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Geographic parthenogenesis and the common tea-tree stick insect of New Zealand

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Abstract

Worldwide, parthenogenetic reproduction has evolved many times in the stick insects (Phasmatidae). Many parthenogenetic stick insects show the distribution pattern known as geographic parthenogenesis, in that they occupy habitats that are at higher altitude or latitude compared with their sexual relatives. Although it is often assumed that, in the short term, parthenogenetic populations will have a reproductive advantage over sexual populations; this is not necessarily the case. We present data on the distribution and evolutionary relationships of sexual and asexual populations of the New Zealand stick insect, *Clitarchus hookeri*. Males are common in the northern half of the species' range but rare or absent elsewhere, and we found that most *C. hookeri* from putative-parthenogenetic populations share a common ancestor. Female stick insects from bisexual populations of *Clitarchus hookeri* are capable of parthenogenetic reproduction, but those insects from putative-parthenogenetic populations produced few offspring via sexual reproduction when males were available. We found similar fertility (hatching success) in mated and virgin females. Mated females produce equal numbers of male and female offspring, with most hatching about 9–16 weeks after laying. In contrast, most eggs from unmated females took longer to hatch (21–23 weeks), and most offspring were female. It appears that all *C. hookeri* females are capable of parthenogenetic reproduction, and thus could benefit from the numerical advantage this yields. Nevertheless, our phylogeographic evidence shows that the majority of all-female populations over a wide geographic area originate from a single loss of sexual reproduction.

Keywords: asexual reproduction, developmental rate, fertility, geographic parthenogenesis, sexual reproduction

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Introduction

The term geographic parthenogenesis refers to a general distribution pattern observed in animal species with mixed reproductive systems. For a given species, parthenogenetic populations (those reproducing without sex) frequently have distinct ranges, that are typically in marginal habitats, at higher altitude and/or latitude than their sexual relatives (Vandel 1928; Lynch 1984). A number of explanations for geographic parthenogenesis have been presented. The hypothesis providing the most universal explanation is that adaptation to marginal habitats occurs more rapidly in the absence of gene flow from central populations, allowing parthenogenetic populations to adapt and expand their range beyond their sexual ancestor (Peck *et al.* 1998). By avoiding sexual reproduction, parthenogenetic populations escape the homogenizing effect of gene flow and therefore may have an ability to more rapidly adapt to marginal environments where they are in low density (Kirkpatrick & Barton 1997; Peck *et al.* 1998). However, it has also been pointed out that many geographic parthenogens evolve via hybridization and/or polyploidy, and a general role for both hybrid and polyploid advantage has been proposed (Kearney 2005; Lundmark & Saura 2006). Further, Haag & Ebert (2004) suggest that where local extinction and recolonization is

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common in marginal habitats, parthenogenetic lineages may replace sexual ones due to inbreeding depression suffered within small sexual populations.

Selective advantage of parthenogenetic individuals might also rest in their potential for long distance colonization. Thus geographic parthenogenesis may result from differential ability to colonize areas following climate warming of interglacial cycles, because a single individual or egg can give rise to a new population following dispersal. For example, the stick insect, *Argosarchus horridus*, is a facultative parthenogen that has probably expanded its range in New Zealand since the last glacial maximum (Buckley *et al.* 2009), and a population on the geologically young Chatham Islands are all-female (Trewick *et al.* 2005). The New Zealand tea-tree stick insect, *Clitarchus hookeri* (White) was accidentally introduced to the United Kingdom where all-female populations have established in the Scilly Isles (Brock 1987). Parthenogenetic reproduction is common in stick insects (Phasmida) where many independent lineages have evolved the ability to reproduce without males (Bullini 1994). Some lineages are obligate parthenogens (with males entirely absent) but many other species are facultative parthenogens and have the ability to reproduce with or without males. The term facultative parthenogen is applied to a species when both sexual and asexual reproduction has been observed, although the ability of individual females to reproduce in both ways has rarely been tested (see Bedford 1978). It is possible that once lost to a lineage, sexual reproduction is not retrievable, in which case parthenogenetic females would be genetically isolated from their sexual siblings.

The theoretical reproductive output per individual for asexual species is twice that for sexual species where a male–female pair produce the same number of offspring as a single asexual female, hence the twofold cost of sex. Thus, a key assumption in most theoretical studies of the maintenance of sexual reproduction is that populations of parthenogenetic animals have a higher intrinsic rate of increase compared with populations of related bisexual animals (Otto & Lenormand 2002). However, some empirical studies have shown that parthenogenetic females do not have higher fecundity than their sexual relatives (Bedford 1978; Lamb & Willey 1979; Lynch 1984; Kearney & Shine 2005). In some cases fecundity may appear high, if for example, many eggs are laid per female, but this can be offset by low egg hatching rate (fertility). Therefore, comparisons of parthenogenetic and sexual females need to differentiate the many factors that contribute to estimates of net fecundity. In addition, the reproductive strategy of the individual is an important factor determining future interactions of sexual and parthenogenetic populations. For example, do individuals reproduce both sexually and parthenogenetically as the opportunity arises? Can females from parthenogenetic populations regain sexual reproduction? It is necessary to determine the details of reproductive behaviour and success in order to understand the origins of geographic parthenogenesis and to predict future distribution patterns (Schwander & Crespi 2009).

