Figure. 1. The first recorded male *Acanthoxyla* individual. This stick insect was found in Penzance, England but the natural range of the genus is New Zealand. (Photo credit: David Fenwick).

Missing New Zealand stickman found in UK

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The recent discovery of a male stick insect in Southern England is a world first: the first male of the genus *Acanthoxyla* ever recorded. He was found half a world away from his species' natural range in New Zealand. *Acanthoxyla* stick insects are common but until now they have all been female. The discovery was made by local experts who collaborate with New Zealand evolutionary ecologists specializing in invertebrate diversity. The original identification of the male as *Acanthoxyla inermis*, the New Zealand unarmed stick insect, was made by Paul Brock (Scientific Associate, Natural History Museum, London) and Malcolm Lee (UK National Phasmid Recorder) based on the shape of the animal, and knowledge of the species introduced to the area (Brock *et al.* 2018). Because this was the first of its kind, no comparative material was available. We were initially sceptical because we are surrounded by eight morphological forms of endemic *Acanthoxyla*, but no male has ever been reported. However, a preserved leg (shed naturally by the male while in captivity) was posted to NZ and mitochondrial DNA sequence data obtained. This confirmed the morphological ID – supporting the very first male *Acanthoxyla* specimen ever recorded anywhere (Figure 1).

The mitochondrial DNA sequence indicates that the stickman's mother was a UK-based *Acanthoxyla inermis* (a smooth species) and he must have arisen via the loss of one chromosome from the normal female complement. Like many insects including grasshoppers, cockroaches and dragonflies, sex determination in stick

Figure 2. A spiny green *Acanthoxyla* feeds on native New Zealand pohutukawa flowers (Myrtaceae). *Acanthoxyla* is an obligate parthenogenetic New Zealand stick insect lineage that includes many different forms, but no males… until the new record in England.

insects involves two X chromosomes for females (XX) and just one X chromosome to make a male (XO). So accidental loss of an X chromosome during egg production can produce 'mutant' males where none have existed before. Remarkably, despite loss of just one copy of a sex chromosome, the resulting males are morphologically indistinguishable from males produced normally by sexual reproduction (in species where this happens). They have the characteristic male traits including "claspers" on the tip of the abdomen that are used to grip the female's abdomen during copulation. However, it appears that males formed this way are usually infertile.

Britain is now home to several stick insect species (Phasmida) from Europe, Asia and the Pacific (Lee 2012). The species that have adapted to living in the wild in the UK are female-only (parthenogenetic) lineages, three of which derive from New Zealand (*Acanthoxyla inermis*, *Acanothxyla geisovii*, and *Clitarchus hookeri*). Most multicellular organisms, including stick insects, reproduce sexually having females and males, but there are some insects that use a combination of asexual and sexual reproduction. Although asexual reproduction can be advantageous in the short term, such as enabling rapid population increase and range expansion, lineages that cannot reproduce via sex are usually short-lived on an evolutionary time scale.

Stick insects are therefore providing the opportunity to compare the evolutionary and ecological outcomes of different reproductive strategies, through the study of lineages that have evolved to reproduce without males and those that can do it either with or without (Scali *et al.* 2003; Milani *et al.* 2010). Reproductive diversity in New Zealand stick insects is typical of the range in phasmids worldwide. The genus *Acanthoxyla*, that comprises many morphologically-distinct lineages, has attracted attention because the entire group lacks males. Each species of *Acanthoxyla* differs in how spiny it is, the presence/absence of abdominal flanges, and the sculpturing of its eggs (Figure 2). There is also plenty of variation in cuticle colour and patterning: the spectrum includes individuals that are almost black, bright green, beige or nearly white, some are uniform, others patterned, and many have prominent dark spines. But every individual (until now) is female and produces viable daughters without fathers.

