

Research Article

A Unique Conjunction: Evidence for Gynogenesis Accompanying Haplodiploid Sex Determination in the Australian Ant *Myrmecia impaternalata* Taylor

Robert W. Taylor ¹, Hirotami T. Imai,² Eisuke Hasegawa ³ and Colin D. Beaton⁴

¹Research School of Biology, The Australian National University, Canberra, Australian Capital Territory 0200, Australia

²Formerly National Institute of Genetics, Mishima, Shizuoka-ken 411-8540, Japan

³Laboratory of Animal Ecology, Department of Ecology and Systematics, Graduate School of Agriculture, Hokkaido University, Sapporo, Japan

⁴Formerly CSIRO, Division of Entomology, Canberra, ACT, Australia

Correspondence should be addressed to Robert W. Taylor; bob.taylor@homemail.com.au

Received 12 June 2018; Revised 10 September 2018; Accepted 6 December 2018; Published 18 December 2018

Academic Editor: G. Wilson Fernandes

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Myrmecia impaternalata is an allodiploid all-female species of hybrid origin. Its parental taxa are confirmed here to be *M. banksi* and *M. pilosula*. We suggest that its queens produce diploid female offspring by gynogenetic parthenogenesis, a process which requires interaction between unreduced maternal oocytes and allospecific sperm cells obtained by copulation with another related species. We propose that *impaternalata* queens almost certainly mate for this purpose with males reared in *impaternalata* nests from eggs laid by *impaternalata* females. Because sex in ants is determined by haplodiploidy (males haploid, females diploid), we posit that these males would in fact not be technically conspecific with the females in whose reproductive systems they developed, since they would each carry the haploid genome of one or other of the hybrid parental species. They would therefore be individually identical karyologically to males of either *M. banksi* or *M. pilosula* and appropriately allospecific to *M. impaternalata*. We postulate that, unlike all other known gynogens, *M. impaternalata* would have no need to maintain parasitic affiliation or sympatry with free-living sperm-donor host species. Its queens are arguably able to produce the required allospecific males by accessing their own genomes. *M. impaternalata* apparently originated by instantaneous speciation when individuals of its parental species first successfully hybridized.

Dedicated to the memory of professor Rossiter H. Crozier FAA (1943-2009)

1. Introduction

The *Myrmecia pilosula* complex includes five related south-east Australian “Jack-jumper” ant species [1]. *M. banksi* Taylor 2015, *M. croslandi* Taylor 1991, *M. impaternalata* Taylor 2015, and *M. pilosula* Fr. Smith 1858 are of interest here.

The cytogenetics and natural history of these taxa have been studied over several decades by HTI, RWT, and associates [1, 2]. Their findings reveal that *M. impaternalata* (Figure 1) is an allodiploid species with unmatched haploid sets of chromosomes ($n=5$ or 14 , $2n=19$), identifying it as a female-only species of hybrid origin [1, 2]. Our researches

also demonstrate that *impaternalata* apparently exhibits gynogenesis, a process in which females of such hybridic species must copulate with males of closely related sympatric species in order to initiate egg development.

We demonstrate by karyological comparison that *M. banksi* ($n=5$, $2n=10$) and *M. pilosula* ($n=14$, $2n=28$) are the hybridic parents of *M. impaternalata* and report evidence that *impaternalata* arguably has eccentric reproductive biology due to the conjunction of gynogenesis and haplodiploid sex determination in its life cycle.

Myrmecine ants generally nest in the soil. Mature colonies typically contain (i) one or several dealate egg-laying



FIGURE 1: *Myrmecia impaternalata*, queen, workers, larvae, and a pupal cocoon in a laboratory observation nest (RWT, CDB).

queens which carry in their reproductive systems a lifetime sperm-bank acquired by mating as young flying adults; (ii) around 100-400 unmated apterous female workers, all daughters of the queen(s); (iii) eggs, larvae, and pupae (Figure 1) variously represented in correlation with an annual vernal season of oviposition by the queens, and (iv) in early summer numbers of winged males and/or winged virgin queens (= gynes): sons and daughters of the queen(s) held for later release to join an annual nuptial flight or mating promenade [1, 3]. The males disperse and die soon after mating and daughter colonies are founded by the newly mated gynes either singly or in small cooperating groups [1, 4].

Sex is determined universally in ants by haplodiploidy and arrhenotoky, the production of paternate diploid females (queens and workers) from fertilised eggs and impaternalate haploid males from unfertilised eggs [4]. Males and their sperm cells are thus genetically identical.

