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A COMPARISON OF FIVE HYBRID ZONES OF THE WETA *HEMIDEINA THORACICA* (ORTHOPTERA: ANOSTOSTOMATIDAE): DEGREE OF CYTOGENETIC DIFFERENTIATION FAILS TO PREDICT ZONE WIDTH

MARY MORGAN-RICHARDS[1] AND GRAHAM P. WALLIS
Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand

Abstract.—Tension zones are maintained by the interaction between selection against hybrids and dispersal of individuals. Investigating multiple hybrid zones within a single species provides the opportunity to examine differences in zone structure on a background of differences in extrinsic factors (e.g., age of the zone, ecology) or intrinsic factors (e.g., chromosomes). The New Zealand tree weta *Hemideina thoracica* comprises at least eight distinct chromosomal races with diploid numbers ranging from 2n = 11 (XO) to 2n = 23 (XO). Five independent hybrid zones were located that involve races differing from one another by a variety of chromosomal rearrangements. The predicted negative correlation between extent of karyotypic differentiation (measured in terms of both percent of genome and number of rearrangements) and zone width was not found. Conversely, the widest zones were those characterized by two chromosome rearrangements involving up to 35% of the genome. The narrowest zone occurred where the two races differ by a single chromosome rearrangement involving approximately 2% of the genome. The five estimates of chromosomal cline width ranged from 0.5 km to 47 km. A comparative investigation of cline width for both chromosomal and mitochondrial markers revealed a complex pattern of zone characteristics. Three of the five zones in this study showed cline concordance for the nuclear and cytoplasmic markers, and at two of the zones the clines were also coincident. Zones with the widest chromosomal clines had the widest mitochondrial DNA clines. It appears that, even within a single species, the extent of karyotypic differentiation between pairs of races is not a good predictor of the level of disadvantage suffered by hybrids.

Key words.—Chromosome evolution, clines, hybrid zone, mitochondrial DNA introgression, population cytogenetics, weta.

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Hybrid zones form where genetically distinct populations meet and produce hybrid offspring (Harrison 1990). Some zones have few hybrids (bimodal) whereas others have many and exhibit high gene flow between taxa (unimodal; Jiggins and Mallet 2000). If the parental genotypes are adapted to different habitats then hybrid zones can form on ecotones, and a mosaic of habitats can result in mosaic zones (Harrison and Rand 1989). However, if there is reduced fitness of hybrids, then a tension zone can develop with parental genotypes occupying the same habitat. In the case of a tension zone, the width of the zone is determined by the balance between the selective disadvantage the hybrids face and the rate of dispersal of the organism (Barton and Hewitt 1985; Barton and Gale 1993).

Frequency clines for unlinked markers that are concordant in shape and position suggest strong selective pressure against hybrids although different evolutionary scenarios may give rise to identical cline shapes (Barton and Gale 1993). Discordance of frequency clines formed by nuclear and cytoplasmic markers at hybrid zones is not uncommon (Harrison 1990; Rieseberg and Wendel 1993). Although mitochondria and chloroplasts are haploid and have a smaller effective population size, these markers are often found to have introgressed further than nuclear markers. Differential introgression may reflect either the fact that cytoplasmic markers are independent of the nuclear genome and therefore are less likely to be linked to genes under selection, or greater selection against hybrid males (Haldane's Rule) because males are more typically the heterogametic sex (Barton 1993). However, Haldane's Rule results in narrow mitochondrial clines in hybrid zones of birds and moths in which the female is the heterogametic sex (Sattler and Braun 2000; Bensch et al. 2002; Dasmahapatra et al. 2002). In addition, differential dispersal or fitness of the two sexes can result in nonconcordant (different width) character clines or noncoincident clines (those with different centers; Carr et al. 1986; Tegelström and Gelter 1990; Butlin and Neems 1994).

The value of studying multiple contact zones has been highlighted by Futuyma and Shapiro (1995). They argue that by studying the same taxa in different situations it may be possible to correlate differences in hybrid-zone structure with characteristics such as age and population structure (Futuyma and Shapiro 1995). Our study compares five independent hybrid zones that involve different combinations of chromosomal races of the same biological species in an attempt to isolate factors of importance in determining zone width (hybrid disadvantage). Our aim is to examine the effect of chromosome rearrangements on fitness, keeping species and ecology constant.

The species chosen for this investigation, *Hemideina thoracica*, is a chromosomally polymorphic but morphologically uniform weta species. Weta is a generic term given to some 50 species of nocturnal orthopteran endemic to New Zealand. *Hemideina thoracica* is found over most of North Island, absent only from the extreme south and regions above 900-m above sea level (Trewick and Morgan-Richards 1995). This species of tree weta is an arboreal, herbivorous generalist, and is found in all forest types within its geographical range including regenerating scrub and suburban gardens (Gibbs 2001).

