

## NOTES AND COMMENTS

### Empirical comparison of microsatellite and SNP markers to estimate introgression in *Apis mellifera mellifera*

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(Received 14 October 2017, accepted 16 May 2018)

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### Online Supplementary Material

### Supplementary Materials and methods

#### Whole-genome sequence data

The first dataset consisted of N=31 drone samples originating from our previous study (Parejo et al., 2016), which were selected to represent the entire range of admixed individuals (e.g. pure *Apis mellifera mellifera*, pure *A. m. carnica*, and a range of hybrids in-between). Detailed information on sequencing and variant calling can be found in the respective publication. Using 3,076,531 SNPs identified from whole-genome sequencing, admixture proportions of the 31 individuals were inferred using ADMIXTURE v1.3.0 (Alexander, Novembre, & Lange, 2009) and a reference sample set of N=334 drone samples (Henriques et al., 2018a). ADMIXTURE was run unsupervised for K=2 with 10,000 iterations and the default termination criterion set to stop when the log-likelihood increases by less than 0.0001 between independent runs.

#### Microsatellites

The second dataset consisted of N=56 *A. m. mellifera* samples, which were tested in 2016 and selected to include a diverse range of introgression proportions (C-lineage ancestry: [0.002-0.502]). All samples have been genotyped at 12 microsatellite loci (A007, A43, Ap33, Ap226, B24, Ap273, Ac306 Ap289, A28, Ap1, A29, A76) (Soland-Reckeweg, 2006) ([www.apigenix.ch](http://www.apigenix.ch)). Ancestries of the 56 samples were inferred together with 355 reference

samples from Switzerland, France, Norway, Sweden and Ireland (*A. m. mellifera*), as well as Austria and Slovenia (*A. m. carnica*) using STRUCTURE v2 (Falush, Stephens, & Pritchard, 2003) with the correlated allele frequency model for K=2. This is the approach of the currently commercially available microsatellite-based hybrid test in Switzerland ([www.apigenix.com](http://www.apigenix.com)).

### **Ancestry-informative SNP panel**

The samples of the two datasets (N=31 and N=56; NTOT=87) with whole-genome sequence information or microsatellite data, respectively, were both genotyped at 117 SNPs using a recently developed ancestry-informative SNP panel for C-lineage introgression in *A. m. mellifera* (Henriques et al., 2018b).

The SNP panel was designed starting from the 144 top-ranked SNPs selected by Muñoz et al., (2015) to identify and estimate introgression in *A. m. mellifera*. Muñoz and colleagues (2015) had ranked by information content >1183 SNPs that had been 2 / 2 genotyped in numerous samples of *A. m. mellifera*, *A. m. ligustica* and *A. m. carnica* using Illuminas GoldenGate Assay. The top-ranked SNPs had been combined into reduced SNP panels to fit the plexes of the GoldenGate Assay genotyped with the VeraCode technology (48, 96, 144, 192, 384 SNPs) (Muñoz et al., 2015). This technology has now been discontinued.

The new 117-SNP assay designed for high sample throughput genotyping using the iPLEX MassARRAY system is validated and extensively tested in different experiments by Henriques et al. (2018b) resulting in a ready-to-use molecular tool to estimate C-lineage introgression in *A. m. mellifera*.

Here, we apply and test this reduced SNP panel in our samples from Switzerland (NTOT=87). Ancestries of the 87 samples were inferred using ADMIXTURE v1.3.0 (Alexander, Novembre, & Lange, 2009). The software was run unsupervised for K=2 with 10,000 iterations and the default termination criterion set to stop when the log-likelihood increases by less than 0.0001 between independent runs.

### **References**

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## Online Supplementary Material 2.

Admixture proportions as estimated by 3.077 M SNPs (whole-genome), 117 SNPs (ancestry-informative panel) and 12 microsatellites.

