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Chromosome rearrangements are not accompanied by expected genome size change in the tree weta *Hemideina thoracica* (Orthoptera, Anostostomatidae)

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Abstract

A comparison of hybrid zones between chromosome races of the tree weta *Hemideina thoracica* (White) found that the chromosomal rearrangements that involved the greatest proportion of the genome apparently had the least effect on fitness of hybrids. In order to further explore the nature of chromosomal rearrangements that differentiate weta races, relative DNA content was compared between 2 races of *H. crassidens* (Blanchard) (known to differ by 2 Robertsonian (Rb) translocations) and 4 races of *H. thoracica*, using flow cytometry. Where Rb translocation was the inferred process (*H. crassidens* 15 *vs* 19; *H. thoracica* 17 *vs* 15′) no change in relative DNA content was detected, as expected. The 19-karyotype of *H. thoracica* was predicted to have more DNA due to a duplication/loss compared to the 17-karyotype of *H. thoracica*, but here too, no change in relative DNA content was detected. In contrast, significantly more DNA was found where less was predicted; the 13-karyotype of *H. thoracica* was thought to have less DNA than the 15′-karyotype, due to a duplication/loss, but in fact this race has significantly more DNA than any of the 3 other races of *H. thoracica* examined. Due to the differential staining of A-T bases by the stain used in flow cytometry, 4′-6′-diamidino-2-phenylindole (DAPI), it is possible the increased DNA estimate for the 13-karyotye is due to a significant shift in A-T base bias throughout its genome. However, it is more likely that the absence of the very small acrocentric autosomes that characterize many of the karyotypes of *H. thoracica*, result from translocations to larger autosomes than from a change in total DNA content and a simultaneous change in base ratio.

Key words

Anostostomatidae, chromosome evolution, hybrid zone, weta, New Zealand, DNA content

Introduction

The role of chromosome rearrangements in speciation is a controversial issue in biology (White 1978, Sites & Moritz 1987, King 1993). The ability of chromosome rearrangements to reduce fertility of heterozygotes provides a means to limit gene flow, but the paradox is that the stronger the barrier caused by the chromosome rearrangement, the lower the probability that novel rearrangements will be fixed in a population. Recently the debate has focused on the role of specific types of chromosome rearrangement that have a greater chance of becoming established in large populations (*i.e.*, the monobrachial centric fusion model; Baker & Bickham 1986, and mutations that suppress recombination, Rieseberg 2001). One of the continuing problems with generalising about the role of chromosome evolution in speciation is the unpredictable effect of chromosomal heterozygosity on fertility. Minor structural changes to chromosomes can disrupt meiosis completely in one species, but a similar change may have no measurable effect in another species (shrews, Wallace 2003; *cf.* humans, Solari 1999).

The Auckland tree weta, *Hemideina thoracica* (White), is subdivided into 8 chromosome races (Morgan-Richards 1997). By comparing the width of hybrid zones where these races meet, a relative measure of the disadvantage the hybrids suffered was obtained (Morgan-Richards & Wallis 2003). Unexpectedly, the karyotype changes that involved large chromosome arms apparently had less effect on fitness than the rearrangements that involved small chromosome fragments. Our failure to predict the relative fertility of hybrids might be due, in part, to a missidentification of the rearrangements that differentiate the races.

The races of *H. thoracica* have diploid numbers between 11 (XO) and 23 (XO) and this variation was previously attributed to 2 types of rearrangement. Robertsonian (Rb) translocations (the fusion or fission of whole chromosome arms at the centromeres) were hypothesised to have differentiated the 23-karyotype *vs* 19-kartotype and the 17-karyotype *vs* 15 and 15′-karyotype (Fig. 1). Rb translocations also differentiate the 2 chromosomal races of a related species, *Hemideina crassidens* (Blanchard) (2n = 19(XO), 2n = 15(XO); Morgan-Richards 2000). Other races of *H. thoracica* that differ in diploid number vary in numbers of very small acrocentric autosomes (11-karyotype *vs* 13-karyotype *vs* 15′-karyotype, and 17-karyotype *vs* 19-karyotype).