[1] Present address: Allan Wilson Centre for Molecular Ecology and Evolution, Massey University, P.O. Box 11-222 Palmerston North, New Zealand; E-mail: xenophon@ihug.co.nz.

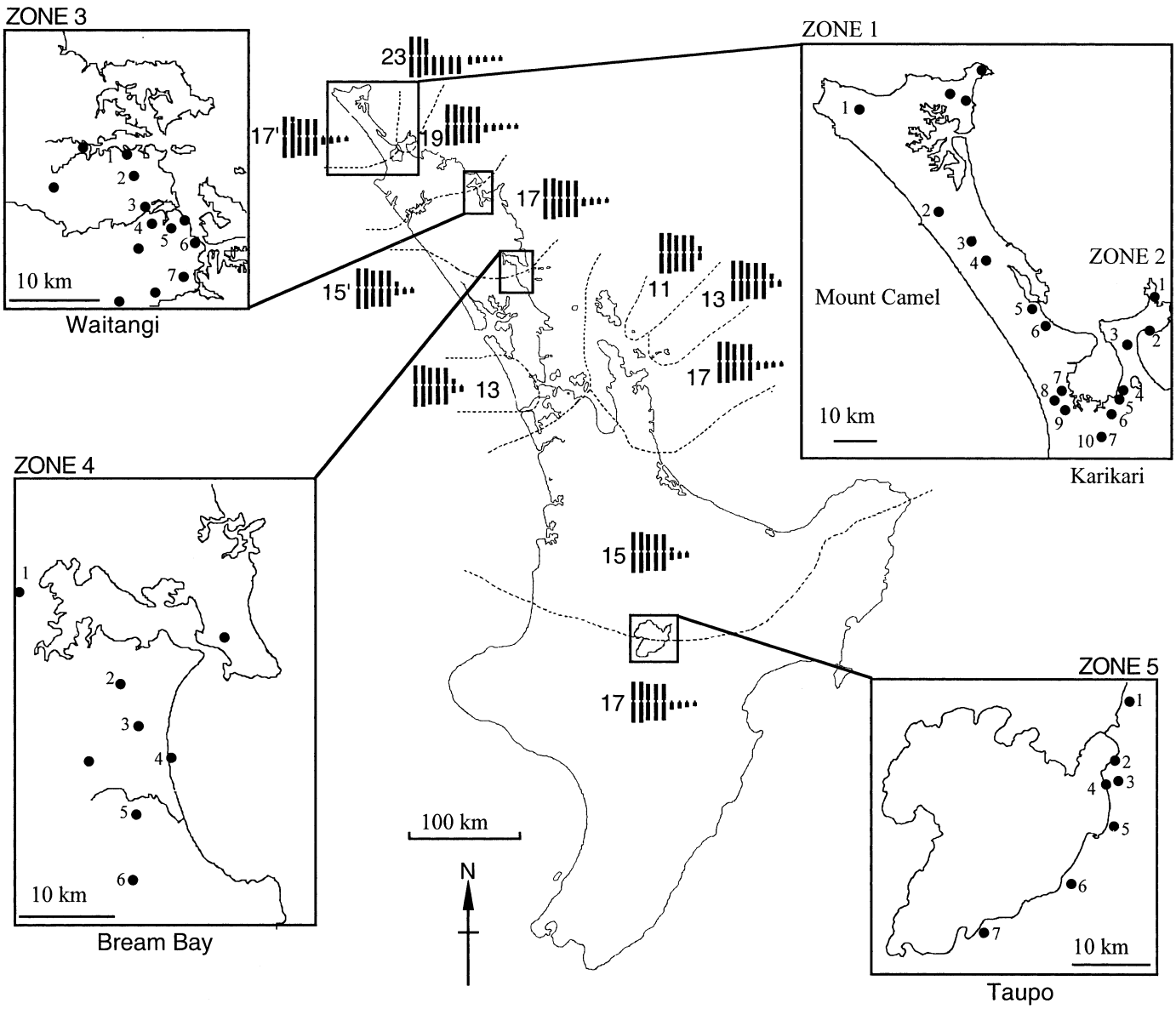

FIG. 1. The distribution of the eight chromosomal races of *Hemideina thoracica* on North Island, New Zealand, showing the haploid karyotype of each race. Dashed lines indicate approximate boundary of each race. Enlargements of five contact zones between these chromosomal races are shown as insets. Sampling locations indicated by spots; only numbered locations were used in cline analyses.

