Molecular Phylogenetics and Evolution 77 (2014) 126–135

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Rapid chromosomal evolution in a morphologically cryptic radiation

Penelope J. Mills *, Lyn G. Cook

The University of Queensland, School of Biological Sciences, Brisbane, QLD 4072, Australia

ARTICLE INFO

Article history:
Received 20 May 2013
Revised 7 March 2014
Accepted 14 March 2014
Available online 26 March 2014

Keywords:
Cryptic species
DNA sequences
Karyotype
Host specificity
Eucalyptus
Speciation

ABSTRACT

Cryptic species occur within most of the major taxonomic divisions, and a current challenge is to determine why some lineages have more cryptic species than others. It is expected that cryptic species are more common in groups where there are life histories or genetic architectures that promote speciation in the absence of apparent morphological differentiation. Chromosomal rearrangements have the potential to lead to post-zygotic isolation and might be an important factor leading to cryptic species. Here we investigate the potential role of chromosomal change in driving speciation in the karyotypically diverse scale insect genus *Apiomorpha*, focussing on four species placed in the same species group (the *A. minor* species group Gullan, 1984). Using mitochondrial and nuclear DNA sequence data, we find that *Apiomorpha minor* is not monophyletic and consists of at least nine cryptic species. Diploid chromosome counts range from $2n = 4$ to $2n = 84$ across the four currently recognized species, and some of the chromosomal variation exists in the absence of other genetic or host use differences, consistent with karyotypic changes being involved in lineage divergence and the generation of cryptic species.

1. Introduction

The molecular revolution in taxonomy and systematics has led to the discovery of many instances of cryptic species—two or more species that are morphologically similar or indistinguishable but have been classified as a single species (e.g., Bernardo, 2011; Bickford et al., 2007; Pfenninger and Schwenk, 2007; Schönrogge et al., 2002). Cryptic species are found throughout most taxonomic groups (Bickford et al., 2007; Pfenninger and Schwenk, 2007) and their prevalence might rival that of yet-to-be-discovered and undescribed morphologically distinct species.

Because taxonomy has traditionally been based on adult morphology (Cook et al., 2010; Lefébure et al., 2006), there are expected to be fewer cryptic taxa in groups where there is strong divergent selection on morphology, for example, resulting from ecological or sexual selection (e.g., three-spined sticklebacks, McKinnon and Rundle, 2002; passerine birds, Barraclough et al., 1995; and birds-of-paradise, Irestedt et al., 2009). Conversely, cryptic species are expected to be more common in those groups in which divergent selection on morphological features has been weak (Bickford et al., 2007).

Cryptic species might also be prevalent when fitness is tied to host specificity rather than gross morphological traits. For example, the 10 *COI* lineages of the cryptic species complex *Astraptes fulgerator* (Hebert et al., 2004) were largely differentiated by the caterpillars' feeding on different host plants and in their having divergent color patterns, whereas adult butterflies exhibited no easily discernible morphological differentiation. Host-specific cryptic species have been frequently documented across many insect orders (e.g., Diptera, Smith et al., 2006; Hymenoptera, Smith et al., 2008; Lepidoptera, Hajibabaei et al., 2006) including groups that contain species with broad distributions and host ranges, such as scale insects (Hemiptera: Coccoidea). High estimates of polyphagy in a number of scale insect families, such as Coccidae (Lin et al., 2010), might be due to the presence of cryptic species complexes that comprise relatively host-specific taxa, such as that found for the pine-feeding armoured scale genus *Chionaspis* (Diaspididae) (Gwiazdowski et al., 2011), the gall-inducing felt scale *Apiomorpha* (Eriococcidae) (Cook and Rowell, 2007) and the pest mealybug *Ferrisia virgata* (Pseudococcidae) (Gullan et al., 2010).

