Characterization of a hybrid zone between two chromosomal races of the weta *Hemideina thoracica* following a geologically recent volcanic eruption

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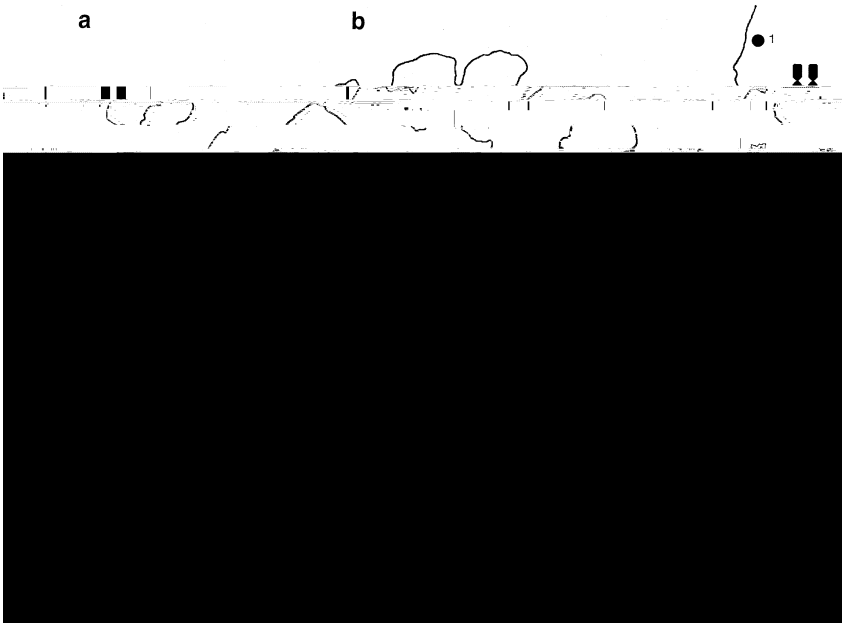
Two chromosomal races ($2n = 17$ and $2n = 15$; XO) of the weta Hemideina thoracica meet at the centre of a volcanic region in North Island, New Zealand. Five independent polymorphic genetic markers showed broadly coinciding, steep frequency clines from north to south across this zone



F . 1 (a) Area of North Island, New Zealand covered by pyroclastic flow (dark shading) and ash (pale stippling) following the most recent Taupo volcanic eruption (about 1850 years ago). (b) Collecting locations of Hemideina thoracica from the shore of Lake Taupo. Diagrams of chromosome markers show the northern race, hybrid, and southern race, from top to bottom, respectively.

ago and the surrounding area has since been subject to sporadic volcanic activity (Healy, 1992). The most recent Taupo eruption occurred about 1850 years ago (estimates range from 1857 to 1813 years ago). This eruption deposited pyroclastic rock over 20 000 km^2 and covered 30 000 km^2 of New Zealand in ash (Fig. 1a) (Wilson & Walker, 1985; Healy, 1992). Thus we know with certainty that plant and animal populations within a radius of about 80 km of Lake Taupo have arrived within the last 1850 years (see also McDowall, 1996).

Tree weta are flightless nocturnal orthopterans (family Anostostomatidae) endemic to New Zealand. The common tree weta species in the northern half of New Zealand (Hemideina thoracica) consists of at least seven chromosomal races (Morgan-Richards, 1997). Weta collected from the extreme south of this species' range have a diploid number of 17(XO) in males and 18(XX) in females. North of, and adjacent to this chromosomal race, weta have diploid numbers of 15(XO) and 16(XX). Weta near Taupo have either four pairs of small acrocentric autosomes (17) or two pairs of small acrocentrics and one pair of small metacentric autosomes (15) (Morgan-Richards, 1997). The difference between the karyotypes can most simply be explained as resulting from a single fission/fusion of small autosomes. No morphological characters distinguishing these chromosomal races have been detected. For this study, tree weta were examined from an area where the 17-karyotype race meets the 15-karyotype race in the Central Plateau of the North Island. Lake Taupo forms a barrier to the west and the eastern shoreline forms a natural north–south transect. Tree weta populations karyotypically monomorphic for the two chromosome complements were known to be separated by at most 40 km (Morgan-Richards, 1997). For this study we used a range of molecular markers to address the following questions. 1. Are there molecular genetic differences between these races? 2. Is there evidence of hybridization or introgression? 3. Do frequency clines for independent markers have the same centre (coincident) and width (concordant)?