|           | Whole-genome (3.077 M SNPs) |           | 117 ancestry-informative SNPs |           | 12 microsatellites |           |
|-----------|-----------------------------|-----------|-------------------------------|-----------|--------------------|-----------|
| Sample_ID | M-lineage                   | C-lineage | M-lineage                     | C-lineage | M-lineage          | C-lineage |
| M1        | 0.833768                    | 0.166232  | 0.839083                      | 0.160917  | -                  | -         |
| M10       | 0.721744                    | 0.278256  | 0.762758                      | 0.237242  | -                  | -         |
| M11       | 0.696076                    | 0.303924  | 0.732541                      | 0.267459  | -                  | -         |
| M12       | 0.652131                    | 0.347869  | 0.647419                      | 0.352581  | -                  | -         |
| M13       | 0.569874                    | 0.430126  | 0.571698                      | 0.428302  | -                  | -         |
| M14       | 0.477073                    | 0.522927  | 0.424935                      | 0.575065  | -                  | -         |
| M15       | 0.412099                    | 0.587901  | 0.419648                      | 0.580352  | -                  | -         |
| M17       | 0.251813                    | 0.748187  | 0.246458                      | 0.753542  | -                  | -         |
| M18       | 0.201527                    | 0.798473  | 0.179544                      | 0.820456  | -                  | -         |
| M19       | 0.172023                    | 0.827977  | 0.00001                       | 0.99999   | -                  | -         |
| M2        | 0.832012                    | 0.167988  | 0.861117                      | 0.138883  | -                  | -         |
| M20       | 0.171783                    | 0.828217  | 0.00001                       | 0.99999   | -                  | -         |
| M21       | 0.053485                    | 0.946515  | 0.00001                       | 0.99999   | -                  | -         |
| M22       | 0.042018                    | 0.957982  | 0.00001                       | 0.99999   | -                  | -         |
| M23       | 0.893369                    | 0.106631  | 0.885053                      | 0.114947  | -                  | -         |
| M24       | 0.896514                    | 0.103486  | 0.959394                      | 0.040606  | -                  | -         |
| M25       | 0.893973                    | 0.106027  | 0.99999                       | 0.00001   | -                  | -         |
| M26       | 0.897572                    | 0.102428  | 0.99999                       | 0.00001   | -                  | -         |
| M27       | 0.00001                     | 0.99999   | 0.00001                       | 0.99999   | -                  | -         |
| M28       | 0.042723                    | 0.957277  | 0.00001                       | 0.99999   | -                  | -         |
| M3        | 0.820399                    | 0.179601  | 0.872257                      | 0.127743  | -                  | -         |
| M33       | 0.127025                    | 0.872975  | 0.00001                       | 0.99999   | -                  | -         |
| M34       | 0.199272                    | 0.800728  | 0.187264                      | 0.812736  | -                  | -         |
| M35       | 0.185363                    | 0.814637  | 0.13997                       | 0.86003   | -                  | -         |
| M36       | 0.147089                    | 0.852911  | 0.00001                       | 0.99999   | -                  | -         |
| M4        | 0.814109                    | 0.185891  | 0.879798                      | 0.120202  | -                  | -         |
| M5        | 0.806082                    | 0.193918  | 0.938224                      | 0.061776  | -                  | -         |
| M6        | 0.803106                    | 0.196894  | 0.882011                      | 0.117989  | -                  | -         |
| M7        | 0.788771                    | 0.211229  | 0.868143                      | 0.131857  | -                  | -         |
| M8        | 0.783093                    | 0.216907  | 0.899767                      | 0.100233  | -                  | -         |
| M9        | 0.755685                    | 0.244315  | 0.700815                      | 0.299185  | -                  | -         |