Host-specific cryptic taxa might arise in sympatry, or parapatry, if divergent selection is acting on areas of the genome associated with adaptation to different hosts or habitats (Feder et al., 2012). Such genomic differences could lead to reproductive isolation if there is strong selection against hybrids and migrants (e.g., Avise, 2000; Mallet and Barton, 1989; Nosil et al., 2012, 2007; Via, 2001; Wu and Ting, 2004). If recombination continues to occur in these genomic regions, reproductive isolation is expected to break down (Felsenstein, 1981). However, rearranged areas (e.g., inversions, translocations) of the genome have reduced rates of recombination (e.g., Ayala and Coluzzi, 2005; Noor et al., 2001;

* Corresponding author.
E-mail address: penelope.mills@uqconnect.edu.au (P.J. Mills).

http://dx.doi.org/10.1016/j.ympev.2014.03.015
1055-7903/

Ortiz-Barrientos et al., 2002; Rieseberg, 2001) compared with collinear regions. Because of the resulting lack of recombination, rearranged areas containing genes associated with adaptation can diverge between different sympatric or parapatric populations (Ayala and Coluzzi, 2005). If genes associated with reproductive isolation and adaptive divergence are found within these rearranged regions, the formation of new species could occur (e.g., Ayala and Coluzzi, 2005; Coluzzi, 1982; Navarro and Barton, 2003; Noor et al., 2001; Rieseberg, 2001).

The Australian scale insect genus *Apiomorpha* Rübsaamen, 1894 exhibits both chromosomal diversity and host specificity. There is extensive chromosome variation within the genus, with chromosome numbers ranging between $2n = 4$ to $2n = 192$ (Cook, 2000), and variation has been reported within eighteen of the described species (Cook, 2000, 2001; Cook and Gullan, 2008). Cryptic taxa have been revealed in the two cases where chromosomally diverse species have been examined using genetic data (*A. munita*, Cook and Rowell, 2007) or species-specific gall development (*A. pharetrata*, Cook and Gullan, 2008). The genus is relatively host-specific, being restricted to a single but widespread and species-rich plant genus *Eucalyptus* (Gullan et al., 2005). The cryptic taxa identified in *A. munita* were found to be restricted to different host sections (a botanical classification rank below genus and subgenus level) within *Eucalyptus* subgenus *Symphyomyrtus* (Cook, 2001; Cook and Rowell, 2007), whereas the two chromosomally differentiated taxa identified in *A. pharetrata* were able to be reared on the same hosts (Cook and Gullan, 2008).

Here we focus on *A. minor* (Froggatt, 1893), a morphospecies with a reported karyotypic range of $2n = 10$, 42 and 84 (Cook, 2000). The sizes of chromosomes within complements indicate that the variation likely results from multiple chromosomal fissions and/or fusions, rather than polyploidy, because chromosomes are larger when chromosome complement is low, and small when there are many chromosomes (Cook, 2000). Such large differences in karyotype are predicted to result in infertile hybrid offspring because, during meiosis, it is highly unlikely that chromosomes will align and segregate correctly (Cook, 2000; Cook and Gullan, 2008). Thus, chromosomal rearrangements might be playing a role in reproductive isolation without concurrent divergence in adult morphology.

We karyotyped specimens from across the known range of *A. minor* and its close relatives, and used DNA sequence data to assess whether the different chromosome forms represent distinct lineages and potentially discrete taxonomic units. Furthermore, we assessed whether chromosomal differentiation might be driving diversification in the group. Host use and geographic distribution were also considered to determine whether these might be a better explanation for lineage divergence.