Five hybrid zones involving six different chromosomal races were located and sampled for this comparative study (Fig. 1). *Hemideina thoracica* has greater allozyme and mitochondrial DNA (mtDNA) diversity in the northern third of its range than in its southern two-thirds (Morgan-Richards 1997; Morgan-Richards et al. 2001a). The northern chromosomal races (17′, 19, 23) are likely to be of Pliocene origin but southern races are probably much younger (Morgan-Richards et al. 2001a). For Zones 1 and 2 it is likely that the chromosome races first made contact during the early Pleistocene when sandbar formation linked a chain of islands and sea levels fell (Ballance and Williams 1992). Lower levels of mtDNA diversity in the southern compared to the northern races suggest that southern races probably originated during the Pleistocene (Morgan-Richards et al. 2001a). Zone 5 is probably the youngest of the five hybrid zones: it formed following a volcanic eruption less than 2000 years ago (Wilson and Walker 1985; Morgan-Richards et al. 2000).

The hybrid zone between two southern chromosomal races of *H. thoracica* centered in the Taupo volcanic region has been investigated using five genetic markers. Three unlinked nuclear markers (two allozyme, one microsatellite) form clines that are between 15 and 22 km wide but the chromosome marker (a Robertsonian [Rb] translocation) forms a significantly narrower cline approximately 5 km wide. The mitochondrial cline, displaced 4 km to the south, is the same width as the chromosomal cline. The nonconcordant character clines through this hybrid zone suggest that the chromosomal rearrangement that differentiates these two races may limit the introgression of the chromosomes involved in

TABLE 1. Predictions of relative zone width for five chromosomal hybrid zones of *Hemideina thoracica* based on the chromosome characteristics of each zone (hypothesized chromosome rearrangements that differentiate the karyotype pairs at each zone and the percentage of karyotype involved in these rearrangements; see Figure 1 for ideograms).

Zone	Location	Number of Chromosomes (XO)	Characteristic elements	Rearrangements that differentiate the races	% complement in rearrangements	Predicted relative zone width
1	Mt. Camel	17′ and 19	1 large metacentric (19) or 1 submetacentric (17′)	1 pericentric inversion or reciprocal translocation		
			1 small acrocentric (19)	1 translocation/duplication	20–35	narrow
2	Karikari	23 and 19	4 medium acrocentrics (23) or 2 large metacentrics (19)	2 Rb translocations	30–35	narrow
3	Waitangi	19 and 17	1 small acrocentric (19)	1 translocation/duplication	1–2	wide
4	Bream Bay	17 and 15′	2 acrocentrics (17) or 1 metacentric (15′)	1 Rb translocation	5–7	medium
5	Taupo	15 and 17	2 acrocentrics (17) or 1 submetacentric (15)	1 Rb translocation	6–8	medium

the rearrangements but have little effect on the introgression of unlinked nuclear alleles (Morgan-Richards et al. 2000). The two Taupo races are differentiated by a single Rb translocation involving relatively small autosomes, but at other contact zones larger chromosomes are involved in Rb translocations and other rearrangements (Morgan-Richards 1997). A comparison of hybrid zones could reveal the relative disadvantage conferred by the chromosome rearrangements. In a number of taxa, the more rearrangements that differentiate any two races, the greater the likelihood of disruptions to meiosis resulting in unbalanced gamete formation and reduced fertility (Searle 1993; Searle and Wójcik 1998; Spirito 1998; Gorlov and Tsurusaki 2000). The likelihood of disruptions to meiosis may depend on the type of chromosomal rearrangement, the number of rearrangements, the quantity of chromosome material involved in the rearrangements, and the genetic background on which they occur, or some combination of these effects. A general prediction is that the more rearrangements and the more material involved, the greater the disruption and reduction in fertility (Spirito 1998; Delneri et al. 2003). Rearrangements that involve simple fission/fusions may permit equal disjunction of genetic material and thus have little effect on fertility. If the same chromosome arms are involved in different fusions (e.g., the Monobrachial chromosome speciation model; Capanna and Corti 1982; Baker and Bickham 1986; Searle 1993) or if there are numerous fission/fusions (Wallace et al. 1992), fertility is likely to be affected. In contrast, inversions involving relatively little of the genome can have quite severe effects on meiosis (White 1978). Comparisons of shrew chromosome hybrid zones clearly show that the number of Rb translocations influences zone width when the genetic background of the races compared is similar (Searle and Wójcik 1998). To test whether greater karyotypic differentiation between races of *H. thoracica* results in greater infertility of hybrids, we compared cline width for chromosome and mtDNA markers across five independent hybrid zones where chromosomal races meet (Table 1).