Materials and methods

Ten sites were searched for tree weta between Huka Falls (site 1) north of Lake Taupo and Parikarangaroa Reserve (site 7) at the southern end of Lake Taupo, forming a transect of 38 km along the eastern shore of the crater lake (Fig. 1b). The land beside Lake Taupo is extensively modified by human activity and searching was successful at only seven sites (Table 1). Nineteen artificial weta hides were made by drilling a hole 9 × 120 mm in a block of wood and tying to shrubs at site 3. Three months later, six of these weta hides were occupied by subadult male H. thoracica. At site 2, night searches were carried out on three sequential nights by scanning branches and trunks of native trees after dusk for 2–3 h. At all other sites weta were collected during the day by extracting them from tree cavities. A total of 87 H. thoracica was collected and analysed (Table 1). Cytogenetic analysis and allozyme electrophoresis were performed as described in Morgan-Richards (1997).

(width and centre) using a metropolis algorithm (1000 iterations), following recommendations given with the program.

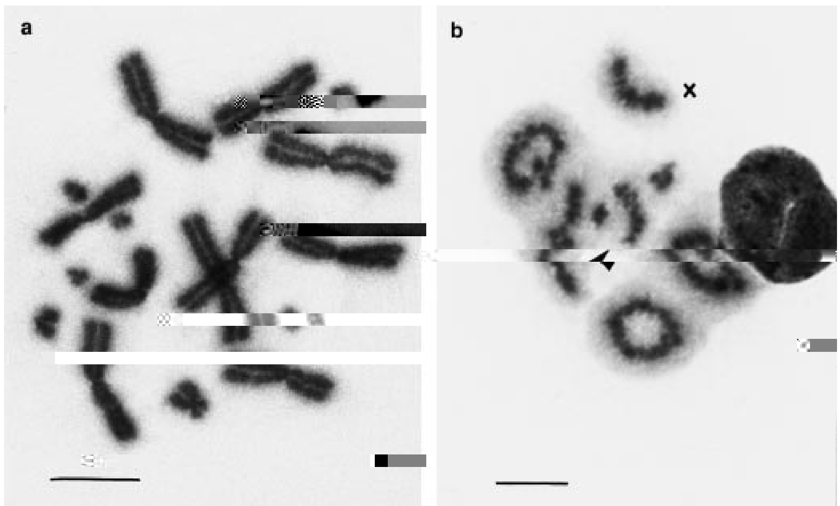
Results

Cytogenetics

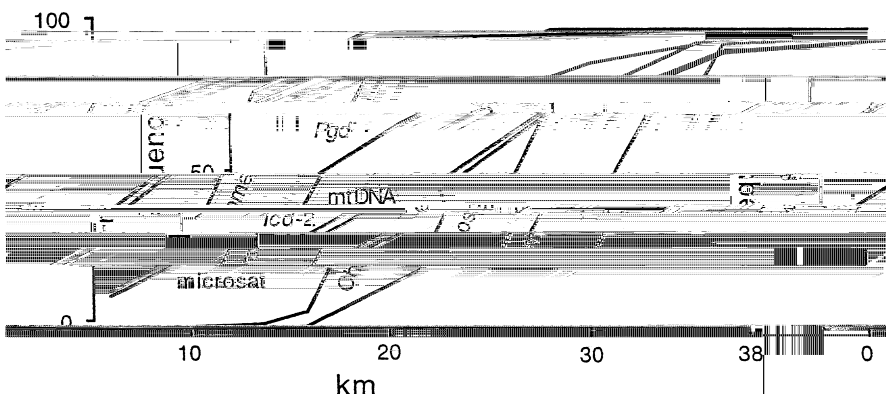
The samples from three northern sites (1, 2, 4; Fig. 1) were monomorphic for the 15-karyotype (coded BB) and samples from the two southern sites (6, 7) were monomorphic for the 17-karyotype (AA) (Table 1). Six chromosomal heterozygotes were found in sites 3 and 5. These six weta all had balanced chromosome complements with five tiny dot chromosomes, one small acrocentric and one slightly larger submetacentric (Fig. 2a); simple heterozygotes (AB) between the 15-karyotype and 17-karyotype. During meiosis in males with normal karyotypes (15 and 17), the 14 and 16 autosomes form 7 and 8 bivalents, respectively, and the X-chromosome forms a univalent. During meiosis in chromosome hybrids, eight chromatid bodies formed in 88% of cells and nine chromatid bodies in 12% of cells (n = 50). Thus, three small autosomes (two acrocentric and one metacentric) apparently form a trivalent in the majority of the meiotic cells (Fig. 2b). One male weta from site 3 (homozygous for the 15-karyotype, BB) had only a single testis. This deformed animal was the only weta from the Taupo region observed with a developmental abnormality.

