

Occurrence of sexual morphs in *Trama troglodytes* von Heyden, 1837 (Hemiptera, Aphididae)

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The root-feeding aphids of the tribe Tramini have been cited as a possible case of ancient apomixis, as there are no substantiated records in the literature of a functional bisexual generation, even in common and well-known European species. The karyotypic variation found in this group, involving considerable structural heterozygosity within species, also pointed to the decay of diploidy that would be an expected consequence of loss of sexuality and absence of meiosis. However, we have now found a small number of oviparae (mating females) and males in populations of the common species *Trama troglodytes*. Fertilized eggs were not obtained, but adult oviparae contained large, yolky eggs. The single adult male was apterous, small and blind, but had fully developed mouthparts and genitalia, and contained mature sperm. Both sexual morphs are described, and the karyotypic variation is reassessed in the light of these findings. Occasional sexual reproduction is in concordance with recent molecular evidence that some recombination occurs in *T. troglodytes* and related species.

KEYWORDS: aphids, Tramini, ancient apomixis, cryptic recombination.

Introduction

Aphids typically have cyclical parthenogenesis, alternating a sequence of thelytokous (all-female, viviparous) generations with a single, usually annual, bisexual generation (of males and mating, oviparous females). The bisexual generation may however be rather easily lost, resulting in continuous, apomictic parthenogenesis, either by some genotypes or populations within a species, or sometimes apparently by the entire species. Even some common and widely distributed aphids seem to be permanent apomicts, but their nearest relatives usually still have a bisexual generation in their life cycle. This suggests that the loss of sexuality in such aphids is an

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evolutionarily recent event, as seems to be the case in most other groups with permanently apomictic species (Suomalainen *et al.*, 1976, Hughes, 1989).

It has been proposed, however, that the loss of sexuality may have been a more ancient event in one aphid group in the subfamily Lachninae, the Tramini (Eastop, 1953; Blackman, 1980a; Normark, 1999). Tramini are large, white, invariably ant-attended aphids feeding on roots of Compositae. They are a Palaearctic group of three genera and about 28 species, and it has been suggested that the group as a whole lacks sexual reproduction (Szelegiewicz, 1978; Blackman, 1980a; Czylok, 1990; Heie, 1995). Cytological studies provided supporting evidence for this conclusion, because all species investigated had unusual karyotypes, with large amounts of constitutive heterochromatin (Blackman, 1980a, 1980b, 1990). In the genus *Trama*, karyotypes appear structurally heterozygous, with considerable variation in number and distribution of heterochromatic segments (Blackman, 1980a) and rDNA sites (Blackman *et al.*, 2000). Aneuploidy and structural heterozygosity might be expected in chromosome sets that no longer undergo meiotic pairing and segregation, and Blackman *et al.* (2000) have suggested that the heterochromatic elements might, in whole or in part, be the remains of the ancestral sex chromosomes.

There are, however, a few isolated reports in the literature of sexual morphs in Tramini. Del Guercio (1899) gave an account of the life cycle of a *Neotrama* sp. (as *Trama radialis*) in Italy, including overwintering eggs, fundatrices, oviparae and apterous males. Mordvilko (1935) stated that he might have reared one male of a *Protrama* sp. (as *Trama radialis*), but again gave no description. Verma (1969) found and described a single male of the Indian species *Trama penecaeca* (Stroyan), the karyotype of which is unknown.

In September 1984, a single colony of *Trama troglodytes* von Heyden on *Centaurea oxylepis* in Poland was found to contain females with an oviparous reproductive system (A. Czylok, unpublished observations). No males were found, and colonies on other host plants in the area consisted entirely of viviparous females, so the existence of a functional bisexual generation could not be established.

Further evidence conflicting with the hypothesis of ancient apomixis in Tramini has recently been reported by Normark (2000), who found very low levels of heterozygosity of the EF-1 α gene in *Trama troglodytes* and *Protrama flavescens* (Koch), compared to substantial divergences between these and other species, indicating that some form of recombination must be occurring at this locus. Both these species are common and widely distributed in Europe. Here we report the occurrence of both males and oviparous females in a population of *T. troglodytes* in southern England.