|           | Whole-genome (3.077 M SNPs) |           | 117 ancestry-informative SNPs |           | 12 microsatellites |           |
|-----------|-----------------------------|-----------|-------------------------------|-----------|--------------------|-----------|
| Sample_ID | M-lineage                   | C-lineage | M-lineage                     | C-lineage | M-lineage          | C-lineage |
| S1        | -                           | -         | 0.99999                       | 0.00001   | 0.989              | 0.011     |
| S10       | -                           | -         | 0.927836                      | 0.072164  | 0.996              | 0.004     |
| S11       | -                           | -         | 0.99999                       | 0.00001   | 0.963              | 0.037     |
| S12       | -                           | -         | 0.972177                      | 0.027823  | 0.991              | 0.009     |
| S13       | -                           | -         | 0.99999                       | 0.00001   | 0.987              | 0.013     |
| S14       | -                           | -         | 0.988134                      | 0.011866  | 0.993              | 0.007     |
| S15       | -                           | -         | 0.99999                       | 0.00001   | 0.924              | 0.076     |
| S16       | -                           | -         | 0.875589                      | 0.124411  | 0.929              | 0.071     |
| S17       | -                           | -         | 0.99999                       | 0.00001   | 0.959              | 0.041     |
| S18       | -                           | -         | 0.919314                      | 0.080686  | 0.763              | 0.237     |
| S19       | -                           | -         | 0.954839                      | 0.045161  | 0.904              | 0.096     |
| S2        | -                           | -         | 0.732292                      | 0.267708  | 0.714              | 0.286     |
| S20       | -                           | -         | 0.99999                       | 0.00001   | 0.882              | 0.118     |
| S21       | -                           | -         | 0.902325                      | 0.097675  | 0.908              | 0.092     |
| S22       | -                           | -         | 0.937467                      | 0.062533  | 0.945              | 0.055     |
| S23       | -                           | -         | 0.940062                      | 0.059938  | 0.96               | 0.04      |
| S24       | -                           | -         | 0.916385                      | 0.083615  | 0.959              | 0.041     |
| S25       | -                           | -         | 0.896222                      | 0.103778  | 0.986              | 0.014     |
| S26       | -                           | -         | 0.935692                      | 0.064308  | 0.982              | 0.018     |
| S27       | -                           | -         | 0.910225                      | 0.089775  | 0.976              | 0.024     |
| S28       | -                           | -         | 0.915911                      | 0.084089  | 0.962              | 0.038     |
| S29       | -                           | -         | 0.951805                      | 0.048195  | 0.968              | 0.032     |
| S3        | -                           | -         | 0.921175                      | 0.078825  | 0.981              | 0.019     |
| S30       | -                           | -         | 0.907743                      | 0.092257  | 0.89               | 0.11      |
| S31       | -                           | -         | 0.890753                      | 0.109247  | 0.962              | 0.038     |
| S32       | -                           | -         | 0.934056                      | 0.065944  | 0.975              | 0.025     |
| S33       | -                           | -         | 0.77296                       | 0.22704   | 0.854              | 0.146     |
| S34       | -                           | -         | 0.88209                       | 0.11791   | 0.954              | 0.046     |
| S35       | -                           | -         | 0.966042                      | 0.033958  | 0.97               | 0.03      |
| S36       | -                           | -         | 0.922025                      | 0.077975  | 0.959              | 0.041     |
| S37       | -                           | -         | 0.64762                       | 0.35238   | 0.759              | 0.241     |
| S38       | -                           | -         | 0.923465                      | 0.076535  | 0.978              | 0.022     |
| S39       | -                           | -         | 0.885865                      | 0.114135  | 0.979              | 0.021     |
| S4        | -                           | -         | 0.99999                       | 0.00001   | 0.931              | 0.069     |
| S40       | -                           | -         | 0.369051                      | 0.630949  | 0.498              | 0.502     |
| S41       | -                           | -         | 0.953665                      | 0.046335  | 0.874              | 0.126     |
| S42       | -                           | -         | 0.929599                      | 0.070401  | 0.944              | 0.056     |
| S43       | -                           | -         | 0.907139                      | 0.092861  | 0.938              | 0.062     |
| S44       | -                           | -         | 0.99999                       | 0.00001   | 0.985              | 0.015     |
| S45       | -                           | -         | 0.671705                      | 0.328295  | 0.702              | 0.298     |
| S46       | -                           | -         | 0.894835                      | 0.105165  | 0.96               | 0.04      |
| S47       | -                           | -         | 0.937088                      | 0.062912  | 0.962              | 0.038     |
| S48       | -                           | -         | 0.962081                      | 0.037919  | 0.989              | 0.011     |
| S49       | -                           | -         | 0.954278                      | 0.045722  | 0.956              | 0.044     |
| S5        | -                           | -         | 0.977618                      | 0.022382  | 0.988              | 0.012     |
| S50       | -                           | -         | 0.838448                      | 0.161552  | 0.886              | 0.114     |
| S51       | -                           | -         | 0.949931                      | 0.050069  | 0.894              | 0.106     |
| S52       | -                           | -         | 0.765782                      | 0.234218  | 0.688              | 0.312     |
| S53       | -                           | -         | 0.93953                       | 0.06047   | 0.97               | 0.03      |
| S54       | -                           | -         | 0.99999                       | 0.00001   | 0.827              | 0.173     |
| S55       | -                           | -         | 0.93538                       | 0.06462   | 0.989              | 0.011     |
| S56       | -                           | -         | 0.993187                      | 0.006813  | 0.964              | 0.036     |
| S6        | -                           | -         | 0.993354                      | 0.006646  | 0.995              | 0.005     |
| S7        | -                           | -         | 0.953464                      | 0.046536  | 0.998              | 0.002     |
| S8        | -                           | -         | 0.948025                      | 0.051975  | 0.994              | 0.006     |
| S9        | -                           | -         | 0.964006                      | 0.035994  | 0.993              | 0.007     |