To explain these differences duplication or loss of very small autosomes, constituting 1-2% of their genome, were hypothesised. The comparison of the width of 5 hybrid zones between *H. thoracica* races, found that the greatest disadvantage was suffered by the hybrids of 2 races that apparently differed only in the duplication or loss of one pair of very small acrocentric autosomes (17 *vs* 19), which form a hybrid zone of <1 km at Waitangi. Hybrids were apparently more successful at zones where races were differentiated by 1 or 2 Rb translocations involving 30-35% of the genome (23 *vs* 19; Morgan-Richards & Wallis 2003).

It is not possible to determine with certainty the direction of the chromosome rearrangements in *H. thoracica*. Evolutionary relationships between the chromosome races were not resolved using DNA sequence data from their mitochondrial genomes (COI & 12S; Morgan-Richards *et al.* 2001). However, the northernmost races (17′, 23 and 19) probably originated during the Pliocene (2 to 7 mya) while the southern races (17, 15, 15′ 13, 11) may have formed during recolonisation/range expansion in the Pleistocene. Therefore, an overall reduction in chromosome number, via a variety of rearrangements is likely in this species (Morgan-Richards *et al.* 2001).

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Table 1. Species, chromosome race, diploid number, sex, and location of tree weta used in this study of relative DNA content. The flow cytometry index is a ratio of the weta nuclei compared to the internal standard; a coefficient of variation (%) based on the ~2500 nuclei measured, is given for each index.

Species	Chromosome race	2n	Sex	Location	Code	Flow cytometry Index	CV (%)
H. crassidens	15	15	XO	Sky Farm	b	3.194	3.02
H. crassidens	15	16	XX	Sky Farm	a	3.556	4.46
H. crassidens	19	19	XO	Lewis Pass	d	3.183	3.00
H. crassidens	19	19	XO	Lewis Pass	c	3.215	3.12
H. thoracica	13	13	XO	Henderson	Hen 31	3.531	4.31
H. thoracica	13	13	XO	Henderson	Hen 32	3.551	3.88
					Hen 32	3.479	3.07
H. thoracica	13	14	XX	Henderson	Hen 30	3.913	2.72
H. thoracica	13	14	XX	Henderson	Hen 33	3.809	3.16
H. thoracica	13	14	XX	Henderson	Hen 33	3.788	2.93
H. thoracica	13	14	XX	Henderson	Hen 34	3.847	2.61
H. thoracica	13	14	XX	Henderson	Hen 35	3.812	2.67
H. thoracica	13	14	XX	Henderson	Hen 36	3.789	2.91
H. thoracica	13	13	XO	Waitakares	Atk 12	3.503	3.15
H. thoracica	13	13	XO	Waitakares	Atk 10	3.542	2.73
					Atk 10	3.499	3.44
H. thoracica	13	13	XO	Waitakares	Atk 13	3.458	2.76
H. thoracica	13	13	XO	Waitakares	Atk 14	3.559	2.84
H. thoracica	13	13	XO	Waitakares	Atk 15	3.574	2.11
H. thoracica	13	14	XX	Waitakares	Atk 11	3.949	2.43
H. thoracica	13	14	XX	Waitakares	Atk 16	3.924	3.42
H. thoracica	13	14	XX	Waitakares	Atk 17	3.934	3.30
H. thoracica	15′	15	XO	Dome Valley	Dom 22	3.317	3.34
					Dom 22	3.295	3.45
H. thoracica	15′	15	XO	Dome Valley	Dom 21	3.344	3.12
					Dom 21	3.307	3.32
H. thoracica	15′	15	XO	Dome Valley	Dom 26	3.245	3.15
					Dom 26	3.317	3.37
H. thoracica	15′	15	XO	Dome Valley	Dom 25	3.268	2.81
H. thoracica	15′	16	XX	Dome Valley	Dom 20	3.741	3.00
H. thoracica	15′	16	XX	Dome Valley	Dom 24	3.713	3.96
					Dom 24	3.584	2.90
H. thoracica	15′	16	XX	Dome Valley	Dom 23	3.677	3.59
H. thoracica	17	18	XX	Paihia	Pai 40	3.680	3.84
					Pai 40	3.496	3.67
					Pai 40	3.631	3.89
H. thoracica	17	18	XX	Paihia	Pai 44	3.492	3.19
H. thoracica	17	17	XO	Paihia	Pai 41	3.183	3.93
					Pai 41	3.178	2.48
H. thoracica	17	17	XO	Paihia	Pai 42	3.212	2.80
H. thoracica	17	17	XO	Paihia	Pai 43	3.151	2.93
H. thoracica	19	20	XX	Mt Te Puke	Puke 50	3.759	3.30
					Puke 50	3.543	3.23
H. thoracica	19	20	XX	Mt Te Puke	Puke 52	3.529	2.86
					Puke 52	3.466	2.71
H. thoracica	19	19	XO	Mt Te Puke	Puke 51	3.345	2.39
					Puke 51	3.255	3.65
H. thoracica	19	19	XO	Mt Te Puke	Puke 53	3.153	2.55