The tea-tree stick insect *Clitarchus hookeri* is endemic to New Zealand. Salmon (1955) reported that this species can reproduce parthenogenetically and sexually. In many locations males and females are found with similar frequency and adults are often found in *copula*, particularly in late summer, whereas at other locations, female *C. hookeri* greatly out-number males (Trewick & Morgan-Richards 2005). The distribution of sexual and parthenogenetic populations of *C. hookeri* has not previously been described, but if the distribution of parthenogenetic populations of this insect matches the classic pattern of geographic parthenogenesis then we expect the asexual populations to occur at the southern limit of this species' range. Many stick insect taxa are complexes of diploid and polyploidy populations, and often hybridization is involved in species formation (Bullini 1994; Scali *et al.* 2003). *Clitarchus hookeri* is implicated as one parental species in the hybrid origin of the genus *Acanthoxyla* (Morgan-Richards & Trewick 2005). *Acanthoxyla* consists of many morphologically distinct lineages, each of which is an obligate parthenogen and some of which may be triploid (Buckley *et al.* 2008). However, there is currently no evidence for polyploidy or hybrid origin in *C. hookeri* itself. Cytogenetics, which provides the most explicit and simple way to test for polyploidy, revealed that all individuals from three sexual and two putative-parthenogenetic populations were diploid ($2n$ = 35(XO)/36(XX); Parfitt 1980; Morgan-Richards & Trewick 2005). Low heterozgosity levels in putative-single copy nuclear loci are also indicative of diploids (Buckley 1995; Buckley *et al.* 2008).

We set out to determine whether *Clitarchus hookeri* have a geographic parthenogenetic distribution, whereby all-female populations occur further from the equator than sexual populations. We address the history of these populations using analyses of mtDNA phylogeographic data. Are the parthenogenetic populations derived from the sexual populations, and if so, how many times? Are the sexual and parthenogenetic populations stable or expanding? Although reproductive outcomes are well studied in certain model organisms, reproductive biology in insects that are the subject of biogeographic and population genetic studies are often deficient. Here we used husbandry and crossing experiments to investigate the reproductive biology of *C. hookeri* with a view to better understanding the interactions

of the alternative strategies, focusing on the key issue of whether *C. hookeri* is a true facultative parthenogen. Do females from bisexual populations employ a mixed mating strategy? Can females from parthenogentic populations reproduce sexually if a male becomes available? We consider whether these alternative reproductive strategies confer short-term fitness advantages by comparing development time of eggs, and fertility.

Materials and methods

Common New Zealand tea-tree stick insect

Clitarchus hookeri is most commonly found on the Myrtaceae species *Leptospermum scoparium* (manuka) and *Kunzea ericoides* (kanuka), collectively known as tea-tree. Adult female tea-tree stick insects are about 8 cm long, the males shorter (~6 cm) and thinner. The exoskeleton is smooth or lightly rugose but lacks sharp spines. Individuals are highly cryptic, often bright green, some with yellow lateral marks and pale blue armpits but body colour may be grey, brown or buff (Trewick & Morgan-Richards 2005). At most locations, individuals of different colours and rugosity are found together. Adult females, feeding in trees, drop eggs at a rate of about one every 17 h (Stringer 1967), and these fall to the ground and remain in the leaf litter until hatching.

Evidence for the current distribution of sexually reproducing populations was obtained from our own records of locations where males of *Clitarchus hookeri* have been collected in the wild (see Appendix S1 in the Supporting Information, available in the online version of the journal) and those in the Museum of New Zealand Te Papa Tongarewa collection. However, much of the pinned material in the Te Papa Tongarewa collection was contributed by John T. Salmon who raised many stick insects in captivity, and these were not included in our study.

Sampling

We collected *Clitarchus hookeri* from sites around New Zealand to obtain a representative sample of their sexual and geographic diversity (Fig. 1, Appendices S1 and S2). Most *C. hookeri* were collected from tea-tree, but a few were also found on *Metrosideros* spp. (native rata and pohutakawa) and Rosaceae (exotic plum, bramble, rose). Identification of the predominate mode of reproduction of individuals at each location was based on the observed sex ratio; sexual reproduction was inferred where the sex ratio was approximately 1:1. Where males were rare or absent the population was identified as putative-parthenogenetic. Only in the Wellington region (including Orongorongo Valley) were males present but uncommon and so interpretation problematic, thus we refer to the females from within the Wellington region as putative-parthenogenetic but recognize this region is distinctive and further work is required.

DNA extraction, amplification and sequencing

Legs from fresh, frozen or alcohol preserved specimens were chopped up for extraction of genomic DNA using the salting-out method (Sunnucks & Hale 1996). We amplified and sequenced a mitochondrial fragment, comprising the three' end of the cytochrome oxidase I (COI), tRNA-Leucine, and cytochrome oxidase II (COII), using primers C1-J-2195 and TK-N-3785 (Simon *et al.* 1994). For older material, two tRNA-Leu primers L2-N-3014 and TL2-J-3034 (Simon *et al.* 1994) were used in conjunction with the previous primer pair to amplify two shorter fragments. Sequences from previous studies using *C. hookeri* are included (Morgan-Richards & Trewick 2005; Buckley *et al.* 2008). Polymerase chain reactions were performed in 20 μL volumes containing: 200 μM dNTPs, 2 mM MgCl, 1 μM primers, and 0.20 U of RedHot DNA polymerase (ABgene) and 1–5 ng template DNA. Amplification cycles consisted of: denaturation at 94 °C for 60 s followed by 35 cycles of 94 °C for 15 s, 50 °C for 15 s and 72 °C for 45 s. Amplified DNA products were treated to Shrimp Alkaline Phosphotase/Exonuclease I digestion prior to sequencing. Cycle sequencing with the PCR primers used Bigdye chemistry (PE) following the manufacturer's protocols, with automated reading on an ABI3730. Consensus sequences were obtained using Sequencher v4.1 (ABI, PE), and aligned using SeAl v2.0a3 (Rambaut 1996). The tRNA-Leu gene between COI and COII was excluded from analyses as this was missing for many specimens due to its use in PCR priming. The sequences were translated to check for stop codons, frame shifts and amino-acid substitutions that might indicate nuclear copies.

