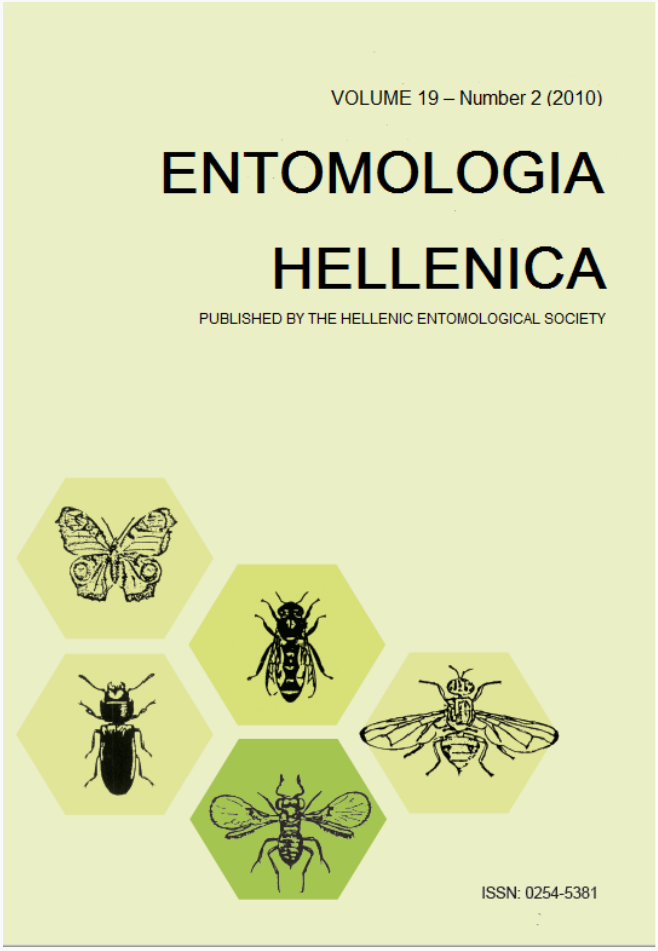
ENTOMOLOGIA HELLENICA

Vol 19, No 2 (2010)



Disparity in chromosomal variation within the Apiomorpha minor species-group

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doi: 10.12681/eh.11575

To cite this article:

Mills, P., & Cook, L. (2010). Disparity in chromosomal variation within the Apiomorpha minor species-group. *ENTOMOLOGIA HELLENICA*, *19*(2), 82–89. https://doi.org/10.12681/eh.11575

ENTOMOLOGIA HELLENICA **19** (2010): 82-89

Disparity in chromosomal variation within the *Apiomorpha minor* species-group

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ABSTRACT

The scale insect genus *Apiomorpha* is one of the most chromosomally diverse of all animal genera, with diploid complements ranging from 4 to 192. There is even considerable variation within many of the 41 described species. For example, variation within the *A. minor* species-group (*A. minor, A. sessilis* and *A. annulata*) shows an extraordinary range with counts from 2n = 4 through to 2n = 84. However, much of this variation is within a single currently recognized species – *A. minor*. In contrast, another species in the *A. minor* species group, *A. sessilis,* has been reported to have only counts of 2n = 4. To determine whether the reported lack of variation within *A. sessilis* was due to limited sampling, we collected specimens from across its known range of more than 1,100 km in eastern Australia. We did not find any additional chromosome counts for *A. sessilis*, confirming the constancy of karyotype in this species. This suggests the question "why does *A. sessilis* have such a conserved karyotype throughout its range while *A. minor* is so diverse?"

KEYWORDS: *Apiomorpha*, holokinetic chromosomes, Eriococcidae, karyotypic variation, cryptic species, *Eucalyptus*.