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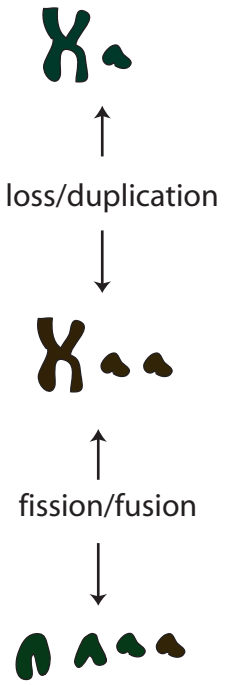


Fig. 1. The 2 types of chromosome rearrangements that were thought to differentiate the chromosomal races of *H. thoracica* and the chromosomes that characterise them (Morgan-Richards 1997).

The aim of this study was to use measures of relative DNA content of weta nuclei to test whether changes in chromosome number were consistent with the hypothesised processes. Duplication or loss of chromosomes was expected to result in changes in total DNA content, whilst Rb translocation was expected to have no significant effect on the total DNA content (Perry *et al.* 2004).

Methods

Tree weta are flightless, arboreal, nocturnal, omnivores that use holes in tree trunks made by wood-boring beetle larvae to hide during the day. Weta were collected during the day by breaking open hollow branches in which they had concealed themselves. A total of 35 weta were collected, representing 4 chromosome races of *H. thoracica* and the 2 chromosome races of *H. crassidens* (Table 1, Fig. 2). Each of the chromosome races was sampled from a single location, with the exception of the 13-chromosome race of *H. thoracica* that was collected from 2 sites within 50 km of each other (Table 1, Fig. 2).

Cytogenetics. — Female weta for cytogenetic study were colchicine-treated by injecting 0.5 ml of 0.05% colchicine in insect saline (0.7% NaCl and 0.02% $CaCl_2$ in distilled water) into the body cavity 13 to 16 h before killing them. Males were not given colchicine treatment as testes have high mitotic and meiotic rates. Weta were killed with ether, their ovarian follicles or testes removed and the animals placed in 100% ethanol. Reproductive tissue was placed in a hypotonic solution (5:1 water:insect saline) for 15 min, then fixed in fresh 3:1 methanol:acetic acid for at least 2 h at 4°C. Chromosome slides were made by the air-drying method and stained with 8% Giemsa's stain (Gurr®) in a Phosphate buffer (pH 6.8), as described in Morgan-Richards 1997.

As observed in most Orthoptera, the sex chromosomes of weta are large and metacentric (Morgan-Richards 1997). The sex chromosomes are paired in females (XX) and single in males (XO). There is no Y chromosome; therefore males have an odd number of chromosomes in their diploid complement.

Flow Cytometry.—Isolation of nuclei from weta tissue for flow cytometry followed the method of Galbraith *et al.* (1983) with some modifications. A 2 to 4 mm fragment of antenna was cut from recently anaesthetised insects and placed, together with the reference, in a plastic petri dish. The internal standard was a quarter of a punched disk of fresh leaf tissue (24 mm^2) from a diploid daisy *Bellis perennis* (L). This standard was chosen for its stability, ease of use and similarity in genome size to that of the experimental animals. A single individual was available fresh for every comparison. The optimal buffers for insects are also optimal for *Bellis perennis*, and there are no confounding issues from heparin (Johnson *et al.* 1999). A few drops of commercial nuclei isolation buffer, UV CyStain precise T solution A (100 ml deionised water, 2.1 g citric acid, 0.5 g Tween-20; PARTEC GmbH, Münster, Federal Republic of Germany) were added, and the tissue chopped finely with a stainless steel razor blade; then an additional 0.5 ml of solution A were added. After approximately 90 s the sample was filtered through a 30-µm filter, and 2.0 ml of Partec Cystain Precise T solution B (100 ml deionised water, 7.9 g dibasic sodium phosphate, 0.5 ml DAPI stock [4.55 mg 4´, 6´-diamidino-2-phenylindole, 10 ml deionised water]) were added. Samples were then analysed for DNA content after at least 90 s of staining. For this, the Partec PA-II Particle Ana-

