

A scientific note on using large mixed sperm samples in instrumental insemination of honeybee queens

Johanna T. Pieplow¹, Jürgen Brauße², Jacob Peter van Praagh³, Robin F. A. Moritz^{1,4,5}, Silvio Erler¹

¹ Institut für Biologie, Molekulare Ökologie, Martin-Luther-Universität Halle-Wittenberg, Hoher Weg 4, 06099 Halle (Saale), Germany, ² Blankenseer Dorfstraße 2, 14959 Trebbin OT Blankensee, Germany, ³ Hassellstraße 23, 29223 Celle, Germany, ⁴ Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa, ⁵ German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

***Corresponding author:** Silvio Erler, Martin-Luther-Universität Halle-Wittenberg, Institut für Biologie, Molekulare Ökologie, Hoher Weg 4, 06099 Halle (Saale), Germany, Tel: +49 (0) 345-5526305; Fax: +49 (0) 345-5527264, E-mail: erler.silvio@gmail.com

Table SI: Genotypes of the drone donating queens (red numbers mark alleles that were known from the single sperm samples).

Queen	A14	A43	A113	HB19	HB-THE-03					
1	218	228	125	141	214	220	113	115	195	195
2	218	220	127	141	222	226	113	119	195	199
3	218	220	127	141	220	222	119	127	191	199
4	218	236	127	141	214	220	123	125	199	201
5	218	236	141	141	214	220	121	123	191	201
6	228	236	141	141	214	220	121	123	195	201
7	218	228	125	141	220	228	117	121	191	197
8	218	228	141	141	214	220	123	127	199	201
9	214	248	127	141	214	222	113	121	195	195
10	218	220	141	141	222	230	111	121	195	197

Supplementary material and methods

All DNA samples were stored at -20°C until genotyping with a set of five highly-polymorphic microsatellite loci [A14, A43 (Estoup et al. 1994), A113 (Estoup et al. 1995), HB19 (Lattorff et al. 2007), HB-THE-03 (Shaibi et al. 2008)] using standard PCR protocols (Shaibi et al. 2008). PCR products were size scored with a capillary DNA sequencer (MegaBACE 1000, GE Healthcare) and the worker genotypes determined.

The sperm mix genotype was a complex mix of allele abundance at each locus. The abundance of each allele within the sperm mix was estimated using the relative mean peak height of the allele (Michelmore et al. 1991) in two replicate analyses. Differences in PCR efficiency of the alleles at the loci A14 and HB-THE3 were corrected. Alleles with shorter fragment length were amplified at higher rates than longer alleles, resulting in biased peak heights (allele abundances). Heterozygote individuals which have an equal abundance of either allele were used to compute a linear regression of PCR efficiency on allele size (A14: $0.1234 \times d + 1$; HB-THE3: $0.4757 \times d + 1$, where d = difference in base pairs between two alleles). After correcting for PCR efficiency, the contribution of every single drone lineage to the total sperm mix was estimated. This was done primarily based on the private alleles of the specific drone lineages and then extended to the other, less discriminative alleles (Table SII). Eventually the frequency of every drone lineage within the semen mix could be determined (Table SII) and deviations from an even distribution was tested with a Kruskal-Wallis test. The mean frequency of each lineage within the mix was compared with its frequency found among the offspring worker pupae using a χ^2 -test. Statistical analyses were conducted using STATISTICA 8.0 (StatSoft). Whether the inseminated queens (as random replicates) had an effect on the representation of the drone lineages among their worker offspring was tested as well with a Fisher's exact test using R (R Core Team 2013). Power analyses were conducted using the R-packages *pwr* and *statmod*.