Introduction

It has been commonly assumed that taxa whose chromosomes possess only a single centromere (monocentric chromosomes) have more restrained diploid counts than taxa with holokinetic chromosomes (chromosomes with multiple attachment sites for spindle fibres) (e.g. Suomalainen 1965). However, this does not always seem to be the case. Some of the most chromosomally diverse animal taxa e.g. the shrew *Sorex* (Hausser et al. 1985, Wojcik 1986, Searle 1986, Halkka et al. 1987, Searle 1988), the house mouse *Mus domesticus* (Nachman and Searle 1995, Britton-Davidian 2000), grasshoppers (White 1978), and *Myrmecia* (Hymenoptera) (Crosland and Crozier 1986, Imai et al. 1990), all have monocentric chromosomes. There are also many examples of taxa that possess holokinetic chromosomes and show modest chromosomal variation e.g. rhabditine nematodes (Blaxter 2000), the family Pentatomidae (Hemiptera) (Mikolajski 1968, Rebagliati et al. 2005, Lanzone and de Souza 2006), the subfamily Triatominae (Hemiptera) (Panzera et al. 1996), and many scale insect families, such as Eriococcidae (Hemiptera) (Cook 2000, Gavrilov 2007). Thus, there does not appear to be a direct link between holocentric chromosomes and high karyotypic variation.

There are several taxa with holokinetic chromosomes that appear to have escaped the constraints on chromosome number ap-

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parent in most taxa with this type of chromosome. Several Lepidoptera genera, such as *Agrodiaetus* (2n = 20 - 268) (Kandul et al. 2004, Kandul et al. 2007), *Lysandra* (2n = 48 - 184) (Delesse 1969, Kandul et al. 2004) and *Plebicula* (2n = 268 - 450) (Kandul et al. 2004) have had many different karyotypes recorded. The scale insect family Eriococcidae, in general, is chromosomally conservative with 70% of examined species having a diploid chromosome count of 2n = 18 (Cook 2000, Gavrilov 2007). However, within the Australian gall-inducing genus *Apiomorpha*, there is a 48-fold difference in karyotypes (2n = 4 up to 2n = ca. 192; Cook 2000). Chromosomal variation among populations within described species of *Apiomorpha* has also been recorded (Cook 2000, Cook 2001, Cook and Rowell 2007, Cook and Gullan 2008).

All species of *Apiomorpha* for which both males and females are known produce sexually dimorphic galls (Gullan 1984, Gullan et al. 1997). The relatively species-specific galls of the adult females are usually many times larger than the small, tubular galls of the males. The adult females of *Apiomorpha* can be very long-lived with respect to other adult insects of similar size. Females have been recorded as living for over five years (Cook and Gullan 2001). Many species of *Apiomorpha* show intraspecific karyotypic variation (Cook 2000, 2001). *Apiomorpha munita* has counts of 2n = 6 to 2n greater than 100, and appears to be a cryptic species-complex of at least five species (Cook and Rowell 2007). Also, *A. thorntoni* has recently been removed from synonymy with *A. pharetrata*, with chromosome differentiation of the two playing a part in the decision (Cook and Gullan 2008). However, further chromosome variation within *A. thorntoni* (2n = 10, 26 and 28) and *A. pharetrata* (2n = 40, 42, 46, 48 and 56) (Cook and Gullan 2008) may indicate the presence of additional cryptic species. A third example of intraspecific variation occurs within the *A. minor* species group. This species group currently includes three species: *A. annulata*, *A. minor* and *A. sessilis* (Gullan 1984). Of the two karyotypically studied species (*A. minor* and *A. sessilis)*, only *A. minor* shows karyotypic variation (2n = 10, 42 and 84; Cook 2000). Even though individuals of *A. sessilis* have been collected hundreds of kilometres apart, the only reported karyotype is 2n = 4 (Cook 2000). However, it is unclear whether the apparent lack of variation in *A. sessilis* is due to the limited sampling undertaken by Cook (2000).

Here, we report on increased sampling of *A. sessilis* from across its recorded (Gullan 1984, Cook 2000) distribution in eastern Australia to determine whether there is previously unsampled karyotypic diversity. Studying both highly variable and chromosomally conservative species is important to help us understand the role that chromosomal evolution might be playing in speciation in this genus.

Materials and Methods

Collections

We used specimens of *A. sessilis* that had been collected previously by Cook, in addition to specimens collected during this study (Table 1). Many sites from eastern Australia were searched for galls of *A. sessilis* across a twenty-year period. By necessity, collection of *A. sessilis* was restricted to eucalypt saplings, branches of mature trees within reach of the collector, and mature trees and branches that had fallen naturally. Galls were still attached to host material when removed from their host. Whenever possible, flowers and fruit were also collected from the host to allow identification of the host to species. Galls and attached plant material were kept in plastic or paper bags until returning to the laboratory.