Materials and methods

As part of ongoing studies of karyotype variation in Tramini, mixed colonies of *Trama troglodytes* and *T. maritima* (Eastop) were collected from roots of *Picris echioides* at Church Ope Cove, Portland, Dorset on 8 September 1999. Colonies were sorted into species in the laboratory, both immature and adult *T. maritima* being readily identified by the presence of siphuncular pores, which are absent in all instars of *T. troglodytes*. Second and third instar larvae were dissected in 1% sodium citrate, prior to fixation and preparation of embryonic tissues for cytological examination. One of the two colonies of *T. troglodytes* examined contained five immature oviparae. The reproductive system of oviparous aphids is easily recognized, even in young larvae, because the germaria are much larger than in viviparae and contain

large, polyploid nurse cells, and because the most developed ovarioles contain yolky oocytes rather than young developing embryos.

A further collection was made at the same site on 19 October 1999, by which time it was judged that any oviparae would have become adult, and in order to make a thorough search for males. Colonies on roots were bagged with plenty of soil and examined in the laboratory. Four out of the six colonies collected were mixed *trogodytes* and *maritima*, the other two being pure *maritima*. There were no alatae or alatiform nymphs in any of the colonies. All the aphids were dissected in 1% sodium citrate.

Chromosome preparations were made from young embryos dissected from immature viviparae and fixed in three parts methanol to one part acetic acid. Embryonic cells were separated by squashing under a glass coverslip in a drop of 45% propionic acid. Preparations were examined with a compound microscope and photographed using phase contrast illumination.

Results

Three out of the four mixed colonies contained oviparae of *T. trogodytes* (table 1), two of which were adult and contained large yolky eggs. One colony contained one immature (4th instar) and one adult apterous male. The testes of these males were fixed, squashed and examined by phase contrast. Spermatogenesis had been completed, and spermatids and mature sperm were present. All the specimens of *T. maritima* were apterous viviparae (table 1).

Viviparae of *T. trogodytes* from each colony were karyotyped. Those from the three colonies containing sexual morphs all had 20 chromosomes in embryonic somatic cell nuclei (as also did a somatic metaphase found in a preparation made from the ovarioles of one of the immature oviparae collected on 8 September 1999). Those from the single colony without sexual morphs had 16 chromosomes.

Descriptions of sexual morphs of *Trama trogodytes*

Oviparous female (two specimens)

In life white, indistinguishable from apterous viviparae. Body broadly oval, 3.1–3.2 mm long. Eye each consisting of three ommatidia. Total antennal length 0.56–0.61 of body length, with proportions of antennal segments III–VI, respectively 100: 44: 70: 43+8. Antennal segment III relatively longer than in viviparae; 0.19 of body length, compared with 0.13–0.14 in viviparae, and 0.80–0.84 of head width

Table 1. Composition of *Trama* colonies from Portland, Dorset on 19 October 1999.

Colony number	<i>Trama trogodytes</i>			<i>Trama maritima</i>		
	Viviparae	Oviparae	Males	Viviparae	Oviparae	Males
1	61	6	0	35	0	0
2	23	4 (1 adult)	2 (1 adult)	103	0	0
3	0	0	0	207	0	0
4	28	0	0	61	0	0
5	0	0	0	47	0	0
6	15	3 (1 adult)	0	135	0	0

across eyes (0.64–0.70 in viviparae). No secondary rhinaria present. Longest hairs on antennal segment III are $80\ \mu\text{m}$ long, about 1.6 times basal articular diameter. Frontal hairs up to $95\ \mu\text{m}$ long. Rostrum 1.5–1.6 mm long, with last segment (IV + V) 0.34–0.35 of length of hind tarsus segment II. Middle femur relatively longer than in viviparae; 0.23–0.25 of body length, compared with 0.19–0.21 in viviparae. Hind tibia without any discernible scent glands (pseudosensoria). Hind tarsus II 0.67–0.69 of length of hind tibia. Subgenital plate apparently no different to that of viviparae, with hairs up to $120\ \mu\text{m}$ long. Other characters as in apterous viviparae.

Measurements of one specimen (in mm): body length 3.18; antennal segments III 0.61, IV 0.25, V 0.42, VI (base) 0.26, VI (terminal process) 0.05; rostrum total length 1.55, last segment 0.32; mid femur 0.72, hind femur 1.02, hind tibia 1.34, hind tarsus II 0.92.