References:

- Estoup, A., Solignac, M., Cornuet, J.-M. (1994) Precise assessment of the number of patriline and of genetic relatedness in honeybee colonies. *P. Roy. Soc. B-Biol. Sci.* **258**, 1-7
- Estoup, A., Scholl, A., Pouvreau, A., Solignac, M. (1995) Monoandry and polyandry in bumble bees (Hymenoptera; Bombinae) as evidenced by highly variable microsatellites. *Mol. Ecol.* **4**, 89-94
- Lattorff, H.M.G., Moritz, R.F.A., Crewe, R.M., Solignac, M. (2007) Control of reproductive dominance by the *thelytoky* gene in honeybees. *Biol. Lett.* **3**, 292-295
- Michelmore, R.W., Paran, I., Kesseli, R.V. (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA* **88**, 9828-9832

- R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Shaibi, T., Lattorff, H.M.G., Moritz, R.F.A. (2008) A microsatellite DNA toolkit for studying population structure in *Apis mellifera*. Mol. Ecol. Resour. **8**, 1034-1036

Table SII: Worker offspring genotypes of each inseminated queen (A-C, n = 32 per queen) with colony identity, identified father and final drone lineage.

worker	A14		A43		A113		HB 19		HB-THE 3		queen/colony	drone	drone lineage
	queen allele	drone allele	queen allele	drone allele	queen allele	drone allele	queen allele	drone allele	queen allele	drone allele			
751-01	218	228	141	141	222	220	121	121	0	0	A	55	7
751-02	218	228	143	141	220	220	119	123	195	201	A	58	6
751-03	218	218	141	141	222	220	119	123	195	201	A	10	4
751-04	230	228	143	141	222	214	121	123	199	201	A	53	8
751-05	218	228	141	141	222	228	121	121	199	191	A	59	7
751-06	218	218	141	141	220	220	121	127	199	191	A	40	3
751-07	230	218	141	141	222	214	121	113	199	195	A	19	1
751-08	218	220	141	141	222	222	121	111	199	197	A	47	10
751-09	218	228	143	141	220	220	121	123	199	201	A	58	6
751-10	218	218	143	141	220	220	121	127	199	199	A	41	8
751-11	230	228	143	141	220	220	119	123	195	201	A	58	6
751-12	218	218	143	141	222	220	119	121	195	191	A	33	5
751-13	218	214	143	141	220	222	119	121	195	195	A	5	9
751-14	218	218	143	127	220	220	119	125	195	199	A	11	4
751-15	218	220	143	141	222	222	121	111	199	197	A	47	10
751-16	230	228	141	141	220	220	119	115	195	195	A	54	1
751-17	230	218	141	141	220	214	119	127	195	199	A	28	8
751-18	218	218	143	141	222	222	119	121	199	199	A	42	2
751-19	230	220	143	141	220	222	121	121	199	197	A	46	10
751-20	218	228	141	141	222	220	121	123	195	201	A	58	6
751-21	218	218	143	125	222	220	119	115	195	195	A	7	1
751-22	230	228	141	141	220	228	121	121	199	197	A	60	7
751-23	230	214	143	141	220	214	119	121	195	195	A	3	9
751-24	230	236	143	141	222	220	121	121	199	191	A	62	5

751-25	230	218	141	141	220	222	121	119	199	199	A	34	3
751-26	230	218	141	141	222	220	121	125	195	199	A	39	4
751-27	230	236	143	141	222	220	119	123	195	201	A	64	4
751-28	230	228	143	141	220	220	121	123	199	201	A	58	6
751-29	218	218	143	141	220	220	119	121	199	191	A	33	5
751-30	230	218	143	141	222	220	121	121	199	197	A	35	7
751-31	230	228	143	141	220	220	121	121	199	201	A	56	6
751-32	218	218	141	127	222	220	121	125	199	199	A	11	4
731-01	218	218	143	141	220	220	117	123	195	201	B	10	4
731-02	218	236	143	141	214	220	113	123	195	201	B	64	4
731-03	218	218	143	141	220	220	117	113	195	195	B	32	1
731-04	218	214	143	141	214	222	113	121	195	195	B	6	9
731-05	218	236	143	141	214	220	113	121	195	191	B	63	5
731-06	218	214	143	127	214	214	113	113	195	195	B	1	9
731-07	218	228	141	141	214	220	113	123	195	0	B	58	6
731-08	218	218	141	141	220	220	113	123	195	191	B	38	5
731-09	218	228	141	141	220	220	117	121	195	195	B	57	6
731-10	218	220	141	141	214	222	117	119	195	199	B	48	2
731-11	218	228	143	141	214	220	113	113	195	0	B	37	1
731-12	218	228	141	125	220	228	117	117	195	197	B	50	7
731-13	218	220	143	141	214	222	113	121	195	197	B	46	10
731-14	218	228	141	141	220	228	117	121	195	191	B	59	7
731-15	218	218	141	141	220	214	117	113	195	195	B	21	1
731-16	218	236	143	141	220	220	117	123	195	201	B	64	6
731-17	218	228	143	141	220	228	117	121	195	191	B	59	7
731-18	218	228	143	141	214	220	113	123	195	201	B	58	6
731-19	218	228	141	141	220	220	117	121	195	201	B	56	6
731-20	218	218	141	125	214	220	113	115	195	195	B	7	1
731-21	218	218	143	141	214	214	117	123	195	0	B	23	8
731-22	218	218	141	141	214	230	113	121	195	197	B	44	10