The *Myrmecia impaternalata* queens and associated males discussed here were unexpectedly collected in January 2011 from two adjacent worker-right nests in the Canberra National Botanic Gardens by RWT and HTI. We then believed that males in this hybrid species had been lost in evolution, having failed to locate any despite numerous targeted excavations of nests at the presumed appropriate season over preceding years in the Canberra area, Australian Capital Territory, and near Armidale, New South Wales (NSW). It is reasonable to conclude that the males in question were reared from eggs produced by the associated queens and that these were their home colonies.

2. Hybridity and Gynogenesis in *Myrmecia impaternalata*

We review here evidence that *M. impaternalata* is a unisexual clonal species of hybrid origin, arguably with a gynogenetic mode of reproduction in which (i) the parthenogenetic production of diploid female eggs by its queens is sperm-dependent, requiring allospecific copulation followed by interaction without fertilisation between their unreduced oocytes and nonconspecific sperm cells and (ii) sperm necessary for this purpose is likely acquired by the gynes through

copulation with technically allospecific males produced from eggs laid by *impaternalata* queens and reared in *impaternalata* nests. In gynogenesis the eggs are not fertilised and female offspring are therefore impaternalate [5–8].

Among vertebrates gynogenesis occurs only in some all-female species of fish, salamanders, and lizards [5]. There are no known gynogenetic birds or mammals [9]. A number of gynogenetic invertebrates have also been reported [7]. All known gynogenetic vertebrates originated as interspecific hybrids [9] but this likely detail has not been confirmed for all relevant invertebrates. Most gynogen taxa are thought to have had multiple origins and to coexist in multiple, genetically identifiable lineages [8, 10].

The unisexual, internally fertilised, live-bearing Amazon molly-fish *Poecilia formosa* (Girard, 1859) from northeast Mexico and southern Texas is a well-known gynogen. Its females obtain the required sperm by parasitic gonopodial copulation with males of several other related *Poecilia* species including its putative female hybridic ancestor *P. mexicana* Steindachner, 1863 [11–14].

Gynogenesis apparently functions to provide targeted oocytes with sperm-derived centrosomes which replace their developmentally eliminated original oocyte centrosomes [15]. The latter are lost during oogenesis in most appropriately investigated eukaryotes [5] apparently to circumvent duplication of the ovarian centrosomes by sperm centrosomes acquired at fertilisation [15]. Sperm cells are the products of cell-lines which require centrosomes for cell division and each eventual spermatozoan normally carries a retained centrosome [5]. Replacement in the females of the lost oocyte centrosomes is essential for later mitotic spindle formation, cell division, and embryonic development. This apparent vital functionality of gynogenesis implies that other insufficiently studied hybrid taxa might also prove to be gynogens: otherwise how (unless they are appropriately atypical) are their lost ovarian centrosomes replaced?

Chromosomes are not involved in gynogenesis: genetic information from male-derived chromosomes is neither accessed nor required [8].

The observations of Ross & Normark [15] confirm that cytoplasmic fusion of the gamete cells rather than some other arrangement like simple contact (as sometimes previously speculated) occurs during gynogenesis.

3. Karyological Evidence

Most known *M. impaternalata* karyotypes [2:179] comprise two unmatched haploid chromosome sets with 5 and 14 chromosomes, respectively (Figure 2(b)).

M. banksi ($2n=10$) has the diploid configuration $2K_B=\{8M, 2A\}$ (Figures 2(a) and 2(a')), and the 5-chromosome set of *M. impaternalata* closely matches one of its haploid sets of chromosomes $K_{Ba}=\{4M, 1A\}$ (Figures 2(a) and 2(c)). Based on this cytological evidence the *impaternalata* $n=5$ haploid set is denoted $K_B=\{4M, 1A\}$ (Figure 2(b)), and affinity with *M. banksi* is clearly indicated.

