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Scientific note

A scientific note on a genetically-determined color morph of the dwarf honey bee, *Apis andreniformis**

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***Apis andreniformis* / color morph / microsatellite**

The dwarf honey bees *Apis florea* (Fabricius, 1787) and *A. andreniformis* (Smith, 1858) both build a single-comb nest from a twig in a shady location. *Apis andreniformis* is distributed west from the Philippines to Myanmar and China and is sympatric with *A. florea* in Southeast Asia (reviewed in Oldroyd and Wongsiri, 2006). Although the two species can be reliably separated by the architecture of the comb, the morphology of the endophallus and hind leg of the male, and worker wing venation (reviewed in Oldroyd and Wongsiri, 2006), variation of worker colour within the two species can lead to confusion (Hepburn et al., 2005). Typically the first two abdominal segments of an *A. andreniformis* worker are black and its scutellum is dark reddish brown (rufous) (Wu and Kuang, 1987). This coloration is reversed in *A. florea* workers which typically have rufous first and second abdominal segments, and a black scutellum. We report here on an *A. andreniformis* worker colour morph which is superficially similar to *A. florea*, and is likely controlled by a single Mendelian locus.

We captured an *A. andreniformis* swarm from Phitsanulok province, Thailand, 17° 01' N, 100°54' E, in 2008. In this swarm 55 workers were not of typical *A. andreniformis* colouration and we suspected that they were drifted *A. florea* workers. The species of these unusually-coloured workers was determined using a diagnostic length polymorphism of the mitochondrial ribosomal RNA large subunit gene (Higgs et al., unpubl. data). All bees but one, which was removed before further analysis, were confirmed as *A. andreniformis*.

Atypical bees were examined under a dissecting microscope. The first and second segments of the abdomen were rufous and very similar to that of *A. florea*, but slightly less red. The scutellum varied from pale tan to very dark rufous (see Fig. S1 in online supplementary material). Thus the workers appeared similar to *A. florea* workers, especially those with a dark scutellum (Fig. S1 in online supplementary material). Unlike the typical *A. andreniformis* queen, which is completely black (Wu and Kuang, 1987), the queen's abdomen had four orange dorsal stripes and the ventral surface was predominately orange.

To determine if the rufous vs. black phenotype is under simple genetic control, all 54 confirmed *A. andreniformis* workers showing the yellow coloration (representing 3.94% of the swarm) and 70 random workers and the queen, were genotyped in a four-locus multiplex PCR reaction (Tab. I). DNA was extracted from a hind leg of each bee with Chelex (Walsh et al., 1991; Oldroyd et al., 1997) and amplified using the microsatellite primers shown in Table I. A further 31 random workers were genotyped at a single locus (*Am*052) that turned out to be diagnostic of the patriline showing the yellow coloration. Reactions were performed in a total volume of 5 μL containing 1 μL of extracted DNA.

For each worker and locus we determined which of the two alleles was the maternal allele by reference to the queen's genotype. Paternal alleles were thus unequivocally inferred by subtraction (Oldroyd et al., 1996). Fourteen patrilines were detected among the workers sampled. All 54 of the rufous colour morph workers were grouped into a single patriline. No individuals of this patriline were found

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Table I. Multiplexed loci and associated primer concentrations. All reverse primers were fluorescently-labeled.

Locus (Solignac et al., 2003)	No. of alleles detected	Primer concentration
*Am*056	3	0.34 μM
*Am*008	2	0.32 μM
*Am*052	3	0.52 μM
*Am*061	4	0.40 μM

in the 101 workers with the typical black coloration. We therefore consider the rufous colour morph to be a recessive trait controlled by a single locus. In this case the queen was homozygous for the trait (as evidenced by her phenotype), and we infer that one of the males with which she mated also carried the trait.

It is unlikely that more than one male carried the allele and that progeny of this male remained undetected in the worker progeny. The probability of not detecting a patriline because two males carried the same microsatellite alleles at all four loci studied is $\prod_i p_j$, where p_j is the frequency of the allele j at the ith locus. We do not have any estimate of population allele frequencies for the microsatellite loci studied, but if we make the highly conservative assumption that the only alleles in the population were those observed in the worker sample, and that each allele is at equal frequency, the non-detection rate = $0.33 \times 0.5 \times 0.33 \times 0.25 = 0.01$ (see Tab. I for the number of alleles detected at each locus). More importantly (for it would suggest a more complex genetic architecture to the trait) it is unlikely that we failed to detect an individual worker which was a daughter of the rufous male, but showed the normal black phenotype. For example, if under a different scenario to that which we propose, only half the workers of this patriline showed the yellow color, then the probability of not sampling one of them in a sample of size n is $(1-k)^n$, where k is the frequency of the patriline (in this case 0.0397) (Boomsma and Ratnieks, 1996). Thus in a sample of size 101, there was only a 1.7% chance that we would fail to detect a worker of the 'rufous' patriline should half of the individuals of that patriline be black.

The gene responsible for the unusual coloration may be that designated *Fl* by Woyke (1995), which is expressed in both queens and workers of *A. florea* and confers a similar phenotype.

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Note scientifique sur un morphe de couleur à détermination génétique chez l'abeille naine, *Apis andreniformis*.

Zusammenfassung – Eine wissenschaftliche Notiz über eine genetisch determinierte Farbvariante der Zwerghonigbiene, *Apis andreniformis*.

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