TABLE 1. Collection details of specimens of *Apiomorpha sessilis* and *A. minor* used in this study. Some specimens from populations Sess1-Sess9, Min1 and Min38 were also used by Cook (2000). The diploid chromosome numbers are under the column titled "2n". Classification of *Eucalyptus* sections as in Brooker (2000).

Species	Population / Code	2n	Latitude (S)	Longitude (E)	State / Territory	Collector[1]	Host eucalypt	*Eucalyptus* section
A. sessilis	Sess1	4	34°43'	150°00'	New South Wales	GPH	*E.* sp.	unidentified
A. sessilis	Sess2	4	36°09'	150°06'	New South Wales	PJG	*E.* sp.	unidentified
A. sessilis	Sess3	4	36°09'	150°06'	New South Wales	PJG	*E.* sp.	unidentified
A. sessilis	Sess4	4	36°11'	150°07'	New South Wales	LGC	*E.* sp.	unidentified
A. sessilis	Sess5	4	37°03'	149°06'	New South Wales	PJG	*E. globoidea*	Capillulus
A. sessilis	Sess6	4	37°03'	149°06'	New South Wales	PJG	*E. globoidea*	Capillulus
A. sessilis	Sess7	4	37°03'	149°06'	New South Wales	PJG	*E. globoidea*	Capillulus
A. sessilis	Sess8	4	33°41'	150°32'	New South Wales	MSV	*E. agglomerata*	Capillulus
A. sessilis	Sess9	4	37°14'	149°56'	New South Wales	PJG	*E. muelleriana*	Capillulus
A. sessilis	Sess10	4	34°16'	150°39'	New South Wales	LGC	*E. oblonga*[2]	Capillulus
A. sessilis	PJM00031	4	27°36'	153°10'	Queensland	RDE	*E.* sp.	unidentified
A. sessilis	Min1	42	30°49'	153°00'	New South Wales	GPH	*E. pilularis*	Pseudophloius
A. sessilis	Min9	84	35°21'	149°14'	New South Wales	LGC	*E. macrorhyncha*	Capillulus
A. sessilis	Min38	10	35°16'	149°06'	Australian Capital Territory	SD	*E. rossii*	Cineraceae

[1]GPH = Greg Harper, PJG = Penny Gullan, LGC = Lyn Cook, MSV = M Vaarwerk, RDE = Robert Edwards, SD = Stuart Donaldson

[2]*E. oblonga* is no longer recognised as a valid species of *Eucalyptus* (Brooker 2000). The host is either *E. globoidea* or *E. sparsiflora* (*E.* section Capillulus).

Chromosome preparation

Ovarian tissue, commonly containing developing embryos, from adult females was used to prepare chromosome squashes. The chromosomes were prepared using a modification of Rowell's (1985) method (Cook 2000). Briefly, tissue was swollen in 70% hypotonic insect saline for about 30 min before fixing in 3:1 ethanol: acetic acid. Tissue was then macerated on a glass microscope slide in a drop of 60% acetic acid and dried on a hot plate at 60˚C. Dried slides were stained with 10% Giemsa stain for about 20 min then washed briefly with distilled water before drying. Slides were viewed under a compound microscope and chromosome counts were determined from at least 20 mitotic cells per individual, when available. Photographs were taken using a high-powered Q-imaging camera (SN: Q20982) attached to an Olympus CX21 compound microscope at 400x magnification. New photographs were also obtained for previously published karyotypes of *A. minor*.

Results

Specimens included in this study were found across a range of approximately 1,100 km from Lake Womboyn (New South Wales) in the south to Daisy Hill (Queensland) in the north (Table 1). Only female specimens of *A. sessilis* were found and thus adult males of *A. sessilis* are still unknown (Gullan 1984, Cook unpubl.). However, the presence of heterochromatic bodies in some developing embryos suggests that male embryos were present. Males of *Apiomorpha* have a haploid set of chromosomes present as a heterochromatic body within most cells (Cook 2000) and these can be clearly seen in preparation of ovarian tissue containing embryos. Almost sixty percent of the galls of adult females of *A. sessilis* collected contained no specimens or a dead female. Additionally, we were unable to obtain clear chromosome counts for two specimens. Chromosome counts were obtained from eleven adult females of *A. sessilis* representing eight populations. All had a diploid count of 2n = 4 (Table 1) (Fig. 1a-c). Individual mitotic chromosomes are larger in *A. sessilis* than in karyotypes of *A. minor* (Fig. 1).