The 14-chromosome haploid set of *M. impaternalata* is putatively denoted $K_P=\{1M, 13A\}$ (Figure 2(b)). Among the known karyotypes of other *pilosula* complex species it most

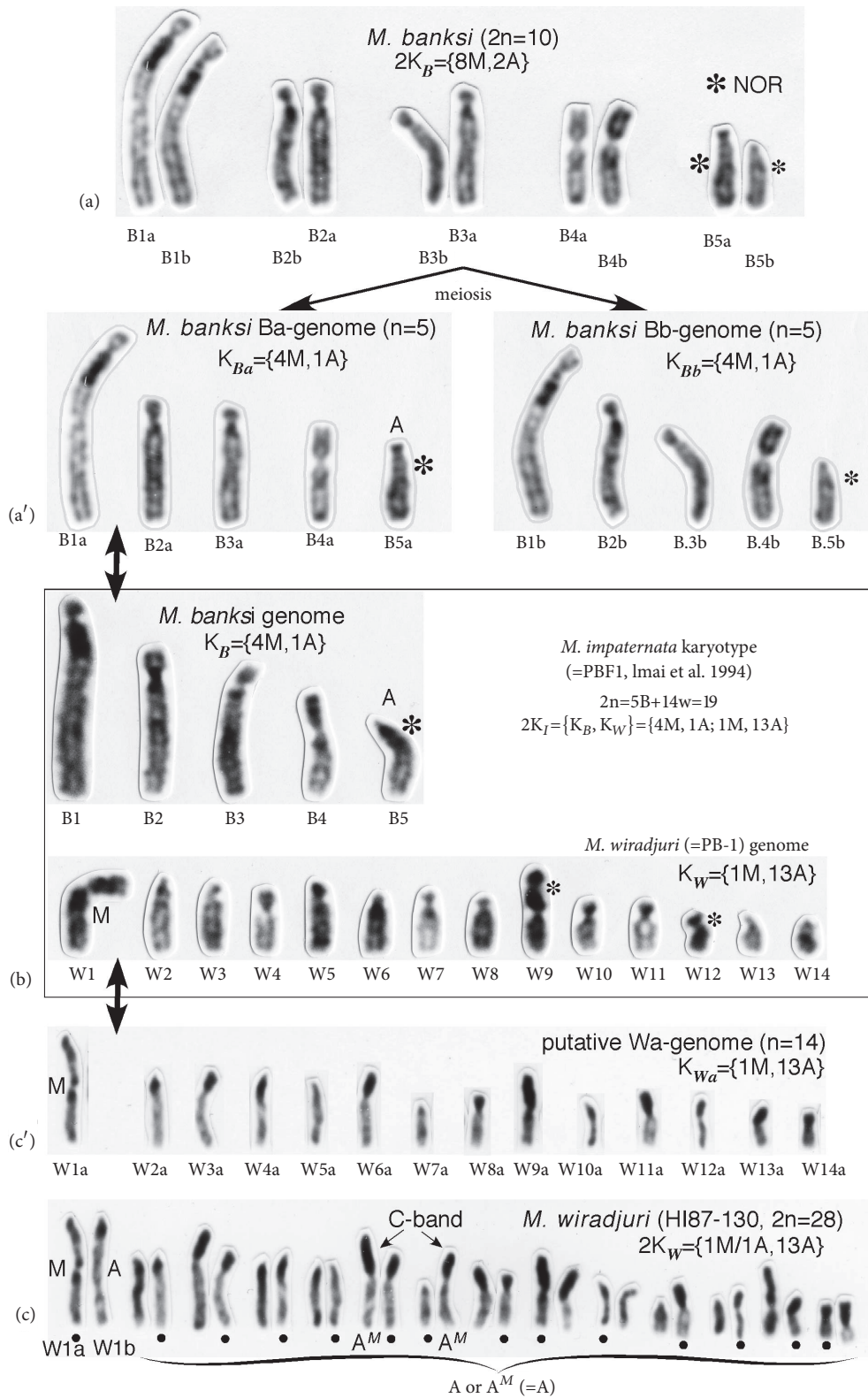


FIGURE 2: Comparison of karyotypes of *Myrmecia banksi* (a, a'), *M. impaternalata* (b), and *M. pilosula* (c, c')(HTI). A = acrocentric, M = metacentric, and NOR = Nuclear Organiser Region.

closely resembles that of an *M. pilosula* colony (accession number HI87-130 [2]) from Wambrook Creek (36°11'S, 148°56'E), NSW, which has the diploid configuration $2K_p = \{1M/1A, 15A^M, 11A\}$ (Figure 2(c)). This karyotype is characterised by one A/M heteromorphic pair of chromosomes resulting from an AM-inversion, with the remaining chromosomes either A or A^M , with heterochromatic arms acquired by C-band elongation. Because heterochromatic arms are highly variable and of no genetic significance, A^M -chromosomes may be considered equivalent to A-chromosomes [16]. The $2K_p$ karyotype can be described as $2K_p = \{1M/1A, 26A\}$ and the two gametes expected from $2K_p$ chromosomes are $K_{pa} = \{1M, 13A\}$ (Figure 2(c')) and $K_{pb} = \{14A\}$. The former is equivalent to the $K_p = \{1M, 13A\}$ haploid set of *M. impaternalata* (Figure 2(b)), the diploid karyotype of which ($2K_I$) is formulated $2K_I = \{K_B, K_P\}$, which is equivalent to $\{4M, 1A; 1M, 13A\}$.