When there is more than one chromosomal rearrangement differentiating races there are two complicating factors that could affect the zone width. First, the proportion of the genome linked to the chromosome rearrangements is larger than for a single rearrangement and therefore the potential for reduced gene flow of neutral genes is stronger, which could narrow the zone further (Spirito 1998). On the other hand, chromosome markers can form staggered clines that would widen the zone (Searle 1993; Searle and Wójcik 1998; Gorlov and Tsurusaki 2000). Frequency clines can be forced apart by selection against double heterozygotes if the selection against double heterozygotes is greater than the sum of the selection suffered by the two single heterozygotes. This synergy increases the width of the zone. At zones involving races differentiated by two or more indistinguishable chromosome rearrangements (such as translocations involving the same-sized chromosome arms), staggered clines would be detected by a lack of animals heterozygous for both markers (Gorlov and Tsurusaki 2000). For *H. thoracica*, two contact zones involve two karyotypic markers (Table 1) and these zones might be wider than expected based on the chromosome rearrangements if the clines are staggered.

Chromosome Rearrangements in Hemideina thoracica

Within *H. thoracica* eight distinct karyotypes have been described (Morgan-Richards 1997). Diploid numbers range from 11 (XO) to 24 (XX). By convention, the diploid number for males is used to name the chromosomal races. There are two distinct karyotypes for both $2n = 15$ and $2n = 17$, distinguished from one another by one autosome which is either metacentric (15′ and 17 karyotype) or submetacentric (15 and 17′; Fig. 1). Where the fundamental number of chromosome arms (NF) remains constant among the karyotypes, the variation in chromosome number has probably arisen via Rb translocations and/or inversions. Variation in NF is due to the addition/loss of very small acrocentric autosomes. Variation in the number of pairs of these very small autosomes differentiates both 15 and 15′ from the 13 race, 13 from the 11 race, and 19 from the 17′ (Zone 1) and 17 races (Zone 3; Fig. 1; Morgan-Richards 1997). Comparisons of the karyotypes of closely related species of pocket gopher (Hafner et al. 1983) and populations of the lizard *Sceloporus grammicus* (Sites 1983) show similar variation in the number of small autosomes. As is the case with *H. thoracica*, the exact mutations involved are not known.

The Hybrid Zones and Predictions

The five hybrid zones of *H. thoracica* included in this study and the chromosome differences that characterize them are presented in Table 1. Zones are numbered 1 to 5 from north to south (Fig. 1). Chromosome rearrangements that distinguish the karyotypes are based on studies of plain-stained mitotic and meiotic cells from homozygotes and heterozygotes (Morgan-Richards 1997; this study). We assume that the five hybrid zones in this study arose through secondary contact of populations that differentiated in allopatry. None of the zones are situated in regions in which there exists or has existed in the recent past an identified change in habitat (Newnham 1999), so all five contact zones may be tension zones. All zones are geographically close (Fig. 1) and exist on a homogeneous ecological background of lowland forest. Although some of this forest has been disturbed recently by human activity it is replaced with patchy regenerating scrub that is also suitable habitat for weta.

Based on the number of rearrangements and proportion of the genome involved in the chromosome rearrangements that differentiate the pairs of races, we made predictions of the relative width of the hybrid zone at each of five contact zones between the six chromosomal races (Table 1). In making these predictions we assumed that: (1) the races do not differ for traits that might affect hybrid zone width other than chromosomal rearrangements, and (2) habitat structure is similar in the five zones. Any differences among races that allow ecological or incompatibility selection in the zones could lead to differences in cline width. The narrowest zones were expected in the north of the species range where three chromosomal races form two contact zones (Zones 1 and 2) on sandbars connecting old islands (Morgan-Richards et al. 2001a). These zones are characterized by races with multiple chromosome rearrangement differences that involve relatively large proportions of their genomes. These differences were expected to result in a relatively high degree of disruption to meiosis in heterozygous individuals and thus strong selection against hybrids, resulting in narrow zones. In contrast, farther south, only single chromosomal rearrangements involving small chromosomes differentiate races, and hybrid zones were expected to be wider (Table 1).

Materials and Methods

Collection of Animals

Hybrid zones were localized by initial broad sampling (Morgan-Richards et al. 2001a). Five contact zones were identified and transects taken perpendicular to the line of contact (Fig. 1). For Zones 1 and 2, transects were on narrow (<15-km), vegetated sandbars, and were made parallel with the shore (Fig. 1 insets). For Zones 3 and 4, transects were made close to and roughly parallel with the eastern coast of North Island. Zone 5 is in the center of a volcanic region and the transect was made along the eastern shore of the flooded crater, Lake Taupo (Morgan-Richards et al. 2000). Weta were collected from holes in trees during the day and transferred alive to the laboratory. Young were raised until large enough for karyotyping.