Discussion

Although *A. sessilis* has been reported from Queensland, New South Wales and Victoria (Gullan 1984), we did not obtain any from the latter state. Despite more than 500 hours of search-time across more than 2,000 km of latitude, very few live individuals were found (Table 1). This difficulty in finding specimens is partly due to the patchy distribution of species of *Apiomorpha* in general (Gullan 1984, Cook and Gullan 2001), but also possibly to the rarity of *A. sessilis*.

This study expanded the current geographic range of karyotyped specimens of *A. sessilis* from across approximately 400 km in Cook (2000) to about 1,100 km. Other species of *Apiomorpha*, *A. bauerleni, A. ovicola, A. variabilis* and *A. withersi*, have also been found to lack variation in karyotype throughout an equivalent geographic range (Cook 2000). In contrast, across a similar range, *A. minor* has at least three very different karyotypes (2n = 10, 42 and 84) (Cook 2000) which were confirmed in this study (Fig. 1d-f). The lack of variation in *A. sessilis* raises the question of why *A. sessilis* has such a conserved karyotype throughout their ranges, whereas *A. minor* is so chromosomally diverse.

It has been suggested that the extent of chromosomal variation within some described species of *Apiomorpha* likely indicates cryptic species because correct segregation during meiosis in hybrid females would be unlikely (Cook 2001). Subsequent studies, using additional data, have determined

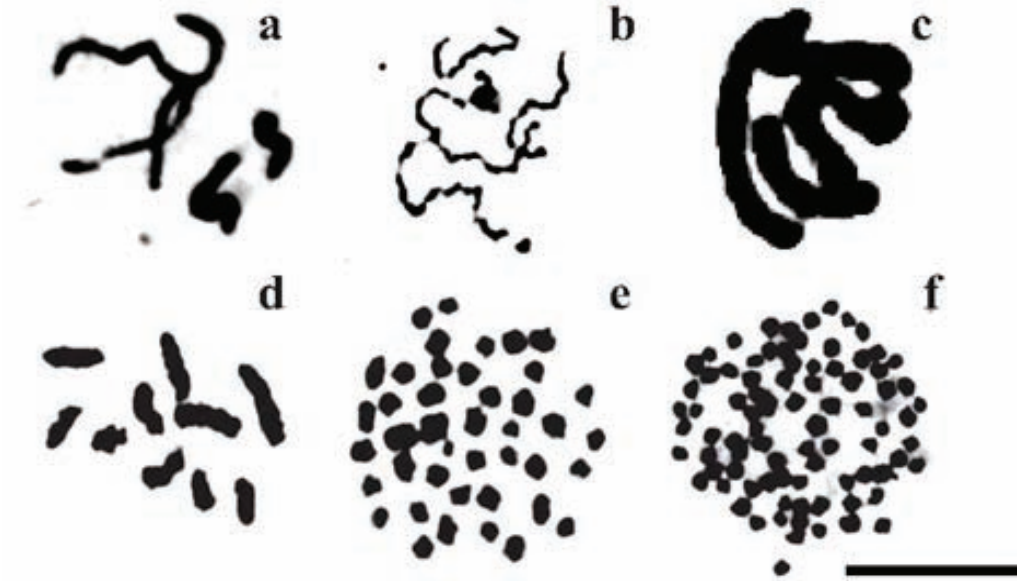

FIG. 1. Chromosome squashes from specimens of the *Apiomorpha minor* species-group. Top: Karyotypes from specimens of *Apiomorpha sessilis* (2n = 4). 1a = Sess10, 1b = Sess9 and 1c = PJM00031. Bottom: Karyotypes from specimens of *A. minor*. 1d = Min38 (2n = 10). 1e = Min1 (2n = 42). 1f = Min9 (2n = 84). Scale bar = 10μm

that some of the intraspecific variation likely indicates cryptic species complexes e.g. in *A. munita* (2n = 6 - 100) (Cook and Rowell 2007), *A. pharetrata* (2n = 40, 42, 46, 48 and 56) and *A. thorntoni* (2n = 10, 26 and 28) (Cook and Gullan 2008). Thus, it is likely that the karyotypic variation in *A. minor* reported by Cook (2000) represents differences among cryptic species, whereas the lack of variation in *A. sessilis* suggests that it is not likely to be a cryptic species-complex. However, these conclusions must be made with caution, due to the small number of individuals that were sampled in this study.

