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High diversity of structurally heterozygous karyotypes and rDNA arrays in parthenogenetic aphids of the genus *Trama* (Aphididae: Lachninae)

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Karyotypes of permanently parthenogenetic aphids of three species of the genus *Trama* show great diversity, particularly in the number and distribution of chromosomal elements containing highly repetitive sequences. Sampling at only a few sites in southern England, chromosome number varied from 14 to 23 in *T. troglodytes*, 9–12 in *T. caudata* and 10–14 in *T. maritima*, with some colonies having individuals of more than one karyotype. This variation was paralleled by differences in the number and distribution of rDNA arrays revealed by *in situ* hybridization. This high intraspecific karyotype diversity contrasts with very low genetic diversity in the same populations, suggesting rapid karyotype evolution. Although *T. troglodytes* feeds on many species of composite plants there was no evidence of any karyotype-associated host race formation.

Keywords: aneuploidy, clonal reproduction, heterochromatin, repetitive DNA.

Introduction

Aphids primitively and typically exhibit cyclical parthenogenesis, alternating a single annual (sometimes biennial) bisexual generation with several unisexual (all-female) generations. The bisexual generation may be lost secondarily, so that reproduction is then exclusively by apomictic parthenogenesis. This often happens within species, particularly in those which have been widely distributed by man. Presumably in such cases the loss of bisexual reproduction is a very recent event, but it is nevertheless often accompanied (or followed) by chromosomal rearrangements, giving rise to aneuploid karyotypes and structural heterozygosity (Blackman, 1980a), suggesting that such changes can occur quite rapidly.

One group of aphids, the tribe Tramini, may have a longer history of parthenogenesis, as functional sexual reproduction has not been observed in any members of this tribe (Mordvilko, 1935; Eastop, 1953). Tramini colonize the roots of composite plants and are obligatorily associated with ants. *Trama troglodytes*, the best-known species in the principal genus *Trama*, shows a remarkable degree of karyotypic diversity, notable especially in the variable distribution of constitutive (C-) heterochromatin (Blackman, 1980a,b, 1990). Large blocks of C-heterochromatin, indicating the presence of tandem arrays of highly repetitive DNA sequences (Lohe & Hilliker, 1995), are a characteristic feature of this group of aphids, comprising about 40% of the genome (Blackman, 1980b).

Another feature of permanently parthenogenetic aphid populations, providing further evidence of rapid karyotype evolution, is the frequent occurrence of a single rDNA array, as opposed to the pair of rDNA arrays usually found on the X chromosomes in species that have regular sexual reproduction. Blackman & Spence (1996) reported such a single rDNA array in the one population of *T. troglodytes* that they examined.

In contrast to the high karyotypic diversity of *T. troglodytes*, a low level of genetic diversity was found for both a nuclear and a mitochondrial gene within this and several other species of Tramini (Normark, 1999). The very low heterozygosity of the elongation factor 1α gene in two species of this group, *T. troglodytes* and *Protrama flavescens*, indicated that some form of recombination must be occurring at this locus. The absence of any records of sexual morphs in these two common species and the structural heterozygosity of the karyotype shown by *T. troglodytes* seem

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to rule out meiosis, and gene conversion or some other form of mitotic recombination has been suggested (Normark, 1999).

Here we report new data on the karyotypes and locations of rDNA arrays in *Trama* (four species) and *Protrama* (two species). We demonstrate similar levels of karyotypic diversity to *T. troglodytes* in two other species of *Trama*, and show that this diversity is paralleled by variation in the number and distribution of rDNA arrays in all three species. We discuss these findings in relation to differences between these taxa at the molecular level.

Materials and methods

Aphid collections

Samples of Tramini were collected from roots of plants of the family Compositae at various locations in southern England in 1996–98 (Table 1). Ant activity at the base of the stem of a plant provided a guide to the presence of aphid colonies. Plants with aphid colonies were uprooted and brought into the laboratory for examination.