Sequence analysis

For phylogenetic reconstruction we used Paup *4.0b10 (Swofford 2002) to implement jModelTest (Posada 2008), Maximium Likelihood (ML) and calculate pairwise genetic distances (absolute and HKY). GENEIOUS (Drummond *et al.* 2009) was used to implement Bayesian analyses using MRBAYES version 3.1 (Ronquist & Huelsenbeck 2005) with GTR + I + G and HKY + I + G models of DNA evolution. Each Bayesian analysis consisted of two parallel runs with four chains for 6 000 000 generations, sampling every 1000 generations and applying a burn-in of 10%. Since ML and Bayesian results were congruent we present a ML tree with

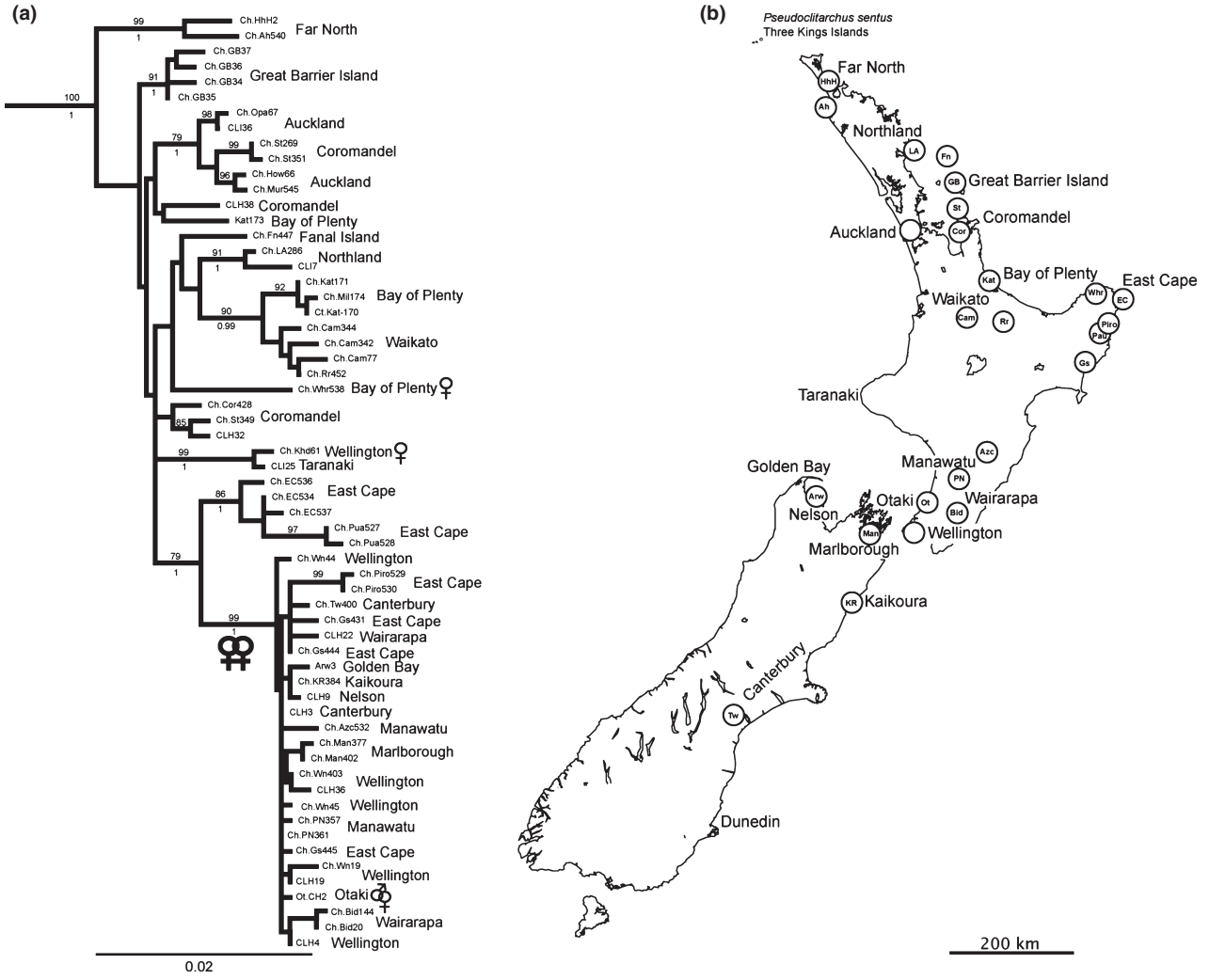

Fig. 1 Phylogenetic relationships of mtDNA haplotypes of the New Zealand common tea-tree stick insect *Clitarchus hookeri*. (a) Maximum Likelihood analysis of 1350 bp COI–COII DNA sequence. Stick insects from putative-parthenogenetic populations are part of a single clade (double female symbols), with two exceptions (single female symbols). Node support shown where Maximum Likelihood bootstraps are above 70% (above branches) and Baysian Posterior Probabilities above 0.96 (below branches). Tree was rooted with three outgroup taxa (*Pseudoclitarchus sentus*, *Acanthoxyla prasina*, *Argosarchus horridus*). (b) New Zealand sampling locations of *Clitarchus hookeri* and regions referred to in the text are shown. Both Auckland and Wellington are regions represented by many sample sites (see Appendix S2).