In *A. munita*, cryptic species appear to be associated with host eucalypts belonging to different sections of *Eucalyptus* subgenus *Symphyomyrtus* (Cook and Rowell 2007). *Apiomorpha minor* and *A. sessilis* appear to be restricted to *Eucalyptus* subgenus *Eucalyptus* (Cook unpublished), with earlier reports of *A. minor* being found also on *Eucalyptus* subgenus *Symphyomyrtus* (Gullan 1984) probably due to misidentification of host plants (Cook unpublished). Although the specimens of *A. minor* and *A. sessilis* used in this study have a similar number of identified host eucalypt species (Table 1), all host species recorded for *A. sessilis* occur within one eucalypt section, *Eucalyptus* subgenus *Eucalyptus* section Capillulus (Table 1). Each of the three host species recorded for *A. minor* are found in different eucalypt sections from each other (Table 1). The larger range of host use in *A. minor* is further indication that there might be multiple cryptic species, and thus *A. minor* needs to be investigated more thoroughly. Increased sampling of *A. sessilis* and *A. minor* throughout their range is still required for further studies. It is also hoped that species boundaries of these two described species, and *A. minor*

in particular, can be tested using chromosome numbers, molecular phylogenies and morphological characters.

Acknowledgements

Thank you to Penny Gullan, Greg Harper, Mark Vaarwerk and Bort Edwards for collecting some of the specimens used for this study. We would also like to thank State Forests of New South Wales and Queensland Parks and Wildlife for issuing permits to collect the specimens used in this research. Thank you also to David Merritt and Cynthia Riginos (UQ), and two anonymous reviewers for giving valuable feedback on the manuscript.

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Διαφορές στη χρωμοσωμική παραλλακτικότητα στο είδος *Apiomorpha minor*

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ΠΕΡΙΛΗΨΗ

Στο γένος *Apiomorpha* παρατηρείται μια από τις μεγαλύτερες χρωμοσωμικές αποκλίσεις στα γένη ζωικών οργανισμών, με τον διπλοειδή αριθμό χρωμοσωμάτων να κυμαίνεται από 4 ως 192. Παρατηρείται επίσης σημαντική διαφοροποίηση και σε πολλά από τα 41 αναγνωρισμένα είδη. Για παράδειγμα, η παραλλακτικότητα στην ομάδα ειδών *A. minor* (*A. minor, A. sessilis* και *A. annulata*) παρουσιάζει ένα εξαιρετικά μεγάλο εύρος που κυμαίνεται από 2n = 4 ως 2n = 84. Ωστόσο, η μεγαλύτερη από αυτή την παραλλακτικότητα υπάρχει στο είδος *A. minor*. Αντίθετα, σε ένα άλλο είδος από την ομάδα ειδών του *A. minor* το *A. sessilis,* έχουν παρατηρηθεί μόνο 2n = 4. Για να εξακριβώσουμε αν η παρατηρούμενη έλλειψη παραλλακτικότητας στο *A. sessilis* ήταν λόγω περιορισμένης δειγματοληψίας, συγκεντρώσαμε άτομα από ολόκληρο το γνωστό εύρος διασποράς στην ανατολική Αυστραλία (> 1,100 km). Δεν βρήκαμε κανένα διαφορετικό αριθμό χρωμοσωμάτων στο *A. sessilis*, επιβεβαιώνοντας τη σταθερότητα του καρυότυπου στο είδος. Αυτό εγείρει την ερώτηση "γιατί στο είδος *A. sessilis* υπάρχει ένας τόσο σταθερός καρυότυπος σε όλο το εύρος διασποράς του ενώ στο *A. minor* είναι τόσο ποικίλος;