731-23	218	236	143	141	220	220	113	121	195	191	B	63	5
731-24	218	218	141	141	0	0	117	127	195	199	B	27	8
731-25	218	218	143	141	220	220	113	123	195	201	B	10	4
731-26	218	248	141	127	214	214	117	121	195	195	B	65	9
731-27	218	236	143	141	220	220	117	121	195	191	B	63	5
731-28	218	220	143	141	214	222	117	119	195	199	B	48	3
731-29	218	220	141	141	214	230	117	121	195	197	B	49	10
731-30	218	218	141	127	214	222	117	113	195	195	B	15	2
731-31	218	214	143	141	220	222	113	121	195	195	B	6	9
731-32	218	218	141	127	214	220	117	119	195	0	B	9	3
732-01	218	218	143	141	220	0	117	125	195	0	C	25	4
732-02	218	218	143	141	220	214	123	113	195	195	C	17	1
732-03	218	218	143	127	220	222	117	119	195	0	C	16	2
732-04	218	218	143	141	220	214	117	115	195	195	C	20	1
732-05	218	218	143	141	220	214	123	127	195	199	C	28	8
732-06	218	236	141	141	220	214	123	121	195	191	C	61	5
732-07	218	218	141	141	220	214	123	113	195	195	C	17	1
732-08	218	214	141	127	220	214	117	113	195	195	C	1	9
732-09	218	248	141	141	220	222	117	121	195	195	C	43	9
732-10	218	228	141	141	220	214	123	121	195	201	C	51	6
732-11	218	228	141	141	220	220	123	121	195	201	C	56	6
732-12	218	236	141	141	220	220	117	121	195	191	C	63	5
732-13	218	236	143	141	220	214	117	121	195	191	C	61	5
732-14	218	0	141	0	220	0	123	121	195	0	C	-	-
732-15	218	0	141	141	220	0	123	117	195	197	C	2	7
732-16	218	0	143	125	220	0	117	0	195	0	C	-	-
732-17	218	218	143	141	220	220	117	121	195	191	C	36	5
732-18	218	236	143	141	220	214	117	121	195	191	C	61	5
732-19	218	218	141	141	220	220	123	113	195	195	C	30	1
732-20	218	220	143	127	220	222	123	119	195	199	C	45	3

732-21	218	218	143	141	220	214	117	125	195	199	C	26	4
732-22	218	218	141	141	220	220	123	0	195	199	C	31	3
732-23	218	218	143	141	220	214	123	123	195	201	C	24	8
732-24	218	228	143	141	220	214	123	121	195	201	C	51	6
732-25	218	228	141	141	220	0	123	121	195	201	C	52	6
732-26	218	228	143	141	220	0	117	121	195	201	C	52	6
732-27	218	248	143	127	220	222	117	113	195	195	C	14	9
732-28	218	218	141	125	220	0	117	115	195	195	C	8	1
732-29	218	218	143	141	220	0	123	121	195	191	C	22	5
732-30	218	214	143	141	220	0	117	121	195	195	C	4	9
732-31	218	218	143	127	220	0	123	125	195	199	C	12	4
732-32	218	218	143	141	220	0	123	0	195	199	C	18	2

0: missing allelic information; -: no clear identification of maternal and paternal alleles, workers were excluded from further analyses