Apterous male (one specimen; figures 1, 2)

In life white with antennae, head, prothorax and first two pairs of legs somewhat pigmented, pale brown. Claspers and valves of aedeagus black. Rather elongate oval, body length 1.64 mm. Eyes completely absent (apparently lost in final moult, as the immature (last larval instar) male found in the same colony had minute triommatidia). Antennae about the same length as body, with proportions of segments III–VI, respectively, 100: 61: 85: 57 + 11. Antennal segment III partially fused with IV on both sides. Primary rhinarium on antennal segment V on one side reduced (diameter $16\ \mu\text{m}$) with two minute accessories; primary rhinaria on other side normal (maximum diameter $52\text{--}56\ \mu\text{m}$). One secondary rhinarium present, on distal part of antennal segment III on one side only. Hairs on antennal segment III

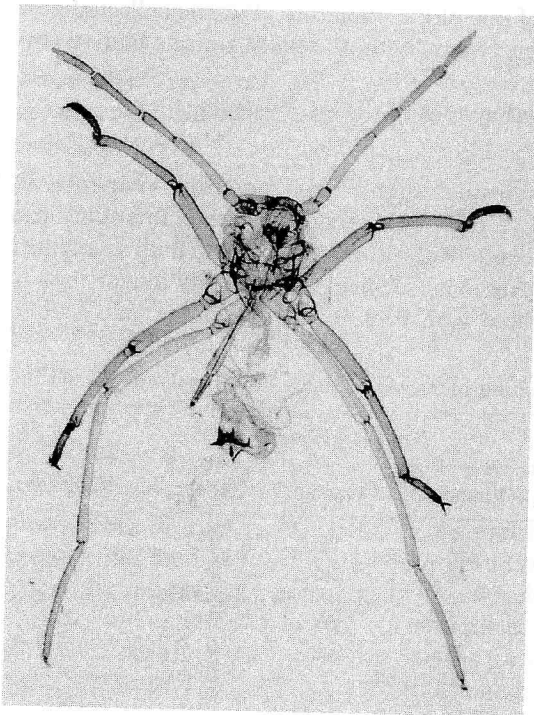


FIG. 1. Slide-mounted preparation of the male of *Trama troglodytes* von Heyden.

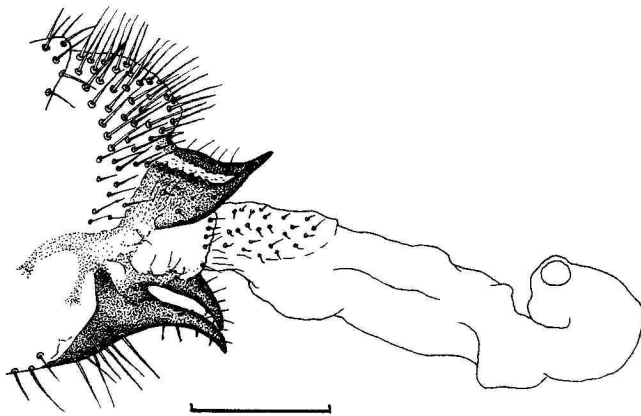


FIG. 2. External genitalia of *Trama troglodytes* von Heyden (bar represents 0.1 mm).

up to $65\ \mu\text{m}$ long, about 1.7 times basal articular diameter of segment. Frontal hairs up to $80\ \mu\text{m}$ long. Rostrum 1.1 mm long, with last segment 0.33 of length of hind tarsus segment II. Hind tarsus segment II 0.8 of length of hind tibia. Anal plate rounded posteriorly, with numerous hairs up to $90\ \mu\text{m}$ long (figure 2). Genital plate sclerotized posteriorly and fused to bases of claspers, with hairs up to $70\ \mu\text{m}$ long. Claspers are strongly sclerotized, united at bases, curved and tapering almost to a point, with a few short hairs even on distal parts. Basal part of aedeagus with two strongly sclerotized valves separated medially and each prolonged distally to a narrow point (figure 2). Dorsal hairs on basal parts of valves up to $30\ \mu\text{m}$ long, apical parts without hairs. Membranous part of aedeagus bearing numerous short hairs ($8\text{--}20\ \mu\text{m}$ long) in a dorsal area near the base.

Measurements (in mm): body length 1.64; antennal segments III 0.46, IV 0.28, V 0.39, VI (base) 0.26, VI (terminal process) 0.05; rostrum total length 1.10, last segment 0.25; mid femur 0.54, hind femur 0.75, hind tibia 0.98, hind tarsus II 0.78.

