

## Odorants Differentiate Australian *Rattus* with Increased Complexity in Sympatry

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**ABSTRACT.** Odorant cues play a critical role in premating isolation among many species. In mammals, they have been most well-studied in rodents, but only in a handful of species. The genus *Rattus* is one of the most species-rich genera of mammals, with a natural distribution from Asia to Australia and a nearly global distribution for a few species that spread through human commensalism. More than one-third of *Rattus* species are the result of a recent and rapid radiation on continental Australia (Sahul) centred on the island of New Guinea. The two most widespread species resulting from this radiation, *Rattus fuscipes* and *Rattus leucopus*, occur sympatrically in the Wet Tropics region of Queensland, Australia. Despite their recent divergence, morphological similarity, and ability to produce fertile offspring in captivity, hybrids of the two species have not been reported in the wild, suggesting that premating isolation mechanisms maintain the species' boundaries. Odorant cues are a plausible mechanism that these species could use to identify mates of the same species, but the chemical composition of their odours has not been characterized. With allozyme data from 166 specimens of the two species we confirmed the absence of gene flow between the species in sympatry. From chemical analysis of preputial glands of 32 males from sympatric and allopatric populations of the two species we identified 120 volatile organic compounds of which 80 were reliably quantitated for statistical analysis. Some of these chemicals have been indicated as signalling compounds in other species of mammals, including seven thiazolines. Among them two (2-sec-butylthiazoline and 2-isopropylthiazoline) have been previously detected in a rodent, the House Mouse, *Mus musculus*, and are involved in social interactions including attracting females. We demonstrate that *R. fuscipes* and *R. leucopus* are quantitatively and qualitatively distinguishable by the chemical composition of their preputial gland secretions. In comparison to allopatric subspecies, sympatric species contained more unique chemical compounds and a higher abundance of compounds overall, suggesting that sympatric populations have more complex and concentrated odours. Together these results indicate that odorant chemistry has evolved rapidly in these two species, with substantial differences among species and subspecies, especially in sympatry. Ultimately, the rapid evolution of chemical signals involved in mate recognition may help to explain the exceptional diversity of species in the genus *Rattus*.

**Keywords:** Rodentia; Muridae; pheromones; preputial gland; species

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**Received:** 3 February 2020 **Accepted:** 28 August 2020 **Published:** 25 November 2020 (in print and online simultaneously)

**Publisher:** The Australian Museum, Sydney, Australia (a statutory authority of, and principally funded by, the NSW State Government)

**Citation:** Rowe, Kevin C., Helena A. Soini, Karen M. C. Rowe, Mark Adams, and Milos V. Novotny. 2020. Odorants differentiate Australian *Rattus* with increased complexity in sympatry. In *Papers in Honour of Ken Aplin*, ed. Julien Louys, Sue O'Connor, and Kristofer M. Helgen. *Records of the Australian Museum* 72(5): 271–286. <https://doi.org/10.3853/j.2201-4349.72.2020.1721>

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