Cytogenetic Analysis

Following euthanasia with ether, reproductive tissue was taken from adult and immature weta of both sexes. Female weta were injected with colchicine (0.05% in insect saline) and left overnight before killing. Ovarian follicles and testes were immersed in hypotonic solution (1:5 insect saline:water) for 20 min then fixed in methanol:acetic acid (3:1) for at least 1 h. Air-dried slides were made and stained with Giemsa (BDH, Dorset, England; 8% in phosphate buffer for 20 min) as described in Morgan-Richards (1997). A total of 357 weta was scored for karyotype using plain-stained mitotic cells, and samples for each hybrid zone ranged from 56 to 88 animals.

Mitochondrial DNA Analysis

DNA was extracted from frozen muscle using a salting-out method (Sunnucks and Hale 1996). Universal insect mitochondrial primers SR-J-14233 and SR-N-14588 (Simon et al. 1994) were used to amplify a 355 base pair (bp) fragment of the small ribosomal subunit (12S) for single-stranded conformational polymorphism (SSCP) analysis to screen for haplotype variants (Trewick et al. 2000). A representative of each SSCP 12S haplotype was then sequenced for a 550-bp fragment of the 3′ end of cytochrome oxidase I (COI) gene. Because mtDNA is inherited as a single nonrecombining unit, it was possible to use different regions of the genome for sorting and sequencing haplotypes. We expected to detect more variation in COI compared to that within 12S because of the smaller size of the 12S fragment used and its slower rate of evolution (Simon et al. 1994). For the cline analysis, SSCP haplotypes were coded into one of two clades based on the COI phylogeny (see below). Universal insect mitochondrial primers C1-J-2195 and L2-N-3014 (Simon et al. 1994) were used to amplify an 800-bp fragment. Polymerase chain reaction products were gel-purified and cleaned using Qiaquick (Qiagen, Victoria, Australia) spin columns. DNA was sequenced using primer C1-J-2195 with Bigdye chemistry (Perkin Elmer, Foster City, CA) following the manufacturer's protocols. Sequences were aligned by eye using SeqEd ver. 1.0.3 (Applied Biosystems Inc., Perkin Elmer, Foster City, CA).

Population Genetic Analysis

Hardy-Weinberg equilibrium was tested using Fisher's exact test implemented in GENEPOP (Raymond and Rousset 1995). Cytonuclear equilibria were not tested because only a single sample was polymorphic for both chromosomal and mitochondrial markers. In that sample (Zone 1, Motutangi) only two of the 16 weta for which there were cytogenetic data had mtDNA markers from the 19 clade (Table 2).

Frequency clines for the chromosomal markers were calculated across each zone (Barton and Gale 1993). It was not possible to distinguish the two chromosome markers at Zone 2 so these were combined. Although the two chromosome markers at Zone 1 could be distinguished, they were also combined for cline analysis. For cline analyses, each mtDNA haplotype was assigned to a race according to the COI phylogeny. Cline widths and centers were estimated with Analyse

TABLE 2. Frequency distribution of karyotypes and mtDNA markers through five hybrid zones between chromosomal races of *Hemideina thoracica.*