Bayesian posterior probabilities and PHYML (Guindon & Gascuel 2003) bootstrap values (1000 replicates) for the major lineages. A minimum spanning network for a subset of haplotypes was constructed without the need of computer software. We used three New Zealand species as the outgroup; the sexual species *Pseudoclitarchus sentus* that is sister to *Clitarchus hookeri*, the obligate pathenogen *Acanthoxyla prasina*, and the facultative parthenogen *Argosarchus horridus* (Trewick *et al.* 2005, 2008).

We used absolute pairwise sequence differences to examine the mismatch distributions among haplotypes within *Clitarchus hookeri*. Time since expansion was not estimated due to lack of information about mutation rate in these insects and the likelihood that time dependency of estimates of the rate of nucleotide substitution would make any inference of unknown value (lazy-j-shaped curve; Ho *et al.* 2005; Burridge *et al.* 2008).

Fertility

Fertility was estimated from hatching rates of eggs laid by females from a number of populations. A comparison of fertility of virgin and mated females used *C. hookeri* females collected from a single location (Lake Karapiro, Waikato). Each adult was held with either zero, one or two males from the same location, for the same 6 week period (Table 1). Copulation was observed where males were present. After 6 weeks all

Table 1 Fertility of mated and virgin females of the stick insect *Clitarchus hookeri* from Waikato. All eggs laid over the same six week period were included

Female code	C337	C339	C342	C344	C338
Number of males held with	1	1	2	2	0
Number of eggs laid	54	93	54	44	112
Number of female offspring	21	35	29	19	107
Number of male offspring	28	49	19	23	0
Fertility (% hatched)	91	90	89	95	96

eggs were collected, counted and held in uniform conditions. The proportion of eggs that hatched, the hatching dates and sex of nymphs were recorded. These eggs provided preliminary estimates of fertility (hatching rate), fecundity (number of offspring produced over a set time period) and sex ratio data.

Rate of development

Adult and late instar *C. hookeri* were collected from Bethells Beach, Auckland every 3 weeks for 4 months. Females were either held with males (mated) or held without males (virgin). All virgin females were collected as late instars and reared while segregated from male stick insects. Eggs were collected from stick insects every three days throughout the summer and held under identical conditions. Upon hatching each nymph was sexed. Deviations from 1:1 sex ratio were tested with chi-squared test (1 d.f.) and mean time to hatching tested with Student's *t*-test. These eggs provided development time and sex ratio data.

Facultative parthenogenesis

To test whether females from sexual populations are capable of parthenogenetic reproduction, late instar females were collected from two locations where males were as common as females (sexual populations in Auckland and Waikato; Fig. 1). Females were reared without males until adult and their eggs were collected and held until they hatched. The resulting nymphs were then sexed using external morphological differences (Stringer 1970). Sub-adults of some phasmid species can mate and store sperm, so even sub-adults may not be virgin. However, in our study, no *C. hookeri* females collected as sub-adults from sexual populations, produced sons.

Obligate parthenogenesis

To test whether females from putative-parthenogenetic populations were obligate parthenogens, females were collected as late instar or nymphs from five locations where males are absent or rare (East Cape, Manawatu, Waiarapa, Wellington and Canterbury; Fig. 1). Once mature, each female was provided with a single adult male from a sexual population (Coromandel or Waikato) and the resulting eggs were collected and kept until they hatched. Copulation was observed in all experiments. The hatching rates of eggs laid before mating were recorded for five females, and post-mating hatching rates were recorded for eight females. All nymphs were sexed and the frequency of males in each case was used to estimate the proportion of offspring resulting from sexual reproduction.

Results

Distribution

We have distribution data of more than a thousand individual *Clitarchus hookeri* from 50 locations but male *C. hookeri* were confirmed from only half of these populations (Fig. 2a, Appendices S1 and S2). All *C. hookeri* collected from South Island were female. Our most southerly location was Peel Forest, Canterbury, where 24 females were sighted but no males. In North Island, only female stick insects were recorded at 17 locations, including all eastern locations (East Cape to Wairarapa; Fig. 2a, Appendix S1). The southern-most male *C. hookeri* were found in Wellington, where males made up 10% of the total sample for this region ($n = 50$; deviating from 1:1 ratio $P < 0.001$ chi squared). *Clitarchus hookeri* were recorded from 17 sites within the Wellington region but males have been found at only five of these. In contrast, at Otaki (Kapiti Coast), Lake Koripiko (Waikato), Coromandel Penninsula, Great Barrier Island and Auckland, ratios of males to females did not depart significantly from 1:1 ($P > 0.05$; chi-squared tests).

Origins of parthenogenetic lineages

We collected adult *Clitarchus hookeri* from 15 locations where both males and females are approximately equally abundant, and from 15 locations where males were absent or rare (Appendix S2). We sequenced one–10 stick insects from each of 30 locations (Figs 1 and 2, Appendix S2). This resulted in a total of 65 *Clitarchus hookeri* mtDNA sequences, (26 sexual, 39 putative-parthenogenetic). We added sequences from outgroup taxa, and 14 *Clitarchus hookeri* sequences from Genbank (Appendix S2). In total 62 unique COI–COII haplotypes were identified from a total of 83 stick insects. Of the 1350 bp of aligned mtDNA COI–COII sequence, 149 sites varied within *Clitarchus hookeri*. Phylogenetic analyses used a reduced dataset consisting of the 62 unique haplotypes with and without the outgroup. jModelTest