Trama caudata was found on roots of *Leontodon hispidus* on chalk downland at two locations in southern England, and *T. maritima* was collected mainly from *Picris echioides* at coastal sites further south and west, including its type locality (Eype Mouth; Table 1). However, neither species is restricted to a particular host plant or habitat, as a colony of *T. maritima* was collected on the roots of *Sonchus* (sample 366) and this species was also found on one occasion on *L. hispidus* at a downland site. *Trama caudata* has also recently been found on *P. echioides* on the English south coast (E. De Boise, pers. comm.). *Trama troglodytes* was found colonizing the roots of a variety of Compositae growing in disturbed locations, especially *Cirsium arvense* and *Artemisia vulgaris*. *Trama rara* appears specific to *Taraxacum* and has previously been found in similar habitats to *T. troglodytes*, but the only colony of this species found for the present study was at a coastal site. The two *Protrama* species included in the study were collected from their normal host plants, to which they appear host-specific.

Chromosome studies

Individual aphids were dissected in 1% sodium citrate to provide embryos for cytological study, then frozen at −80°C for DNA analysis. Embryos were fixed in 3:1 methanol/acetic acid, squashed in 45% propionic acid under an 18-mm square coverslip and the spread cells examined under phase contrast. Good prometaphase and metaphase spreads were photographed and their locations marked.

The method of using fluorescence *in situ* hybridization to reveal rDNA arrays has already been described (Blackman *et al.*, 1995; Blackman & Spence, 1996). Cells photographed by phase contrast were relocated after *in situ* hybridization and re-photographed using fluorescence microscopy. The location of rDNA arrays was observed in 5–10 prometaphase cells per slide.

Results

Karyotypes

The range of chromosome numbers previously reported for *T. troglodytes* (14–22; Blackman, 1980a) was found again in the 1996–98 samples, with additionally one sample having 23 chromosomes (Table 1). On the basis of gross morphology alone, at least nine different karyotypes were present in the 17 colonies examined and, as in previous work, there was no evidence of host plant races, several karyotypes being encountered on more than one host, and some colonies containing individuals of more than one karyotype. No C-banding was attempted, as this makes the preparations unsuitable for the *in situ* hybridization technique that we used. However, the distribution of heterochromatin was often observable in prophase nuclei, and conformed to the pattern previously recorded for this species, i.e. 10 mainly euchromatic elements of similar length (sometimes two of these were fused end-to-end), plus 4–13 mainly heterochromatic elements of varying size (mostly small). It has previously been shown (Blackman, 1980b, 1990) that the 10 euchromatic segments usually have heterochromatin positioned telomerically or subtelomerically, with the only interstitial location marking the point at which a tandem fusion is thought to have occurred (Fig. 1a,b).

The karyotypes of *T. caudata* and *T. maritima* are quite different in general form from *T. troglodytes*. *Trama caudata* from the two sites sampled consistently had six euchromatic elements, three being about twice as long as the others, with very little subtelomeric heterochromatin, plus 3–6 heterochromatic elements of various lengths, often very short (Fig. 1c). *Trama maritima* from coastal sites consistently had seven euchromatic elements, four being about twice as long as the others, with no discernible subtelomeric heterochromatin, plus 3–7 heterochromatic elements of various lengths (Fig. 1d). However, an individual examined from the colony found at Old Winchester Hill (no. 343) had eight euchromatic elements, of which only three were long. A single tandem fusion or dissociation could explain the difference between these two karyotypes. The three