Zone 1, Mt. Camel Sampling site		17′ contacts 19-karyotype Code	Km from site 1	% 19-karyotype	*n*	% mtDNA	Haplotypes	*n*
Pandora's Track	1	Pnd	0	0	6	0	a(2) b(6)	8
Wahakari	2	Whk	19.5	0	5	0	g(3)	3
Lake Bulrush	3	Bul	29	0	5	0	c(2) g(2)	4
Lake Waihopo	4	Wai	34.5	0	4	0	d, e, f, g	4
Hauhora Heads	5	HhH	47	45.84	12	0	h(13)	13
Motutangi Swamp	6	Mot	52.5	31.25	16	14.29	g(9) l(6) m, n, o, P, R(2)	21
Paparore	7	Papa	65.75	22.73	11	0	k(12)	12
Avocado Stop	8	Avo	68.25	0	7	0	g(8)	8
90-mile beach road	9	NmR	71	100	14	37.5	g, j(9) Q(6)	16
Quarry Road	10	Qry	80	100	8	83.33	i(2) Q(10)	12
Zone 2, Karikari Sampling site		23 contacts 19-karyotype Code	Km from site 1	% 19-karyotype	*n*	% mtDNA	Haplotypes	*n*
Matai Bay	1	Mat	0	0	8	0	a(5) b, d, e(2)	9
Whatuwhiwhi	2	Whw	5.5	9.37	8	0	e(12)	12
Lake Waiporohita	3	Wp	10.5	2.94	17	0	d, e(15)	16
Tahanga Road	4	Tah	20	86.54	13	100	F(13)	13
Pukewhai	5	Pkw	22	81.25	8	100	F(9)	9
Arawhata	6	Arw	25.75	97.92	12	100	F(13)	13
Quarry Road	7	Qry	31	100	8	83.33	c(2) F(10)	12
Zone 3, Waitangi Sampling site		19 contacts 17-karyotype Code	Km from site 1	% 17-karyotype	*n*	% mtDNA	Haplotypes	*n*
KeriKeri Inlet	1	KkI	0	0	4	0	A(5)	5
Mt. Te Puke	2	Puke	3.25	0	13	0	B(11) C, D	13
Haruru Falls	3	Hrr	5.75	11.12	9	0	D(10) E	11
Puketona	4	Ptk	8	100	9	100	h(10)	10
Paihia Walkway	5	Wwy	9.5	100	15	100	h(13) f, g	15
North End Oromahoe	6	ENO	13.75	100	4	100	h(2)	4
Paihia Inlet	7	Pai	17	100	2	100	h(4)	2
Zone 4, Bream Bay Sampling site		17 contacts 15′-karyotype Code	Km from site 1	% 15′-karyotype	*n*	% mtDNA 52	Haplotypes	*n*
Otaika Valley	1	Otk	0	0	7	0	a(10)	10
Flyger Road	2	Fly	15	0	6	66.66	b(2) D(2) I, J	6
Prescott Road	3	Prst	19.5	0	17	100	D(17)	17
Uretiti	4	Urt	24	45.46	11	100	D(13)	13
Ahuroa River	5	Ahu	30.75	95.45	11	100	C, D(8) E, G(3)	13
Waipu	6	Wpu	37	95.83	12	100	D(10) G(3)	13
Zone 5, Taupo Sampling site		15 contacts 17-karyotype Code	Km from site 1	% 17-karyotype	*n*	% mtDNA	Haplotypes	*n*
Huka Falls	1	Hk	0	0	9	0	a(9)	9
Rainbow Point	2	Ta	7.6	0	30	0	a(32)	32
Airport	3	Ap	9.6	12.5	12	0	a(10)	10
Five-mile Bay	4	5m	9.7	0	6	16.67	a(5) B	6
Mill Road	5	Mill	15.3	78.57	7	0	a(8)	8
Hinemaiaia River	6	Hin	22.1	100	9	100	B(11)	11
Parikanangaroa	7	Pak	38	100	2	100	B(2)	2

1.30, a software package for the analysis of hybrid zones (Barton and Baird 1998). Cline width is defined as the inverse of the maximum slope and the center is where the cline slope is steepest (Barton and Gale 1993). Variation in allele frequencies among sites was assumed to be caused by a combination of sampling error and a smooth frequency cline and therefore F_{ST} was set to zero. Setting F_{ST} to zero also compensates for variation in sample size. Estimations of cline width and center were also made using $F_{ST} > 0$, but this parameter had little effect on the confidence intervals and did not alter the conclusions drawn. Confidence intervals were based on the points that were 1/7.4 as likely as the maximum-likelihood estimates obtained by randomly varying the parameters (width and center) using a metropolis