These karyological similarities suggest that the founding parents of *M. impaternalata* were individuals of *M. banksi* and *M. pilosula* (or of their close ancestors) with the latter karyotypically close to the Wambrook Creek series.

The above cytological details (with minor differences in C-banding) apply to the $2n=19$ *impaternalata* samples reported previously from the Canberra area, from the southern NSW localities Mayfield and Charleyong and from near Armidale in north-eastern NSW [2:179]. A reported 18-chromosome sample (HI 85-373) from Canberra is part of this karyological series, but is believed to have secondarily lost an A-chromosome [2].

Imai et al. [2:179] listed two *impaternalata* nest series from Yowrie, NSW, with $2n=15$ and karyotypes $2K = \{K_B, K_P\} = \{4M, 1A; 4M, 6A\}$. These have the *banksi* $K_B = \{4M, 1A\}$ haploid chromosome set, but their *pilosula*-equivalent component differs from that discussed above and illustrated here. Importantly this might indicate that past hybridization between *banksi* and *pilosula* with origination of alternative hybrid entities has successfully occurred more than once and that there could be more than one existing allodiploid *banksi/pilosula* hybrid clone present in south-eastern Australia.

Because sex determination in ants involves arrhenotoky, the reproductive arrangements in *M. impaternalata* differ significantly from those of *Poecilia formosa*. Unlike all other known gynogens *impaternalata* females (queens and probably at times workers), being hymenopterous insects, regularly lay unfertilised haploid eggs which develop as males (as in all investigated ants). In *M. impaternalata*, these would each carry one or other of the two parental haploid sets of 5 or 14 chromosomes, since they are derived from hybrid maternal cells with the $2n=5+14$ karyology illustrated in Figure 2. Such males effectively represent those of the two parental species, *M. banksi* and *M. pilosula*, which initially hybridized to originate *M. impaternalata*. They would not therefore be technically conspecific with the *impaternalata* females in whose reproductive systems their eggs developed. These males would be expected individually to be morphologically identical to those of either *M. banksi* or *M. pilosula*, but that has yet to be confirmed.

4. Evidence from Sperm Samples

HTI has compared sperm cells recovered from (i) testes of a male from one of the *impaternalata* nests discussed above (Figure 2(b)), with (ii) others dissected from the spermathecae (sperm storage organs) of several dealate *impaternalata* queens from the same nests (Figure 2(c)) and (iii) sperm obtained from the testes of an *M. banksi* male collected from a nest near Nowra NSW (Figure 2(a)) [1].

The *banksi* spermatozoa were all morphologically normal, as illustrated, while most of those from the *impaternalata*-associated male and the *impaternalata* queen (Figures 3(b) and 3(c)) were apparently abnormal, with reduced head structure, and presumably incapable of effecting fertilisation. A few morphologically normal sperm cells were observed in the *impaternalata*-derived samples: their functionality, if any, is unknown.

Common identity is confirmed by the similarly degenerate condition of these separate spermathecal and testicular sperm samples. They indicate that copulation with sperm transfer had occurred between *impaternalata* females and *impaternalata*-reared males and that the subject sperm is unlikely to have been obtained by parasitic copulation with another free-living related species such as *M. banksi*, *M. pilosula*, or the regularly sympatric *M. croslandi* (where the sperm cells would need to be functional for conspecific fertilisation and so unlikely to be degenerate). *M. croslandi* has the remarkable karyology $n=1$ or sometimes $n=2$, yielding $2n=2, 3, \text{ or } 4$ [1, 17, 18] which might preclude it from such affiliation.

The degenerate structure of *impaternalata*-derived spermatozoa might indicate evolutionary modification to remove their capacity for fertilisation (which could otherwise complicate the gynogenetic process [14]), while retaining cellular ability to effect gynogenesis. Males in gynogenesis do not transmit genes and are therefore not directly subject to natural selection. The adaptive evolution of degenerate sperm cells suggested here would necessarily be affected by the positive natural selection of *impaternalata* queens whose male offspring produce degenerate sperm. Causal correlation of sperm degeneracy with gynogenesis in this case seems evident.