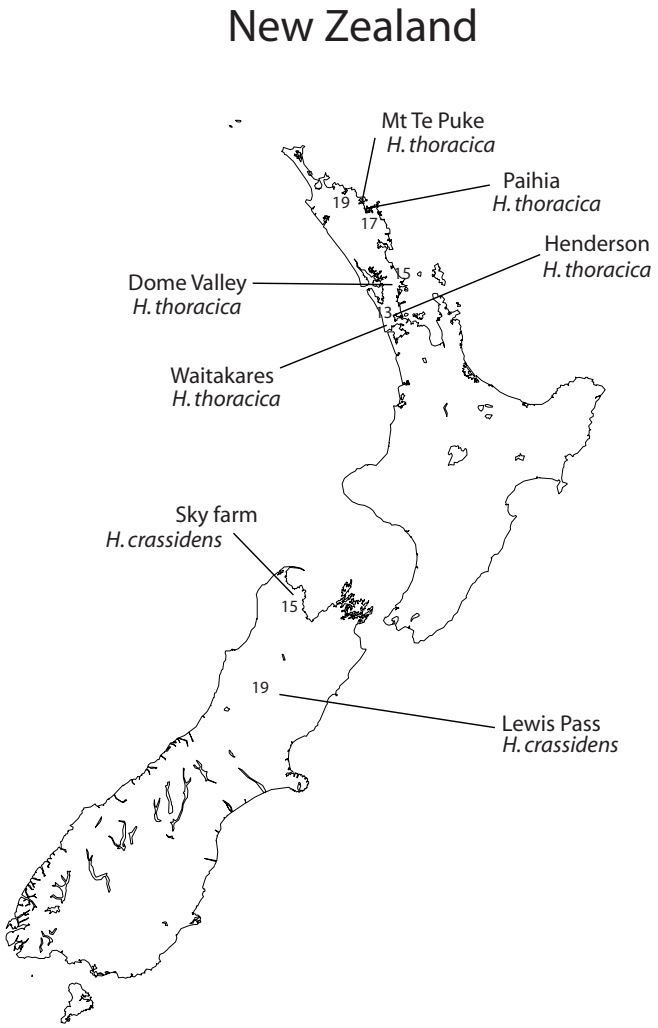


Fig. 2. The 7 locations in New Zealand where tree weta (*Hemideina* sp.) were collected to compare their relative DNA content.

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lysing System (PAS) was employed, using filter combinations of UG 1, TK420, TK590, and GG435 and a mercury arc lamp (HBO 100 W/2). Five thousand nuclei were measured for each weta, plus internal standard (≈2500 from each). DNA was stained with DAPI, which is a nonintercalating dye that binds preferentially to A-T bases: it is therefore not appropriate to convert DNA measurements into picograms. Johnston *et al.* (1999) show that, in general, genome size estimates using DAPI agreed well with those obtained using other fluorochromes. Genome sizes are presented as the ratio of the mean nuclei size of the insect compared to the reference and therefore are only comparable within this study.

A variety of tissue types from the weta were tried, including tibia, intestine and malpighian tubules, before the antenna was selected for flow cytometric analysis. Cells from the antenna of adult weta were seldom in stage G2 of mitosis and so this tissue gave a single peak of nuclei in G0/G1. Antenna tissue has the added advantages that it is unlikely to be contaminated with cells from parasites or food plants and samples can be taken without killing the insect. All flow-cytometry results presented are from antenna tissue taken before colchicine treatment, and used within 1 h of collection. Analysis of variance (one-way ANOVA) was performed separately for the two sexes (planned comparisons, model II) using SPSS version 11.0.2.