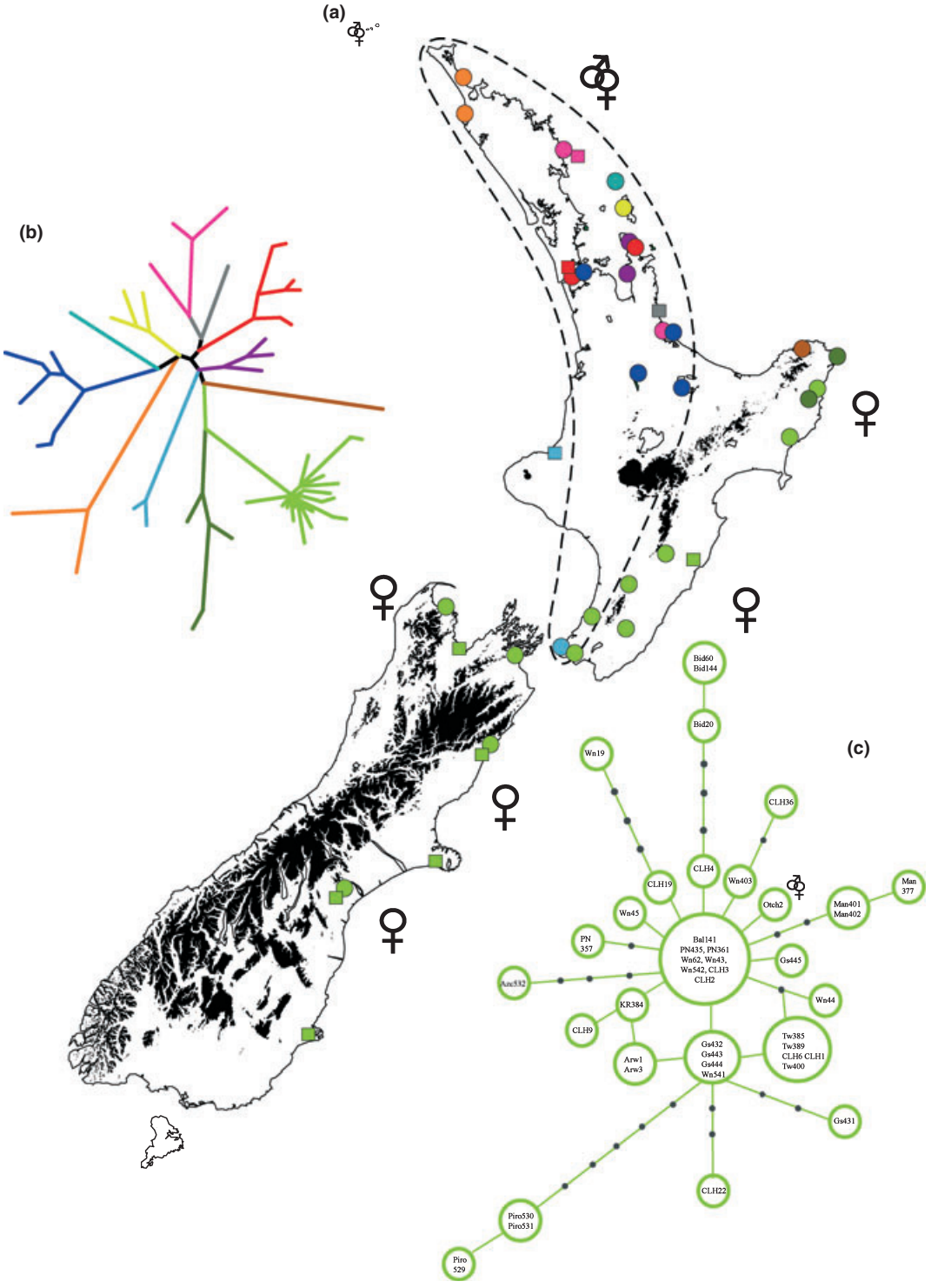

Fig. 2 Reproductive and genetic variation in the New Zealand common tea-tree stick insect *Clitarchus hookeri*. (a) Distribution of sexual and putative-parthenogenetic populations reveal a pattern of geographic parthenogenesis. Dotted line encloses sites where males have been recorded (1087 records from museum material and our sampling; Appendix S1). Only locations where *C. hookeri* were collected for genetic study are indicated: ○ = our data; □ = Buckley *et al*. 2008; colours represent distinct genetic clades as shown in B. (b) Unrooted Maximum Likelihood analysis of all 62 unique mtDNA haplotypes of *C. hookeri* (1350bp COI-COII). (c) Minimum spanning network of 25 haplotyoes from the parthenogenetic clade. Codes for the 37 stick insects are given in Appendix S2, haplotype circles scaled by sample size, inferred missing haplotypes indicated with small closed circles.

(Posada 2008) selected GTR+I+G model of DNA evolution. Inclusion of outgroup had little effect on ingroup relationships (Figs 1a and 2b). Maximum pairwise genetic distances were between sequences from stick insects in the most northern and eastern North Island locations (pairwise maximum of 39 differences, HKY85 0.03 divergence, between Ah-540 and Bid-144). In rooted trees, haplotypes from northern locations (Houhora Heads and Ahipara) were resolved as sister to the rest of the *C. hookeri* diversity (Fig. 1). The majority of locations were represented by a single individual, so genetic diversity was not compared among populations. Individuals from the same location (16 locations, 60 stick insects) usually group together in the phylogenies, but not exclusively (13/16).

Most haplotypes from putative-parthenogenetic populations (31/33) fall in one shallow monophyletic clade (Figs 1a and 2b). These haplotypes share substitutions at five nucleotide sites (almost exclusively). This parthenogenetic clade also includes one location where males are common (Otaki) and one location where males are uncommon (putative-parthenogenetic; Wellington). Of the 13 Wellington stick insects sequenced, one (Khd-61) has a haplotype outside the main parthenogenetic clade. Haplotypes from East Cape stick insects (where no males were found) fall in the parthenogenetic clade, but in the nearby Bay of Plenty (70 km west) a single female at Whanarua Bay had a haplotype outside this clade (Fig. 1a).