Table 1 Collection and karyotype data for sampled species of Tramini 1996–98

Ident. no.	Date	Locality	Host plant	Species	No. of individuals examined	Chromosome no(s)	No. of rDNA arrays (no. of individuals)
304	3/10/96	Ham Lands, Surrey	*Artemisia vulgaris*	*Protrama flavescens*	1	≈42	1
4424	19/5/98	Feltham, Middlesex	*Artemisia vulgaris*	*Protrama flavescens*	1	≈42	1
4438	10/6/98	Kew, Surrey	*Artemisia vulgaris*	*Protrama flavescens*	1	42	1
303	3/10/96	Ham Lands, Surrey	*Cirsium arvense*	*Protrama radicis*	1	≈50	1
344	20/5/97	Old Winchester Hill	*Leontodon hispidus*	*Trama caudata*	1	10	1
346	20/5/97	Martin Down, Hampshire	*Leontodon hispidus*	*Trama caudata*	1	12	2
347	20/5/97	Martin Down, Hampshire	*Leontodon hispidus*	*Trama caudata*	3	12	1
4427	19/5/98	Martin Down, Hampshire	*Leontodon hispidus*	*Trama caudata*	1	9	1
4433	19/5/98	Martin Down, Hampshire	*Leontodon hispidus*	*Trama caudata*	1	11	2
343	20/5/97	Old Winchester Hill	*Leontodon hispidus*	*Trama maritima*	1	13	1
366	5/7/97	Eype Mouth, Dorset	*Sonchus*	*Trama maritima*	2	11,12	1
369	5/7/97	Eype Mouth, Dorset	*Picris echioides*	*Trama maritima*	1	11	1
374	5/7/97	Eype Mouth, Dorset	*Picris echioides*	*Trama maritima*	1	12	1
376	19/7/97	Church Ope Cove	*Picris echioides*	*Trama maritima*	3	13	2
403	22/10/97	Borstal, Portland	*Picris echioides*	*Trama maritima*	1	10	1
405A	22/10/97	Church Ope Cove	*Picris echioides*	*Trama maritima*	1	13	1
405D	22/10/97	Church Ope Cove	*Picris echioides*	*Trama maritima*	1	13	2
407	22/10/97	Perryfield Quarry	*Picris echioides*	*Trama maritima*	2	11	1
412	22/10/97	Cheyne Weare	*Picris echioides*	*Trama maritima*	2	14	3
368	5/7/97	Eype Mouth, Dorset	*Taraxacum*	*Trama rara*	3	12	3
301	3/10/96	Ham Lands, Surrey	*Cirsium arvense*	*Trama troglodytes*	1	20	1
312	3/10/96	Shepperton, Middlesex	*Crepis taraxacifolia*	*Trama troglodytes*	1	14	3
316D	15.10/96	Shepperton, Middlesex	*Cirsium arvense*	*Trama troglodytes*	1	14	3
316E	15/10/96	Shepperton, Middlesex	*Cirsium arvense*	*Trama troglodytes*	1	19*	4
318A	15/10/96	Shepperton, Middlesex	*Artemisia vulgaris*	*Trama troglodytes*	1	15	2
318B	15/10/96	Shepperton, Middlesex	*Artemisia vulgaris*	*Trama troglodytes*	1	14	3
319A	15./10/96	Feltham, Middlesex	*Cirsium arvense*	*Trama troglodytes*	1	18	1
319C	15/10/96	Feltham, Middlesex	*Cirsium arvense*	*Trama troglodytes*	1	21	2
322	22/4/97	Robertsbridge, Sussex	*Achillea millefolium*	*Trama troglodytes*	1	18	1
332B	1/5/97	Shepperton, Middlesex	*Cirsium arvense*	*Trama troglodytes*	1	20	2
332C	1/5/97	Shepperton, Middlesex	*Cirsium arvense*	*Trama troglodytes*	1	17*	3
335	1/5/97	Shepperton, Middlesex	*Artemisia vulgaris*	*Trama troglodytes*	1	17*	3
336B	1/5/97	Shepperton, Middlesex	*Cirsium arvense*	*Trama troglodytes*	1	17*	1
338	1/5/97	Wraysbury, Middlesex	*Crepis taraxacifolia*	*Trama troglodytes*	2	20,23	1
389B	17/10/97	Goczałkowice, Poland	*Tanacetum vulgare*	*Trama troglodytes*	1	16	1
4417A	28/4/98	Shepperton, Middlesex	*Crepis taraxacifolia*	*Trama troglodytes*	1	14	3 (+3 faint)
4421B	28/4/98	Wraysbury, Middlesex	*Cirsium arvense*	*Trama troglodytes*	1	17*	1

All locations are in the UK, apart from 389B.
*Presence of an autosomal fusion in *Trama troglodytes* (column 7) — see text.

individuals from the single population of *T. rara* examined all had embryonic cells with 12 chromosomes, with one element more than twice as long as any other. Previous preparations of *T. rara* with 13 chromosomes have also had this one very long chromosome. The 13-chromosome karyotype previously studied had nine euchromatic elements, plus four shorter heterochromosomes (Fig. 1e), and the present sample is within this general plan.

