk2007Black-nSpots44OnlyBRG-RRed-nSpots50BRG-B4FranceBourgogne2013OnlyBNA-RUSAGeorgia2007Red-nSpots50OnlyOnlyOnlyOnlyOnlyWAS-RUSAWashington2007Red-nSpots45Only </td <td>JP-B4</td> <td>-</td> <td>and other cities</td> <td></td> <td>Black-4Spots</td> <td>58</td>  | JP-B4       | -        | and other cities |          | Black-4Spots    | 58          |
| NOV-BnRussiaNovosibirsk2007Black-nSpots<br>only44BRG-RFranceBourgogne2013Red-nSpots<br>only50BRG-B4Black-4Spots<br>only50ENA-RUSAGeorgia2007Red-nSpots<br>only45WAS-RUSAWashington2007Red-nSpots<br>only40   |             |          |                  |          | only            |             |
| BRG-R<br>FranceFranceBourgogne2013Red-nSpots<br>only50<br>onlyBRG-B4FranceBourgogne2013Black-4Spots<br>only50<br>onlyENA-RUSAGeorgia2007Red-nSpots<br>only45<br>onlyWAS-RUSAWashington2007Red-nSpots<br>only40<br>only   | NOV-Bn      | Russia   | Novosibirsk      | 2007     | Black-nSpots    | 44          |
| BRG-R<br>FranceFranceBourgogne2013Red-nSpots<br>only50<br>onlyBRG-B4ENA-RUSAGeorgia2007Red-nSpots<br>only50<br>onlyENA-RUSAGeorgia2007Red-nSpots<br>only45<br>onlyWAS-RUSAWashington2007Red-nSpots<br>only40<br>only   |             |          |                  |          | only            |             |
| FranceBourgogne2013onlyBRG-B4Black-4Spots50ENA-RUSAGeorgia2007Red-nSpots45WAS-RUSAWashington2007Red-nSpots40   | BRG-R       |          |                  |          | Red-nSpots      | 50          |
| BRG-B4     Black-4Spots only     50 only       ENA-R     USA     Georgia     2007     Red-nSpots only     45 only       WAS-R     USA     Washington     2007     Red-nSpots only     40 only  |             | France   | Bourgogne        | 2013     | only            |             |
| ENA-RUSAGeorgia2007Red-nSpots45WAS-RUSAWashington2007Red-nSpots40  | BRG-B4      | -        |                  |          | Black-4Spots    | 50          |
| ENA-RUSAGeorgia2007Red-nSpots45WAS-RUSAWashington2007Red-nSpots40only001001001001  |             |          |                  |          | only            |             |
| WAS-RUSAWashington2007Red-nSpots40only   | ENA-R       | USA      | Georgia          | 2007     | Red-nSpots      | 45          |
| WAS-R USA Washington 2007 Red-nSpots 40  |             |          |                  |          | only            |             |
| only   | WAS-R       | USA      | Washington       | 2007     | Red-nSpots      | 40          |
| Ully   |             |          | -                |          | only            |             |
| BIO-R Biological Red-nSpots 100  | BIO-R       |          | Biological       |          | Red-nSpots      | 100         |
| France control 2012 only   |             | France   | control          | 2012     | only            |             |
| BIO-B4 population Black-4Spots 100   | BIO-B4      | -        | population       |          | Black-4Spots    | 100         |
| (BIOTOP) only  |             |          | (BIOTOP)         |          | only            |             |

# Table S2 Sequenced pools of individuals representative of the world-wide diversity and the four main color pattern forms of *H. axyridis*. Related to STAR Methods.

The colour pattern forms Red-nSpot, Black-nSpots, Black-4Spots and Black-2Spots correspond to the forms f. *succinea*, f. *axyridis*, f. *conspicua*, and f. *spectabilis*, respectively. Because of the hierarchical dominance between color form alleles (i.e. Black-2Spots > Black-4Spots > Black-nSpots > Red-nspot), a population pool sample

including individuals of a single colour pattern form is characterized by a high proportion of the allele associated to that form but also contains alleles associated to other more recessive forms if present in the population. Only population pool samples with Red-nSpots individuals contain 100% of Red-nSpots alleles.