The sexual populations are paraphyletic with respect to the parthenogenetic clade. Thus, sexual reproduction appears to be the ancestral state, and the majority of southern parthenogenetic populations are most simply inferred as being derived from north-east locations. The diversity found within putative-parthenogenetic populations requires three independent origins. However, two apparently parthenogenetic lineages are represented by single females in Wellington (Khd-61), and Bay of Plenty (Whr-538), and these could represent undersampled sexual populations adjacent to parthenogenetic populations or translocated individuals.

The relationships of 25 haplotypes (from 45 stick insects) that were part of the main parthenogenetic clade are presented as a minimum-spanning network (Fig. 2c). This network is not fully resolved due to homoplasy involving three nucleotide sites. The central haplotype was found in individuals from four locations; Wellington (3), Manawatu (3), Kaikoura (1), Christchurch (1). Other haplotypes within this clade differ by no more than seven substitutions from the central haplotype. In a frequency plot of pairwise genetic distances (Fig. 3) sexual populations have a more 'ragged' mismatch distribution typical of a fairly stable population (although subdivision is likely in this species). Pairwise differences between haplotypes from putative-parthenogenetic stick insects have a unimodal distribution typical of populations undergoing growth (recent or current), with secondary peaks resulting from the divergent haplotypes found in putative-parthenogenetic populations at East Cape.

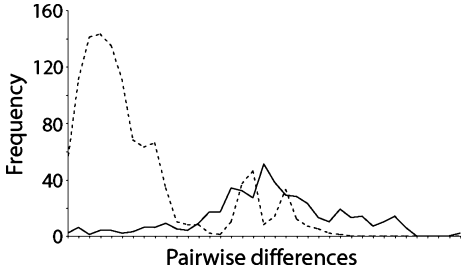

Fig. 3 Mismatch distribution for the stick insect *Clitarchus hookeri* mtDNA haplotypes using absolute differences in pairwise comparisons. Distributions are shown for sexual (solid line) and putative-parthenogenetic (dashed line) populations.

Fertility

Virgin *Clitarchus hookeri* females produced only female offspring whether they originated from a sexual population (three populations) or a putative-parthenogenetic population (five populations). Fertility (percent of eggs that hatched) of virgin females from putative-parthenogenetic populations (74%; $n = 318$ eggs) did not differ significantly from that of virgin females from sexual populations (85%; $n = 186$ eggs).

Hatching success was recorded for 357 eggs laid by five adult female stick insects, from one population, over the same 6 week period. Hatching rate per female ranged between 89% and 96% (Table 1). The unfertilized eggs had a similar hatching rate (96%) to the fertilized eggs (89% – 95%) but the virgin female produced more offspring overall as she laid more eggs in the same time period. The four mated females produced equal numbers of male and female offspring (Table 1, $P > 0.05$; chi-squared test).

Rate of development

Time from laying to hatching is known for 550 nymphs hatched from eggs laid by mated female *C. hookeri*, and for 19 nymphs from four virgin females. The majority of eggs ($n = 550$) hatched within 110–150 days of laying, with a minimum time from laying to hatching of just 14 days and maximum 246 days (mean = 83.3 days). The majority of the fertilized eggs (mated-mothers) hatched earlier than the unfertilized eggs (virgin-mothers; mean = 135.1 days; Fig. 4). Although the timing of later-hatching fertilized eggs overlapped with the hatching

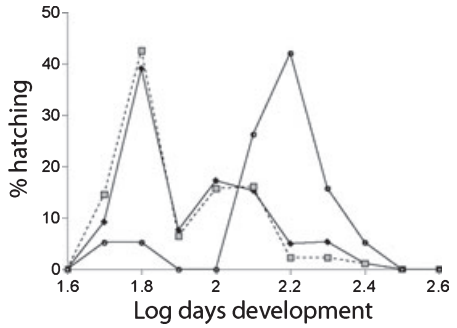

Fig. 4 A comparison of time to hatching for eggs from virgin and mated *Clitarchus hookeri* from a sexual population. Male nymphs from mated = filled diamonds, female nymphs from mated = open squares dashed lines, female nymphs from virgin stick insects = open circles.

of unfertilized eggs, there was no evidence that the proportion of female nymphs changed at this time (Fig. 4). Eggs resulting from sexual reproduction, on average had a faster rate of development than eggs laid by virgin females ($P < 0.0001$).

Faculative parthenogenesis

A total of 1490 *Clitarchus hookeri* nymphs hatched and were sexed to compare reproductive strategies. All females sourced from sexual populations were capable of parthenogenetic reproduction. All nymphs hatching from eggs laid by females kept without males were female ($n = 315$), with one exception. Wild females were collected as late instars, they were virgins and thus their offspring can only be the result of parthenogenetic reproduction. A single male hatched from an egg laid by a captive virgin mother. Although this fatherless male was raised until an adult and then held in captivity with conspecific females he was not observed mating and did not father any sons. A total of 745 nymphs were hatched and sexed from females from sexual populations held in captivity with males from the same location. The sex ratio of these nymphs did not differ significantly from 1:1 (366 female: 379 male; $P > 0.05$; chi-squared test).

Obligate parthenogenesis?