Species of the genus *Protrama* have karyotypes very different from those of *Trama*, with many small chromosomes that are difficult to count. Interphase nuclei have large chromatin bodies (chromocentres) indicating that there is much heterochromatin, but it is unclear how this is distributed in the karyotype. Samples collected for the present work had similar chromosome numbers to those previously recorded (Blackman, 1980a), and one of the three samples of *P. flavescens* definitely had $2n=42$. *Protrama radicis* was observed to have $2n$ about 50, but could have had up to 60 chromosomes, with many small heterochromosomes, as previously recorded.

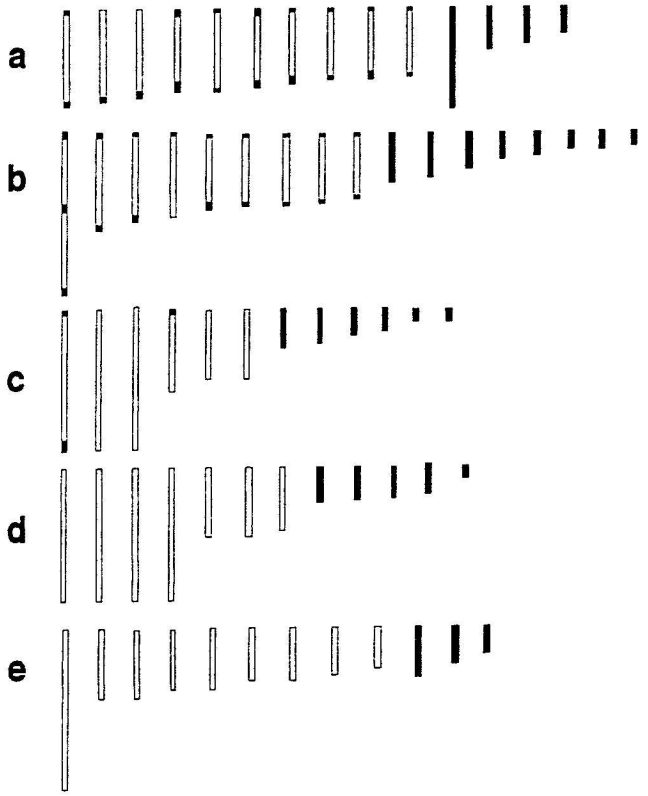

Fig. 1 Karyotypes of *Trama* species. (a) *T. troglodytes* with 14 chromosomes; (b) *T. troglodytes* with 17 chromosomes, two euchromatic elements having undergone tandem fusion; (c) *T. caudata* with 12 chromosomes; (d) *T. maritima* with 12 chromosomes; (e) *T. rara* with 12 chromosomes. The number and size of euchromatic sections (white) is relatively constant, but the number, size and position of heterochromatic sections/chromosomes is highly variable (see text and Table 1).

Number and distribution of rDNA arrays

Trama caudata had 1–2 rDNA arrays, *T. maritima* had 1–3 arrays and *T. troglodytes* 1–6 arrays (Table 1). The single sample of *T. rara* had three arrays. The number of rDNA arrays varied independently of chromosome number, both within species and across the genus. All four samples of *Protrama* (two species) had a single array.

The locations of the rDNA arrays could be determined in prometaphase cells by comparing preparations hybridized to the rDNA probe with the same cells photographed by phase contrast (Fig. 2). In *P. flavescens* there is one large, brightly fluorescing array that cannot be assigned to any particular chromosome at metaphase, but is clearly associated with the heterochromatin body in prophase nuclei (Fig. 2a). In *T. rara* most of the arrays were on one chromosome, with faint signals at two locations on other chromosomes (Fig. 2b). Both the number and locations of rDNA arrays in *T. troglodytes* varied greatly (Fig. 2c–f). Arrays could be all concentrated on a single small heterochromosome (Fig. 2c), or distributed evenly or unevenly, but generally at telomeric or subtelomeric locations, on short or longer elements (Fig. 2e–f). One common pattern, found in populations on three different host plants at Shepperton (Table 1; nos 312, 316D, 318B), had three separate arrays, one at the end of a long, mainly euchromatic element, and the others at both ends of a shorter, mainly heterochromatic element (Fig. 2d).