2. Materials and methods

2.1. Sampling

Apiomorpha minor, like other species of *Apiomorpha*, is patchily distributed (Gullan, 1984), rarely collected and relatively poorly represented in museum collections, e.g., there are no specimens held in the Queensland Museum despite the species occurring in that state. Additionally, *Apiomorpha* have high rates of parasitism in the field (LGC, PJM unpublished). *Apiomorpha minor* has been recorded as occurring down the eastern seaboard of Australia from as far north as Fraser Island (Qld) to near Melbourne (Vic.) in the south (Gullan, 1984), a distance of about 1600 km. We collected 158 adult specimens of *A. minor* from across its known range over a 20-year period (Supplementary Table S1). Forty-four adult specimens of other members of the *A. minor* species group Gullan, 1984 (*A. annulata* Froggatt, 1930, *A. nookara* Mills, MacDonald, Rigby and Cook 2011, and *A. sessilis* (Froggatt, 1895)) were also collected. Two individuals of *A. maliformis* (Fuller, 1897) and eight specimens of *A. variabilis* (Froggatt, 1893) (*A. strombylosa* species group Gullan, 1984) were used as outgroups for phylogenetic analyses. All specimens were identified to morphological species using Gullan (1984). Ten specimens did not match any described species but were similar to *A. nookara*. Host plants were identified using Brooker and Kleinig (2006) and classified to section according to Brooker (2000).

2.2. DNA extraction, sequencing and alignment

DNA was extracted from individual specimens using one of three methods: the salting-out method of Sunnucks and Hales (1996) (collections prior to May 2004), a Qiagen DNeasy Tissue Kit (cat. no. 69506) following the manufacturer's instructions (collections between May 2004 and December 2006), or a modified version of the CTAB/chloroform protocol found in Doyle and Doyle (1990) (collections after December 2006). Either whole specimens or 100–200 mg tissue were placed in a 1.5 mL microfuge tube and (if removed from 100% ethanol) left to air dry. 600 μL CTAB buffer and 5 or 10 μL Proteinase K was added. The tubes were incubated at 55 °C for at least 3 h. After the lysis incubation, 300 μL chloroform was added, and the tubes were gently rocked for 15 min. After spinning at 13,000 rpm for 5 min, the aqueous layer was transferred to a new tube, and 600 μL 100% ethanol was added. After vortexing, tubes were spun for 10 min at 13,000 rpm. The supernatant was removed and the DNA pellet was washed twice with 70% ethanol, spinning at 13,000 rpm for 1 min after each wash. The pellet was left to air dry, before being resuspended in 50, 100, or 200 μL Qiagen AE (elution) buffer. Cuticles were removed after the lysis incubation and stored in 70% ethanol until being mounted on slides. After resuspension, DNA extracted using the CTAB/chloroform protocol was quantified on an agarose gel and diluted to about 50 ng/μL.

Two gene regions were amplified: the 5′ region of nuclear small subunit ribosomal DNA (*18S* rDNA) and the 5′ region of mitochondrial cytochrome oxidase subunit 1 (*COI*). Details of the primers used for the two gene regions are given in Table 1. Each 25 μL PCR reaction included 13 μL H_2O, 5 μL MangoTaq PCR buffer (5×), 2 μL dNTP (2 mM), 1.5 μL $MgCl_2$ (50 mM), 0.5 μL forward primer (10 μM), 0.5 μL reverse primer (10 μM), 1 unit of MangoTaq (Bioline Australia) and 2 μL of DNA template. PCR cycles for DNA amplification used an initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, primer anneal-

Table 1
Primers for each gene region used in this study.

Primer	Direction	Sequence	Reference
SSU rDNA (18S)			
2880	F	5′-CTGGTTGATCCTGCCAGTAG-3′	Tautz et al. (1988)
B-	R	5′-CCGCGGCTGCTGGCACCAGA-3′	von Dohlen and Moran (1995)
Cytochrome Oxidase subunit 1 (COI)			
F108-COI-Apio	F	5′-ATAGGWATATCTATAAGTATAATTAT-3′	Mills et al. (2011)
PcoF1	F	5′-CCTTCAACTAATCATAAAAATATYAG-3′	Park et al. (2010)
C1-J-2183 (Jerry, reverse)	R	5′-CCAAAAAATCAAAATAAATGTTG-3′	Simon et al. (1994)