Females from putative-parthenogenetic populations who mated with males from sexual populations did not produce offspring of both sexes in equal numbers; less then 3% of the offspring were male ($n = 391$ nymphs). From the 10 male offspring hatched we infer approximately 5.1% of offspring overall were the result of sexual reproduction. However, these ten sons have just three mothers (two from Wellington and one Gisborne) yielding estimates of sexual reproduction for these of 8.4%, 19% and 50% respectively (Table 2). Male offspring from these three stick insects hatched earlier than the majority of their sisters, even though the mothers had unrestricted access to adult males during the period of egg laying. Other females from putative-parthenogenetic populations ($n = 9$) produced 246 daughters and no sons (Table 2), although all these females were observed mating.

The proportion of male offspring was used to estimate rate of sexual reproduction. This estimate could be biased by differential death of male or female embryos, however, even if all eggs that failed to hatch had been male this would still not yield a 1:1 sex ratio.

Discussion

The stick insect *Clitarchus hookeri* has a range that spans North Island and extends into South Island New Zealand. The most southerly record of *C. hookeri* is from Dunedin city (Buckley *et al.* 2008). The species may occur throughout South Island but is encountered much less frequently than in North Island. We have found no male *C. hookeri* in South Island (total females sighted = 44, from five locations) and the only putative record of one is a pinned specimen collected by J.T. Salmon 1944, enigmatically labelled 'Price's Bush Parthenogentic' (Museum of New Zealand Te Papa Tongarewa). A site frequently visited by Salmon called Prices Bush is near Christchurch and this male might have been of parthenogenetic origin by the loss of an X chromosome. The absence of males in natural populations in South Island requires confirmation, but it is likely that sexual populations are either rare or entirely absent from South Island. In North Island, males are rare and patchily distributed in the southern third and rare or absent in the eastern region, whereas males and females occur in equal numbers elsewhere to the north (Fig. 2a, Appendix S1). Overall, the distribution of *C. hookeri* males is consistent with the predictions of geographical parthenogenesis, although there is not a simple latitude divide because of the absence of males in the east of the range, the rare males in the Wellington region, and the sexual population in Otaki. It is possible that the distribution of males may have been influenced by human mediated transportation and additional records from sites away from suburban gardens and human plantings would help test this. Urban centres such as Wellington are most likely to be influenced by accidental introduction.

Origins of parthenogenetic populations

With the inclusion of an outgroup, the phylogeny of *C. hookeri* reveals individuals from the central or northern North Island as sister to all others (Trewick *et al.*

Table 2 Male offspring are rare when females from putative-parthenogenetic populations of *Clitarchus hookeri* are mated with conspecific males from sexual populations

Female stick insect	Eggs laid post-copulation	Female offspring	Male offspring	Total	Fertility (% hatching)
Peel Forest Tw-437	18	16	0	16	88.9
Wellington Wn-487	50	39	0	39	78.0
Wellington WV-486	46	38	4	42	91.3
Wellington Otr-403	113	91	4	95	84.1
Waiarapa Bid-490	12	11	0	11	91.7
Waiarapa Bid-489	27	24	0	24	88.9
Manawatu PN-435	54	45	0	45	83.3
Manawatu PN-438	35	22	0	22	62.9
Gisborne Gs-431	39	31	0	31	79.5
Gisborne Gs-432	49	37	0	37	75.5
Gisborne Gs-439	9	6	2	8	88.9
Gisborne Gs-443	22	21	0	21	95.5
Total	474	367	10	391	82.5

2005; Buckley *et al*. 2008; this study). The pattern of genetic diversity observed in nuclear and mtDNA markers suggests that the sexual populations in the north of the species' range have most probably given rise to the southern sexual and parthenogenetic lineages. Our phylogenetic analysis of mitochondrial DNA sequences suggest that putative-parthenogenetic populations of *C. hookeri* from the north-east location of East Cape to south-east Canterbury (a distance of approximately 800 km) constitute a single derived clade (with one exception). This implies a single origin of the majority of parthenogenetic populations. The shape and mismatch distribution of haplotypes in this parthenogenetic clade is characteristic of recent population expansion (Slatkin & Hudson 1991). Eleven haplotyes belonging to this clade (including the central haplotype) are found in Wellington, where males are rare. The twelfth haplotype (Khd-61) from Wellington does not belong to the main parthenogenetic clade but groups with a stick insect from Taranaki. This exception was a female stick insect, collected in a Wellington suburban garden, and may represent an independent origin of parthenogenesis, dispersal or human transfer.

We have shown that females from sexual populations can reproduce parthenogenetically, so it is possible for any sexual population to give rise to a parthenogenetic lineage, and thus multiple origins of parthenogenetic populations is feasible. It is therefore striking that almost all of our samples of *C. hookeri* from putative-parthenogenetic populations are part of a single, shallow monophyletic clade.

Initial experiments crossing females from putative-parthenogenetic populations with males from sexual populations reveal that while some females can apparently recover limited sexuality when males are available, female offspring still dominate the first generation hybrids (ratio of 1:39 males to females). While female *Clitarchus hookeri* from sexual populations are capable of producing as many viable egg as those from parthenogenetic populations, it appears that the progeny resulting from unfertilized eggs might not have the capacity to regain complete sexuality in a single generation. Thus, while the species might be described as a facultative parthenogen this cannot be said for all individuals. The popular conception of a strict dichotomy between facultative and obligate parthenogenesis may be artificial in *C. hookeri* just as it is in other stick insects (Bedford 1978). For example, females from parthenogenetic populations of the mediterranean stick insect *Bacillus rossius* produce only 10% male offspring when mated with conspecific males from sexual populations (Bullini 1968 as cited in Bedford 1978).