Allozyme and microsatellite analysis

Two of the 25 allozyme loci surveyed were polymorphic: Pgd and Icd-2. Both of these loci showed a cline in allele frequencies over the chromosome contact zone (Table 1, Fig. 3). None of the samples deviated significantly from Hardy–Weinberg expectations for any of the variable markers. No deviation from linkage disequilibrium was detected in any sample for the seven possible pairwise comparisons of polymorphic autosomal markers. The sex-linked microsatellite locus showed a cline in allele frequencies over the chromosome contact zone remarkably similar to the Icd-2 cline (Table 1, Fig. 3).



F . 2 Cell divisions in chromosomally heterozygous H. thoracica from site 3. (a) Mitotic cell from a hybrid female (2n = 17, XX) showing four pairs of large autosomes, a pair of large X chromosomes, five dot chromosomes, a small acrocentric and a slightly larger submetacentric (bottom centre). The submetacentric is homologous to the small acrocentric plus one of the five dot chromosomes. (b) Meiotic cell from a hybrid male (2n = 16, XO) showing three large autosome ring bivalents, one large autosome rod, an X univalent, two dot bivalents and probable trivalent (indicated by arrowhead). Scale bar equals 10 μm.



F . 3 Fitted tanh curve showing the clinal transition of five genetic markers through the hybrid zone of Hemideina thoracica beside Lake Taupo. Location is measured as absolute distance from site 1.

mtDNA

Two haplotypes were identified by single stranded conformational polymorphism (SSCP) of 355 bp of 12S: one found only in the northern sites and one found only in the southern sites (Table 1), with the exception of site 4 which was polymorphic. Sequencing of these mtDNA fragments from four weta (two of each haplotype) revealed a difference of four nucleotide substitutions (three transitions, one transversion).

Cline coincidence and concordance

Two sites contained chromosomal heterozygotes and only one site contained both mtDNA haplotypes. In contrast, four sites were polymorphic for Pgd and five polymorphic for Icd-2 and the microsatellite. The most northerly samples in our transect were polymorphic for all three nuclear loci, although wider surveying of the species suggested that more northerly populations are monomorphic (Morgan-Richards, 1997). The widths of the clines for Pgd, Icd-2 and the microsatellite locus do not differ significantly (Table 2). These three clines,

inheritance of haploid mtDNA would be expected to result in a narrower cline than that for biparental nuclear markers, as observed. In addition, if female weta did not disperse as far as males this also would result in a narrower mtDNA cline compared to the nuclear markers. However, the cline width estimate for the chromosomal markers is also significantly narrower than that for the three nuclear genes, indeed very similar to mtDNA cline width. The chromosome cline would not be expected to be narrower than the nuclear gene clines unless the chromosomal markers were linked to or causing hybrid disadvantage. The centres of the frequency clines for the five markers vary from 5.3 to 17.5 km south of site 1. Only Icd and the microsatellite have clines that are both coincident and concordant. Disparity of the cline centres with respect to various markers may result from differential selection of alleles (e.g. Shaw et al., 1993), perhaps exacerbated by differential migration (e.g. Cathey et al., 1998) or movement of the tension zone (e.g. Arntzen & Wallis, 1991) resulting in a nonequilibrium situation.