Results

For *H. crassidens* (15-chromosome race) the X chromosome is approximately 11% of the male genome. This is consistent with estimates from measuring plain stained chromosomes that are between 11.5% (15-chromosome race) and 10% of the male genome (19-chromosome race; Morgan-Richards 1995, 2000). For *H. thoracica*, estimates of the size of their X chromosome based on flow cytometry, range from 9.9% to 12% of the male genome (Fig. 3). These figures are similar to estimates from measurements of plain stained chromosomes of between 9.4% and 13.2% (Morgan-Richards 1997). The relative DNA content of the 2 races of *H. crassidens* is within the range of *H. thoracica* (Table 1).

The chromosomal rearrangements involved in the formation of the 2 chromosome races of *H. crassidens* (15 and 19) have been well established with the study of meiosis in F1 hybrid males from inter-racial crosses (Morgan-Richards 2000). In *H. crassidens*,2 Rb translocations have resulted in diploid numbers of 15(XO) 16(XX) and 19(XO) 20(XX). Translocations of this type, which involve the fission of metacentric chromosomes at the centromere to produce 2 acrocentric chromosomes (or *vice-versa*), would not be expected to alter the total DNA content. The relative DNA indexes obtained for the 2 races of *H. crassidens* were consistent with expectation (Table 1).

The 4 chromosomal races of *H. thoracica* represented in this study differ in relative DNA content (ANOVA: males df = 3, F = 57.3, $p < 0.001$; females df = 3, F = 18.8, $p < 0.001$,). The 13-chromosomal race has relatively more DNA than the other 3 chromosomal races, which do not differ significantly from each other (ANOVA: males df = 2, F = 4.7, p = 0.059; females df = 2, F = 2.6, p = 0.19). The 15′ and 17-chromosome races differ by a hypothesised single Rb translocation; however the 13 and 15′, 17 and 19 pairs were previously thought to differ from each other by a single duplication/loss of a small acrocentric autosome (Fig. 1). On average the 13-karyotype has relatively more DNA than the other karyotypes; this difference ranges from approximately 5% of the female genome (between 14(XX) and 16(XX)) to 11% of the male genome (between 13(XO) and 17(XO)).

Discussion

Rb translocations are not expected to change the total DNA content and my predictions have been met; thus the hypothesis for the chromosome rearrangement that differentiates the 15′ from the 17-karyotype is supported. And the DNA content of the 2 chromosome races of *H. crassidens*, which differ by 2 Rb translocations, also conforms to expectations. In contrast, the prediction that relative DNA content would be lower in the 13-karyotype and higher in 19-karyotype, compared to 15′ and 17, has not been met. This

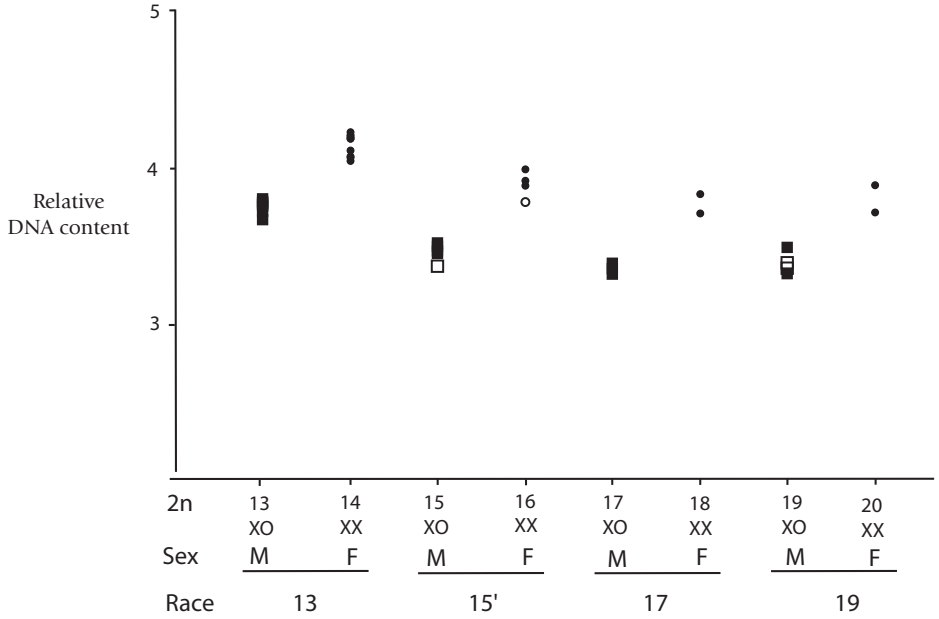


Fig. 3. Relative DNA content based on flow cytometry of 35 tree weta (*H. thoracica* (filled shapes) and *H. crassidens* (unfilled shapes) from 6 different chromosomal races, males (XO) squares, females (XX) circles.