Samples of *T. caudata* had rDNA arrays on either one or two very small heterochromosomes (Fig. 2g,h). In those individuals of *T. maritima* with a single array, this could be on a very short heterochromosome (Fig. 2i), or at the end of a longer heterochromosome (Fig. 2j). *Trama maritima* with 13 chromosomes and two rDNA arrays had these arrays at interstitial locations on two mainly heterochromatic elements of similar length (Fig. 2k). In one metaphase cell these two elements were aligned in parallel (Fig. 2l), suggesting that they might be homologues. Figure 2 (m,n) shows two other features of the distribution of heterochromatin and rDNA sites in *Trama*: clumping together (ectopic pairing) of heterochromatic elements in late prophase (Fig. 2m) and, at an earlier stage of prophase (Fig. 2n), association of all or most heterochromosomes, whether or not they have rDNA arrays, with the nucleolus.

Discussion

It has been shown previously that aphids from sexually reproducing populations typically have two homologous rDNA arrays in their diploid genomes, and that in parthenogenetic aphid lineages one of these is typically reduced (Mandrioli *et al.*, 1999) or lost entirely (Blackman & Spence, 1996). This is consistent with the hypothesis that meiosis is involved in the maintenance of diploidy in eukaryotic genomes, and that the loss of meiosis permits that diploid structure to decay (Birky, 1996).

In this study we add new examples of rDNA array loss in permanently parthenogenetic aphids. More remarkably, we show for the first time that the decay of diploidy at the rDNA array in parthenogenetic aphids may take the form of multiplication rather than reduction, with several instances of three rDNA arrays, across three species, and isolated instances of four and even six arrays in *T. troglodytes* (Table 1).

The decay of diploidy at the rDNA array is accompanied by a similar decay of diploidy of the karyotype as a whole (at least in the heterochromatic regions), with structural heterozygosity characterizing every karyotype examined. A parallel molecular–phylogenetic study of