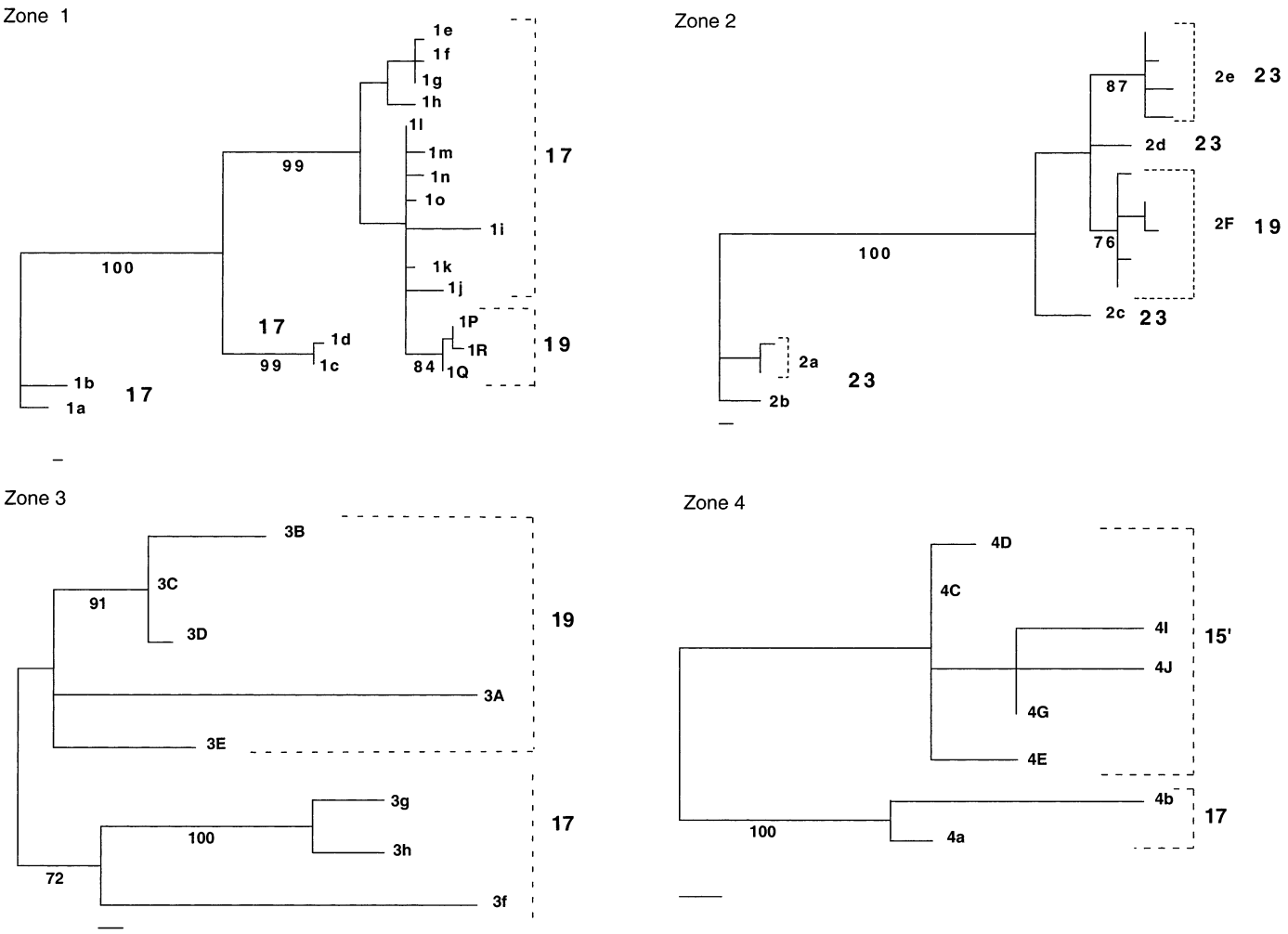

FIG. 2. Maximum parsimony trees for mtDNA sequence data (550 bp of cytochrome oxidase I) from each of four hybrid zones (Zone 5 had only two haplotypes). Terminal branches are labeled according to 12S-SSCP haplotypes. Numbers below branches are bootstrap values (>70) obtained from 1000 replicates. Scale line = 1 substitution.

algorithm (1000 iterations), following recommendations given with the program (Barton and Baird 1998).

To assign mtDNA haplotypes to the alternative chromosome races, the COI sequence data was phylogenetically analyzed. PAUP*4.0b (Swofford 1998) was used to implement phylogenetic reconstruction using maximum parsimony (MP). Unweighted and unrooted settings were used. We used 1000 bootstrap iterations and presented the resulting tree. Only bootstrap values that represent good support for branches (≥ 70; Hillis and Bull 1993) are included on the phylograms (Fig. 2). At each of the five zones, one bipartition of the tree divided haplotypes that defined the majority of weta from the two chromosomal races.

RESULTS

Cytogenetics

Chromosome heterozygotes were found at between one and five sites per zone (Table 2). The polymorphic populations did not deviate significantly from Hardy-Weinberg equilibrium (genotypes available on request). All heterozygous weta were chromosomally balanced (without additional or reduced chromosomal material), with the exception of three weta from Zone 4, which all had small additional chromosomes.

Cline width estimations for the chromosomal markers ranged from 0.3 to 47 km (Table 3). Thus, the narrowest (cline 3) and the widest (cline 1) differed in width by two orders of magnitude (Fig. 3). The zones with the greatest proportion of their genome involved in the chromosome rearrangements (Zones 1 and 2) were expected to show the narrowest clines as these hybrids should suffer the greatest disadvantage. However, these zones have chromosome clines that were wide relative to the other zones (47 km and 10 km; Tables 3 and 4, Fig. 3). The zone in which the karyotype differentiation appears to involve the least chromosome material (Zone 3) has the narrowest chromosome cline (0.3 km; Table 3). Thus the predicted relationship between the degree of karyotypic differentiation and cline width was not found (Table 4). The two zones in which the chromosomal races are differentiated from each other by two chromosomal markers (Zones 1 and 2) had wide clines when the markers were combined in the cline analyses. The width of these zones

TABLE 3. Estimates of cline width and center for chromosome and mtDNA markers through five hybrid zones between chromosomal races of *Hemideina thoracica.* CI, 95% confidence interval approximated with maximum-likelihood estimates.