Interestingly, the sexual population of *C. hookeri* at Otaki (Kapiti Coast, N.I.) is also part of the main parthenogenetic clade, and might represent an ancestral sexual population or an invasion by males from further north into an all-female population. At Otaki the stick insects were collected from a plum tree in a suburban garden and it is feasible that male *C. hookeri* could have arrived recently via accidental human transportation. An investigation involving biparentally inherited markers will help to distinguish these possibilities.

Facultative parthenogen but not mixed mating

Facultative parthenogenetic reproduction is common in stick insects (Bedford 1978). We have shown that *C. hookeri* females from sexual populations can reproduce parthenogenetically (without males), but only do so if they do not have access to males. Female stick insects from bisexual population that had mated, produced an equal number of male and female offspring. This would not be expected if a significant proportion of their offspring arose from unfertilized eggs. Therefore it is unlikely that these insects employ a mixed mating strategy within sexual populations. In contrast,

females from parthenogenetic populations produce only a few male offspring after copulating. A single male offspring hatched from the egg of a virgin mother (1 of 315 parthenogenetic offspring). This male may have arisen by the loss of an X chromosome during cell division (non-disjunction), a mechanism recorded for other stick insect species with the same XO/XX sex-determination mechanism seen in *C. hookeri* (Parfitt 1980; Morgan-Richards & Trewick 2005). Such fatherless males might have the capacity to restore sexuality to parthenogenetic diploid populations (as suggested for *Bacillus rossius*) but males of virgin-birth of the triploid, hybrid species *Carausius morosus* have all been sterile due to abnormal sperm and failure to transfer sperm during copulation (Pijnacker 1987).

Reproductive advantage

Although in theory reproduction without males provides an immediate numerical advantage we should not automatically assume that parthenogenetic individuals have a reproductive advantage. In fact, some comparisons of closely related sexual and parthenogenetic animals have found the opposite to be true, although variation in ploidy level and hybrid origin have confounded some studies. Thus, where reproductive success is lower in parthenogentic individuals this has often been attributed to hybrid breakdown or developmental problems associated with polyploidy. For example, asexual geckos that have lower fecundity than sexual conspecifics are of hybrid origin (Kearney & Shine 2005), whereas comparisons of population growth of asexual freshwater snails with their sexual relatives actually compare triploids with diploids (*Potamopyrgus antipodarum*; Jokela *et al.* 1997). Studies of stick insect species such as *Bacillus rossius* and *Clitarchus hookeri* have the advantage of direct intraspecific comparison without hybrid origin or polyploidy being involved. At least ten stick insects species are known to reproduce via both fertilized and unfertilized eggs, but in three of these species the hatching rate of unfertilized eggs is significantly reduced (Bedford 1978). Sexual and parthenogenetic individuals of the mediterranean stick insect *Bacillus rossius* are sympatric in one location but their fertility and fecundity rates are similar (Bullini 1965; Scali 1968, 1969, 1970 cited by Bedford 1978). Confirmation with additional replicates is required to determine whether the trend observed here that parthenogenetic reproduction neither reduces the total number of eggs laid by *C. hookeri* nor lowers the hatching success of these eggs, is significant. Mated and virgin females from a sexual population of *Bacillus rossius* produced the same number of eggs but the eggs from unmated females had a slightly lower hatching rate (82%) compared with fertilized eggs (89%). Similarly, the fecundity of unmated *Bacillus rossius* females of parthenogenetic origin was lower than unmated females of sexual origin. There is no evidence that delayed development affects the fecundity of *C. hookeri,* but unfertilized eggs of *C. hookeri* did hatch significantly later than fertilized eggs from the same population. A similar delay has been reported for other stick insect species (*Clitumnus extradentatus*; Bergerard 1958 cited in Lamb & Willey 1879, *Bacillus rossius*; Bedford 1978) and may be due to the transition of haploid eggs converting into diploid embryos. Finally, Bedford (1978) suggested that slower development may be an advantage where winters are longer and colder and this may benefit the southern parthenogenetic populations of *C. hookeri.*

Geographic parthenogenesis

Competing hypotheses seek to explain the repeated pattern seen in the distribution of asexual taxa where these are more common further from the equator, at higher altitude or in generally more marginal habitats compared with their sexual relatives. In New Zealand, the common stick insect *C. hookeri* broadly fits this pattern of geographic parthenogenesis. *Clitarchus hookeri* probably has neither a hybrid nor polyploid origin (Parfitt 1980; Morgan-Richards & Trewick 2005; Buckley *et al.* 2008) and therefore explanations involving hybrid or polyploid vigour can be excluded (Kearney 2005; Lundmark & Saura 2006). The roles of colonization, adaptation, and local extinction in the distribution of all-female poulations of *C. hookeri* have yet to be identified (Peck *et al.* 1998; Haag & Ebert 2004). However, it appears the transition from sexual reproduction to parthenogenesis has established only a few times in *C. hookeri*. The main parthenogentic lineage shows evidence of population expansion, and parthenogenetic reproduction does not appear to reduce fertility in *C. hookeri*, although development rate is significantly reduced. If north to south forest expansion through New Zealand after the last glacial maximum was complete about 10 000 years ago (McGlone 1985; Alloway *et al.* 2007), it is likely that parthenogenetic *Clitarchus hookeri* reached their present distribution by about this time too. The substantial lag this implies in arrival of males in southern and eastern populations may be due to poor recovery of sexuality, numeric advantage of parthenogens, or adaptive advantage of parthenogens at range limits.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Appendix S1 Records of *Clitarchus hookeri* used to infer distribution of the species and its two reproductive strategies.

Appendix S2 The New Zealand stick insect *Clitarchus hookeri* used in the genetic analysis of geographical parthenogenesis, collection location, codes, and genbank numbers.

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