A hybrid origin for the genus involving the ancestor of a related endemic bisexual species, *Clitarchus hookeri*, has been inferred from a

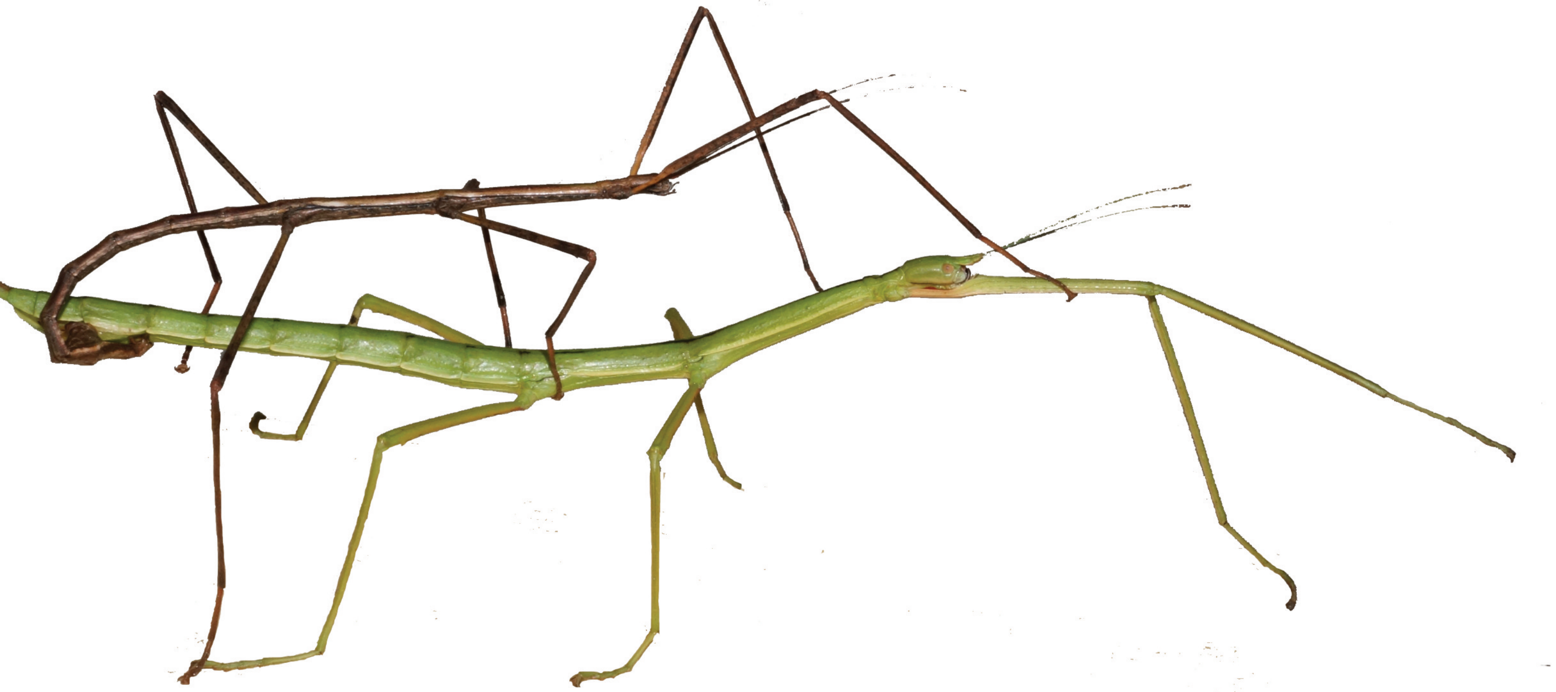

Figure 3. A pair of the New Zealand common tree-tree stick insect *Clitarchus hookeri* in copula. The female is descended from an individual from the Tresco, Scilly Island parthenogenetic population, but the male in this experiment came from a natural sexual population in northern New Zealand.

combination of genetic markers (Morgan-Richards & Trewick 2005; Buckley *et al*. 2008; Morgan-Richards *et al*. 2016). It has also emerged that many lineages of *Acanthoxyla* are mosaic triploids having cells with either two (diploid) or three sets of chromosomes (Myers *et al*. 2013), indicating the original combination of parental genomes. This association of polyploidy and hybrid origin has been identified for many organisms, including stick insect lineages in Europe and North Africa.

Another New Zealand stick insect species that has established in the UK is *Clitarchus hookeri* (Figure 3). In its native range this species has some populations that reproduce sexually, and some populations comprising only females that reproduce parthenogenetically (Morgan-Richards *et al.* 2010). Together they present a classic pattern called geographic parthenogenesis, where sexual populations occupy more hospitable and environmentally stable regions towards the equator and the all-female lineage southwards (in the southern hemisphere) to cooler, younger habitat (Figure 4). Genetic data show that the New Zealand parthenogenetic lineage is derived from a sexual population and whilst experiments show that once formed the parthenogenetic lineages have limited ability to return to sexual reproduction when males are available.