The circumstances of copulation in *M. impaternalata* are unknown. We posit that mating probably occurs between males and alate gynes in their home nests prior to dispersal of the gynes and that males might not normally depart the nests at all. They would likely also be short-lived after mating, as usual in male ants, thus with very brief seasonal, exclusively in-nest, presence and availability for discovery. This could explain their evident rarity.

5. Genetic Analysis

Genetic comparison of adult males from the subject colonies to investigate their possible duality was attempted by EH who amplified and sequenced DNA from the inappropriately preserved remains of 2 of the 5 specimens dissected for sperm by HTI. The results were limited because of the inadequate

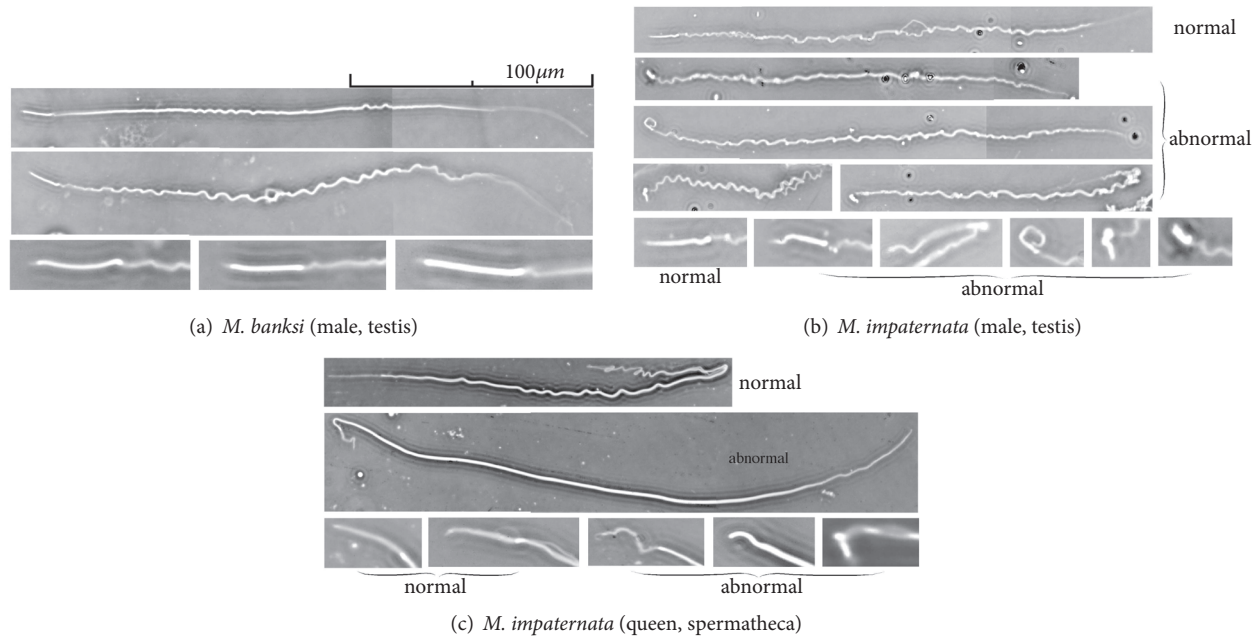


FIGURE 3: Morphological comparison of sperm cells from a *Myrmecia banksi* testis (a), an *M. impaternata* testis (b), and an *M. impaternata* queen spermatheca (c) (HTI).

material. Further work will be prioritised when more males become available.

6. Special Features of *M. impaternata* Biology

Poecilia formosa and other gynogenetic animals are dependent for reproduction on related free-living sperm-donor species and are thus unable to extend their geographical ranges or potential evolutionary survival beyond those of their sympatric hosts [19]. They must also confront various other biological disadvantages [20]. If our hypothesis is correct, these restrictions would not apply to *M. impaternata*. It would have no apparent requirement for sperm parasitism of free-living consociates because its gynes arguably have the in-built capacity themselves to produce allospecific males destined to become the gynogenetic partners of their female offspring.