Discussion

Trama troglodytes is a common European aphid and it is somewhat surprising that its sexual morphs have not been found before. However, males are small, whitish and superficially resemble immature viviparae, and the identify of oviparae was only ascertained after dissection. Sexual morph production is presumably seasonal, and mating occurs underground. The eggs, although quite large, would not be readily observed and are probably looked after during winter by ants. The main method of overwintering is undoubtedly by continued parthenogenesis, and from the findings reported here it seems likely that only certain genotypes produce sexual morphs (in this case a 20-chromosome genotype), and that even those genotypes also continue to produce viviparae through the winter.

The male is small, which is often the case in Lachninae. The male external genitalia resemble those of Stomaphidini in having long claspers and paired projections of the aedeagal valves (see Sorin, 1965). This resemblance is interesting in view of the fact that phylogenetic studies (Czylok, 1990) place Tramini and Stomaphidini as sister groups within the Lachninae. The male of *Protrama penecaeca* described by Verma (1969) had long claspers, but no mention was made of any aedeagal projections. Males of other Lachninae, where known, have quite different external

genitalia. Normark's (2000) recent molecular phylogenetic analysis of Lachninae confirms the relationship between Tramini and Stomaphidini, but also places the species *Tuberolachnus salignus* (Gmelin) and *Nippolachnus piri* Matsumura in the same clade; males of these species and related ones are either unknown or their genitalia have not yet been described.

Apterous females of *T. troglodytes* have eyes represented only by triommatidia, and other *Trama* have the compound eyes very reduced and variably developed if not absent, indicating that they are practically functionless in these root-feeding aphids. Even so, the total loss of the triommatidia at the final moult in the male is interesting, suggesting some positive advantage in eye loss such as the need to reallocate resources. The male of *P. penecaeca* has triommatidia in the adult stage (Verma, 1969). The rostrum of the *T. troglodytes* male is fully developed, as in the male of *P. penecaeca*, but unlike Stomaphidini, where the males do not feed and are aristrate. The scarcity of secondary rhinaria on the antennae of the *T. troglodytes* male, and the absence of the scent glands on the hind tibia of oviparae that are characteristic of some groups of aphids, are not unusual features in Lachninae.

The anomalous karyotypes of Tramini were thought to provide positive evidence for ancient asexuality in Tramini. Aneuploidy and structural heterozygosity are found frequently in aphids which have lost the sexual part of their life cycle (Blackman, 1980b), and the Tramini seemed to be an extreme example of loss of diploidy in the absence of meiotic pairing and recombination. Much of the variation seen within and between populations of *T. troglodytes* involves the number and arrangement of heterochromatic sections of the karyotype, consisting largely of repetitive DNA. The idea that the predominantly heterochromatic elements might be relict X chromatin is no longer tenable, as it is clear that the sex determination process, involving loss of half the sex chromatin to produce XO males (Blackman, 1987), is still functional in a population that has 10 of these elements. Studies of the cytology of sex determination will be difficult in this species, unless populations with a higher incidence of male production can be found. Given the large variation in number and size of heterochromatic elements in parthenogenetic females, it is also unlikely that male embryos can be distinguished cytologically and the functional X chromosomes thus identified. Studies of spermatogenesis, especially spermatological divisions and metaphase I and anaphase I of meiosis, would be informative, but these stages occur in earlier larval instars than have so far been found.

In the absence of hard evidence, one may surmise that the largely heterochromatic elements in the female karyotype of *T. troglodytes* comprise one or more pairs of X chromosomes and a variable number of additional elements that may be classed as accessory or 'B' chromosomes. Multiple X chromosomes and X chromosome/B chromosome systems are known in another aphid genus, *Euceraphis*, where there is always an obligatory annual sexual generation (Blackman, 1988). However, in *Euceraphis* the X/B systems are remarkably stable throughout the genus, and similar systems even seem to occur in related genera, contrasting with the extreme variability found within and between populations in *Trama* spp. In other aphid genera with considerable karyotype variation such as *Amphorophora*, karyotype evolution has involved change in the number of autosomes while the X chromosomes have remained stable (Blackman, 1980b). The difference may be a reflection of the low incidence of sexuality in *Trama*.

Even a low incidence of sexuality would nevertheless over many generations have a significant effect on genetic variation, and could explain the low level of

heterozygosity at the EF-1 α locus found by Normark (1999), and why deleterious mutations do not seem to be accumulating in this species (Normark and Moran, in press). The finding of sexual morphs in *T. troglodytes* suggests that they may occur at similarly low frequency in other species of Tramini, most of which are less well known.

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