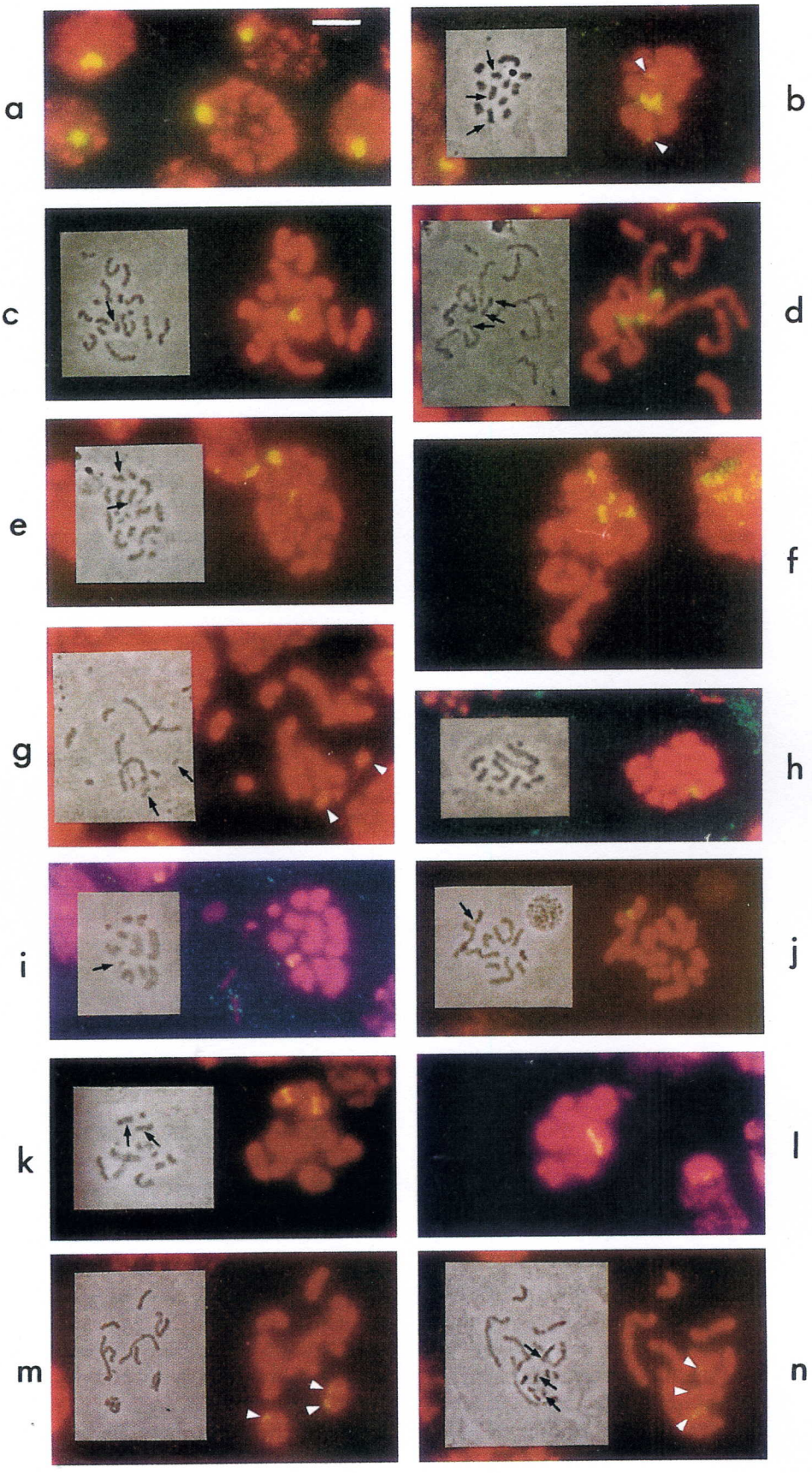

Fig. 2 Location of rDNA arrays in Tramini, revealed by *in situ* hybridization of *Drosophila* probe pDm 238 to somatic prometaphase chromosomes in squash preparations of embryonic tissues. Inset black-and-white photographs show same nuclei viewed by phase contrast prior to hybridization, with black arrows pointing to locations of rDNA arrays. White arrowheads point to weakly fluorescing rDNA arrays. Identification numbers refer to Table 1. (a) *Protrama flavescens* (304); (b) *Trama rara* (368); (c) *T. troglodytes* (319A); (d) *T. troglodytes* (318B); (e) *T. troglodytes* (332B); (f) *T. troglodytes* (316E); (g) *T. caudata* (346); (h) *T. caudata* (344); (i) *T. maritima* (343); (j) *T. maritima* (374); (k) *T. maritima* (376); (l) *T. maritima* (376); (m) *T. maritima* (412); (n) *T. maritima* (412). Bar in (a) represents 5 μm.

these lineages found that aphids having widely different karyotypes often shared the same DNA sequence at two loci, leading to the inference that karyotype evolution has been rapid in species of *Trama* in comparison to the per-locus rate of nucleotide substitution (Normark, 1999). A high rate of karyotype evolution has previously been inferred for other aphids in culture (Blackman & Spence, 1996) or in recently introduced populations (Sunnucks *et al.*, 1996).

One factor that may help to explain both the release from diploidy and the rapid rate of karyotype evolution is the fact that the chromosomes of aphids and other Hemiptera are holocentric; that is, the centromeric activity is dispersed along the length of each chromosome rather than concentrated at one point. As a consequence, dissociations, tandem fusions and other chromosomal changes can arise and be transmitted to daughter cells at successive cell divisions. In organisms with localized centromeres, any cells with changes that result in acentric or dicentric chromosomes will tend to be eliminated, as such chromosomes will not segregate normally at mitosis. A high level of karyotypic diversity in association with large variation in the number of rDNA sites has been reported in the plant genus *Rhynchospora* (Cyperaceae), which also has holocentric chromosomes (Vanzela *et al.*, 1998).

The observed changes mostly involve chromosomal elements that are mainly or wholly heterochromatic, the number and size of euchromatic elements being relatively stable within species. That chromosome breakpoints usually occur in heterochromatin is well-established for other organisms, e.g. much of the karyotype evolution in mammals involves pericentric heterochromatin. C-heterochromatin is known to be particularly variable in certain other organisms, such as grasshoppers (John, 1983).