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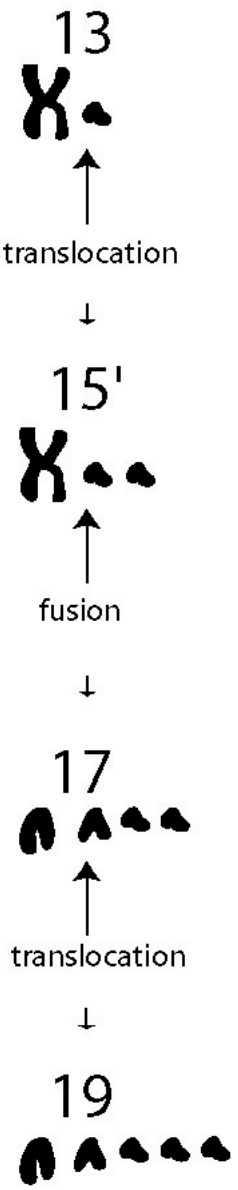


Fig. 4. The chromosomes that characterize each of 4 races of *H. thoracica* and the revised hypothesised rearrangements that produced the chromosome variation.

does not provide evidence that the small acrocentric autosomes are lost or duplicated, as has been previously suggested (Morgan-Richards 1997). Rather the rearrangements are more likely to be translocations/fissions of these small acrocentric autosomes and their incorporation into the larger metacentric autosomes (Fig. 4). Which larger autosomes are the recipient of the translocations could be determined using banding studies and fluorescent labelling of karyotypes using probes from other chromosome races.

Strikingly, the chromosome race that was predicted to have the least DNA (13-karyotype) in fact has relatively more DNA per nucleus than the 4 other chromosome races of *H. thoracica* examined. Increase in DNA content without major chromosomal change is common and usually attributed to increased quantities of noncoding repetitive DNA (Gregory & Hebert 1999). Intraspecific continuous increases in DNA content have been associated with climatic gradients and age-of-occupancy gradients (Jockusch 1997, Marescalchi *et al.* 1998). The trend seen in this dataset of relatively more DNA with fewer chromosomes (Fig. 3) and from more southerly locations, is worth further investigation.

Because DAPI differentially binds to AT bases, it is possible that the 13-karyotype has the same quantity of DNA as the races with more chromosomes, but its AT:GC ratio is skewed by 5-11%, with more AT bases. Likewise, it is possible that the 15', 17 and 19-karyotypes differ from each other in their DNA content, but that changes in AT ratios give them the same DAPI absorption. Variation in GC content of coding genes has been studied among species but not within species, and as coding genes typically make up less than 50% of the genome of insects, and satellite (junk) DNA can be GC rich, it is unclear how important this is to the GC ratio of the whole genome. The presence or absence of the small acrocentric autosomes in the weta karyotypes appear to have little effect on the total number of AT bases as measured by DAPI absorbtion.

It should be remembered that each race was sampled from one or two locations and the relative DNA content estimates may not be representative of the entire chromosome race. More variation was detected among individuals within a sample than within individual weta. Therefore, it would be beneficial to increase the number of individuals studied for each race by collecting from additional locations.

The hybrid zone between the 19-chromosomal race and the 17-chromosomal race is the narrowest of 5 zones studied in this species and therefore where the hybrids suffer the greatest disadvantage. We are now closer to understanding the nature of the chromosome rearrangements that separate these 2 races, but why translocations involving small autosomes should have a greater effect on fitness compared to Rb translocations involving larger chromosome arms (as seen at the 19-23 hybrid zone) has yet to be answered. In addition, the potential role of other genetic differences at this hybrid zone has still to be determined.

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