The difference in cline width for nuclear genes vs. chromosomes probably results from a semipermeable barrier that disadvantages only the chromosomes involved in the rearrangements and linked markers. Observations from chromosomally heterozygous weta suggested that the small acrocentric chromosome and a dot chromosome are together homologous with the small submetacentric chromosome, forming a trivalent at meiosis (Fig. 2b). This observation is compatible with the notion that the chromosomal rearrangement that differentiates the two karyotypes is a centric fission or fusion (Morgan-Richards, 1997). Such a change may result in little disadvantage to chromosomal heterozygotes as seen in some taxa (shrews (Rogatcheva et al., 1998), mice (Gropp & Winking, 1981; Wallace et al., 1998)) although even in these species the genetic background can determine whether such rearrangements reduce fertility (Hauffe & Searle, 1998). Shaw (1981) reviewed five examples of chromosome hybrid zones in orthopteroid insects and concluded that chromosomal rearrangements offer minimal isolation via mechanical impairment of meiosis. In a number of studies where chromosomal markers have been used to identify hybrid zones, selection against hybrids has been explained by a combination of genetical effects rather than purely from selection against chromosomal heterozygotes (Harrison, 1990). However, at this geologically recent contact zone it appears that introgression of nuclear markers is occurring. On reconstitution of parental karyotype (AA or BB) in F_2, nuclear genes are able to introgress more easily. There may be no barrier to the eventual genetic homogenization of these two races, with the exception of the distinct karyotypes, and thus little chance that this hybrid zone will lead to the formation of distinct species. Within the species as a whole, little concordance of allozyme and chromosome markers was found (Morgan-Richards, 1997) supporting the notion that within this species, it is the chromosome heterozygotes themselves that explain most of the reduced fitness of hybrids.

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References

ARNTZEN, J. W. AND WALLIS, G. P. 1991. Restricted gene flow in a moving hybrid zone of the newts Triturus cristatus and T. marmoratus in western France. Evolution, **45**, 805–824.

BARTON, N. H. AND BAIRD, S. J. E. 1998. ANALYSE (version 1.10). Edinburgh: http://helios.bto.edsac.uk/evolgen/Mac/Analyse/

CATHEY, J. C., BICKHAM, J. W. AND PATTON, J. C. 1998. Introgressive hybridization and nonconcordant evolutionary history of maternal and paternal lineages in North American deer. Evolution, **52**, 1224–1229.

COATES, D. J. AND SHAW, D. D. 1984. The chromosomal component of reproductive isolation in the grasshopper Caledia captiva. III Chiasma distribution patterns in a new chromosomal taxon. Heredity, **53**, 85–100.

FEL-CLAIR, F., LENORMAND, T., CATALAN, J., GROBERT, J., ORTH, A., BOURSOT, P. ET AL. 1996. Genomic incompatibilities in the hybrid zone between house mice in Denmark: evidence from steep and non-coincident chromosomal clines for Robertsonian fusions. Genet. Res., **67**, 123–134.

GROPP, A. AND WINKING, H. 1981. Robertsonian translocations: cytology, meiosis, segregation patterns and biological consequences of heterozygosity. ,

zone in the common shrew (Sorex araneus) using microsatellites. Hereditas, **125**, 159–168.

MCDOWALL, R. M. 1996. Volcanism and freshwater fish biogeography in the northeastern North Island of New Zealand. J. Biogeogr., **23**, 139–148.

MORGAN-RICHARDS, M. 1997. Intraspecific karyotype variation is not concordant with allozyme variation in the Auckland tree weta of New Zealand, Hemideina thoracica (Orthoptera: Stenopelmatidae). Biol. J. Linn. Soc., **60**, 423–442.

ORDISH, R. G. 1992. Aggregation and communication of the Wellington weta Hemideina crassidens (Blanchard) (Orthoptera: Stenopelmatidae). N. Z. Entomol., **15**, 1–7.

RAYMOND, M. AND ROUSSET, F. 1995. GENEPOP, a population genetics software for exact tests and ecumenicism. J. Hered., **86**, 248–249.

ROGATCHEVA, M. B., ODA, S.-I., AXENOVICH, T. I8.966008.966114.633685.198rganduTj8.966008.967089.981531.779Tm(,)Tj6.59006.2280.00860TD(S.AUL.1(N/(-u.I016(r86.25T.)-.1.6(