In the UK a parthenogenetic population of *Clitarchus hookeri* is established on the Isles of Scilly. This almost certainly originated from accidental introduction as eggs accompanying New Zealand plants shipped to the Tresco Abbey gardens in the early 1900s. Work is continuing to discover whether this UK population was derived from a sexual or parthenogenetic New Zealand population. By comparing population samples from around New Zealand using population genetic markers we will be able to infer the likely origin and thus likely reproductive strategy of the ancestor. In addition, eggs from the *Clitarchus hookeri* population on Tresco have been taken to New Zealand and raised in captivity for three generations. Once adult, the females are given local *Clitarchus hookeri* males who are happy to mate with them. The ability of the females to use the sperm for sexual reproduction is being monitored to assess the capacity of females that have resulted from a known number of parthenogenetic generations to regain sexuality. This will help answer fundamental questions in biology about the costs and benefits of sexual reproduction in animals.

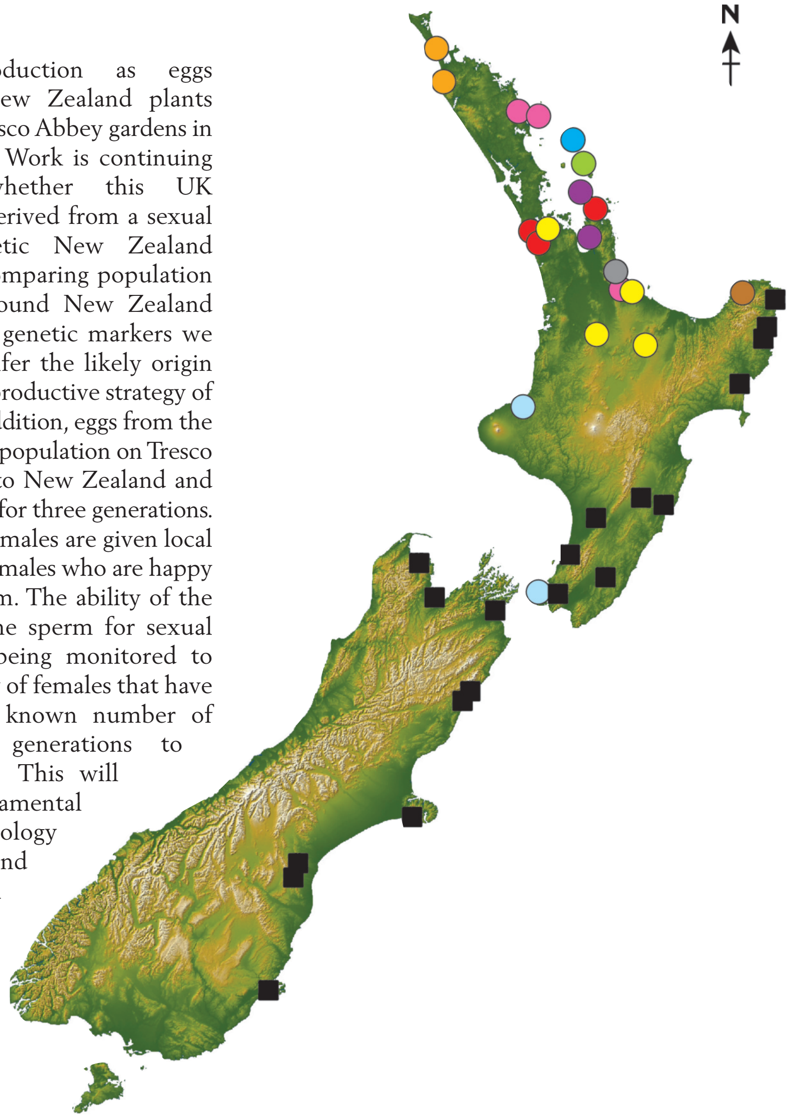

Figure 4 *Clitarchus hookeri* shows a pattern of geographic parthenogenesis in New Zealand with higher genetic diversity in northern (toward the equator) sexual populations and less diverse southern all-female populations. Sexual populations are indicated by circles, and colours indicate different genetic lineages. Black squares show parthenogenetic populations. Cooler, younger habitat was most probably colonised since the last Pleistocene glacial, and achieved more rapidly by parthenogenetic individuals with their numerical advantage.

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