Changes in heterochromatin are presumably as a general rule selectively neutral, and therefore unlikely in themselves to lead to any significant genetic change that would affect the phenotype. Nevertheless, one might perhaps expect particular clonal lineages to prefer or perform better on particular host plant species, in which case one would presumably find karyotypes acting as markers for such lineages. There is, however, no evidence of any association between karyotype and host plant in the most polyphagous species, *T. troglodytes*. Considering the extent of karyotypic diversity in this aphid, this seems to provide strong evidence that no host race or biotype formation is occurring. The absence of host-associated genotypes in this species is also supported by DNA sequence data (Normark, 1999). Mitochondrial DNA haplotype diversity (which is very low) does not correlate with host use, each haplotype being found on several hosts. *Trama troglodytes* thus seems to be a good example of a parthenogen with a general purpose genotype, in agreement with the ideas of Lynch (1984) that the parthenogenetic lineages most likely to survive are those that are tolerant of the widest range of host plants and environmental conditions.

It might also be argued that a complex trait, such as specialization on a particular host plant, is unlikely to evolve in the absence of bisexual reproduction. Significant genetic changes do seem to have occurred in some pest aphid species that were presumed to be permanently parthenogenetic. For example, the corn leaf aphid, *Rhopalosiphum maidis*, has karyotypic variants associated with barley (10-chromosome form) and maize/sorghum (8-chromosome form), although pest populations of this species throughout the world are entirely parthenogenetic (Brown & Blackman, 1988). However, a bisexual generation of *R. maidis* has now been found to occur in Pakistan (Remaudière & Naumann-Etienne, 1991), so it is possible that the variation in host plant preference traits was originally generated by recombination, and that clonal lineages starting out with different host adaptations subsequently diverged in karyotype. Other traits known to have arisen in permanently parthenogenetic aphid populations, such as resistance to insecticides or ability to colonize resistant crop varieties, are simple (usually single-locus) rather than complex, and have resulted from artificially intense selection in sheltered monocultural systems.

If there is no bisexual generation in *Trama* there are presumably no functional X chromosomes. There seems a strong possibility that some or all of the hypervariable heterochromatic elements in these aphids are derived from redundant X chromatin. Aphids typically have XX/XO sex determination, and the X chromosomes frequently have more heterochromatin than the autosomes (Blackman, 1985). In *Trama*, rDNA arrays are almost always located on one or more of the heterochromatic elements, and in other aphids rDNA arrays

or nucleolar organizing regions (NORs) are typically located on the X chromosomes. In aphids which retain a bisexual generation, there are sometimes heterochromosomes that conform to the definition of B chromosomes; that is, they are supernumerary to the normal chromosome complement and have no role in sex determination, but nevertheless associate with and behave similarly to the functional X chromosomes at meiosis (e.g. in *Euceraphis*; Blackman, 1976). In *Maculolachnus submacula*, an aphid in the same subfamily as *Trama*, there are two small heterochromatic elements which behave like the X chromosomes in male meiosis I (Blackman, 1990), and one of these has the single rDNA array that is found in this species (Blackman & Spence, 1996), suggesting that it may have originally been part of an X chromosome.

In one sample of *T. maritima* (376) collected in July 1997 there were rDNA arrays at similar (interstitial) positions on two chromosomes that might be X homologues (Fig. 2l). As this might be indicative of a viable bisexual generation we returned to this site in October of the same year, but found only parthenogenetic females.

Further investigation of the nature of karyotypic variation and genome evolution in *Trama* will require isolation and characterization of the major repetitive DNA sequences in the heterochromatin of each species, to see how homogeneous this is, and to map the locations of particular sequences on chromosomes by *in situ* hybridization. In a preliminary study, one of us has isolated a highly repetitive 950 bp sequence (TtR) from *T. troglodytes*, which has high sequence homology to repeat units in other *Trama* species, but not to *Protrama*. The sites to which this sequence hybridized *in situ* were always within heterochromatic elements of the *T. troglodytes* karyotype (J.M.S., unpublished data), but varied greatly within this species in their number and disposition, as well as in the proportion of total heterochromatin accounted for. A significant proportion of the heterochromatin did not hybridize with the TtR probe, and must therefore consist of one or more quite different repeat units, as yet unidentified.

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