7. Instantaneous Speciation of *M. impaternata*

The future of a newly mated ant gyne depends on her ability to establish a foundation nest and produce, by oviposition of fertilised diploid eggs, a first brood of workers able to secure her survival and future reproduction. *M. impaternata* as a species must have been originated by a successful colony-founding gyne of either *M. banksi* or *M. pilosula* which had been fertilised by a male of the opposite species. Her daughter workers and gynes would have had the unmatched allodiploid karyology illustrated in Figure 2, compromising any normal reproductive future (for example, their gametes would be a statistical 50:50 mix of those of the two parental species). The chance inheritable parthenogenetic production

of unreduced diploid eggs and allospecific males by one such hybrid gyne would have originated *M. impaternata* as a newly evolved hybridic species. We recognise this event as an example of instantaneous biological speciation [21].

The evidential production by *impaternata* queens of two types of males representing those of its parental species is posited as a simple consequence of hybridization in the presence of arrhenotoky.

8. Concluding Remarks

Unisexuality, hybridity, interspecific mating, and matrilineal inheritance are readily observed in *Poecilia formosa* [11–13, 22]. *Myrmecia impaternata*, of which we have excavated many field nests and observed several laboratory colonies (Figure 1), offers no such convenience. Colony composition, general behaviour, and reproductive organisation appear fully consistent with those of other species of the *M. pilosula* complex, except for the rarity of males [1]. Without our fortuitous knowledge of its allodiploid karyology, discovered by HTI in the course of a general cytological survey of Australian ants [1, 2], the eccentric nature of this remarkable species might never have been recognised.

There could well be other similarly cryptic undiscovered cases of gynogenesis among ants and arrhenotokous social wasps and bees. They would be characterised by allodiploid chromosomes and the likely production of two classes of males with differing karyology related to that of associated queens and workers and to the hybridic parental species. Such clones are theoretically likely to be unstable and short-lived [8].

Voucher specimens of all species mentioned above are in the Australian National Insect Collection, Canberra.

HTI's karyological investigations followed the procedures detailed by Imai [23]. The text follows established protocols for the recognition of hybrid organisms as taxonomic species [24, 25].

9. Future Directions

The male specimens reported here were lost following karyological study by HTI in Japan and so were not available for projected morphological analysis by RWT and CDB. When further male-right *impaternata* colonies are discovered, priority should be given to analysis of genetic markers in queens, workers males, and larvae, especially to test our hypothesis that males will be of two types, respectively, matching those of *M. pilosula* and *M. banksi*. Karyology and gross morphology of male specimens should also be investigated, and whether both kinds of hypothecated males survive to adulthood, and if so whether both engage in copulation.

Karyological analysis will require laboratory culture of colonies to obtain chromosomes from dividing pupal brain cells (see [23] for details).

Adult or pupal males of *M. banksi* and *M. pilosula* for comparison with males from *M. impaternata* nests can readily be obtained from parental colonies in the field at appropriate seasons (for details and distributions of these species see [1, 2]).

Microscopic checking of spermathecal sperm dissected from *impaternata* colony queens would indicate whether they had mated and whether degenerate sperm are the norm in these arrangements. The regular presence of degenerate sperm would strongly support our hypothesis.

Future investigators should be alert to the possible presence in nature of other separately evolved *pilosula-banksi* hybrid populations.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding this publication.

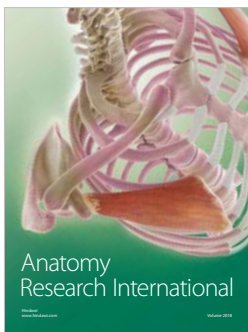
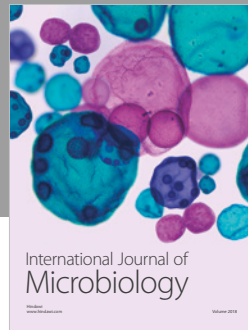
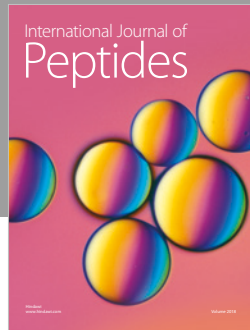
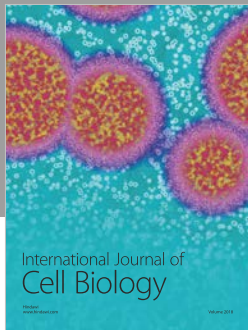
Acknowledgments

We thank Kathy Horton, Hanna Kokko, Ajay Narendra, Ralph Rawlinson, Barry Richardson, Ingo Schlupp, Wee Tek Tay, Wendy Taylor, Jackson Taylor Grant, Bruce Halliday, David Somerfield, Max Whitten, Edward O. Wilson, Jochen Zeil, and two anonymous referees for helpful comments.

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