	Chromosome cline				mtDNA cline			
Zone	Width (km)	CI	Center	CI	Width (km)	CI	Center	CI
1	47.28	(44.0–51.5)	61.81	(60.7–63.0)	25.98	(23–29.3)	72.66	(71.6–73.8)
2	10.32	(9.7–11.1)	16.52	(16.1–17.0)	10.35	(9.4–11.5)	16.37	(15.6–16.9)
3	0.53	(0.3–1.3)	6.00	(5.9–6.4)	0.31	(0.01–0.88)	6.90	(5.7–8.0)
4	8.13	(7.4–8.9)	25.32	(24.9–25.7)	1.67	(0.5–4.4)	14.71	(13.9–14.9)
5	5.29	(4.9–5.8)	13.40	(13.1–13.7)	6.19	(5.4–7.1)	17.51	(17.0–18.1)

might be relatively wide if the independent chromosome markers formed clines that were staggered rather than coincidental. At Zone 1, the two chromosomal markers could be clearly distinguished from one other, but weta heterozygous for only one of the two chromosome markers were very rare, providing no evidence for staggered chromosome clines at this zone. At Zone 2, two Rb translocations differentiating the chromosomal races involve chromosome arms of very similar size, creating markers indistinguishable by plain-staining. It was not possible to sample the center of Zone 2 because fire eradicated weta habitat in 1995 (Fig. 3). Thus, with the available data from Zone 2 it is not possible to distinguish between two concordant, coincident chromosome clines and two clines that are slightly staggered.

Mitochondrial DNA

A total of 44 different mtDNA haplotypes was detected by SSCP from 395 weta (Zone 1,18; Zone 2, 6; Zone 3, 8; Zone 4, 10; zone 5, 2). Many haplotypes were found at just a single site (private), so to classify the mtDNA haplotypes into markers that could be used to measure cline width, haplotypes at each zone were divided into two groups according to phylogeny (Fig. 2), each characteristic of one chromosomal race. Each 12S haplotype identified by SSCP was sequenced for 550 bp of COI from up to nine individual weta. Some additional variation was detected by sequencing multiple individuals with the same SSCP haplotype, but in each case the sequences differed by only one to three substitutions and were sister taxa.

At Zone 1, 18 unique COI sequences were obtained from the 18 SSCP haplotypes (Fig. 2a; Table 2; upper- and lowercase letters distinguish the haplotypes that characterize the two sides of the zone). Only two of the 18 haplotypes were found at more than one site. At Zone 2, 14 unique COI sequences were obtained from six SSCP haplotypes (Fig. 2b). Two SSCP haplotypes were common (2e and 2F), one or other being found at all seven sites, and four additional haplotypes were private. Eight unique COI sequences were obtained from the eight SSCP haplotypes of weta from Zone 3 (Fig. 2c). One of these haplotypes was common (3h) in four southern sites but three sites had only private haplotypes. At Zone 4, eight unique COI sequences were obtained (Fig. 2d); one was common (4D) but six were private. Only two SSCP haplotypes were observed within Zone 5, corresponding to the two karyotypes.

Estimated widths of the five mtDNA frequency clines ranged from 0.3 to 26 km (Table 3). The narrowest clines were at Zones 3 and 4 (these two did not differ significantly in their width estimates although the slopes of the clines appear different; Fig. 3). At Zone 3 no samples had mtDNA haplotypes from both clades and therefore the cline estimate is limited by the sampling resolution. Similarly, at Zone 2, a single sample at the southern end of the transect was polymorphic for mtDNA. If a less conservative approach to coding the mtDNA haplotypes were taken, this cline would be much narrower and limited by the sampling resolution. The widest mtDNA cline was at Zone 1, which also had the widest chromosome cline. The mtDNA cline was narrower than the chromosome cline at Zones 1 and 4. For the three other zones (2, 3, and 5) the clines for the two markers did not differ significantly in width (concordant; Fig. 3), although the estimate of the mtDNA cline at Zone 3 is limited by sampling and therefore might be narrower than the chromosome cline. Chromosome and mtDNA clines were coincident (their centers were at the same locations) at Zones 2 and 3 (Fig. 3). The displacement of the cline centers for Zones 1, 4, and 5 was 10.8 km, 10.6 km, and 4.1 km respectively. However, in terms of the cline widths these displacements varied from approximately 0.25 times (Zone 1) to 1.3 times (Zone 4) the width of the actual clines.

DISCUSSION

This comparison of five independent hybrid zones within a single species revealed a number of notable features. First, although chromosome cline widths varied by two orders of magnitude, they show the opposite pattern of zone widths to that predicted by degree of karyotype differentiation. The narrowest chromosome cline was found where karyotype differences involved about 2% of the genome and the widest where they involved 20–35% of the genome. Second, chromosome and mtDNA clines did not show a consistent pattern of concordance and coincidence among zones (only three zones had clines of the same width and only two had clines with the same centers). Third, mtDNA markers introgressed no farther than nuclear markers at any of the five zones.

Variation in Chromosome Cline Widths among Five Zones

At Taupo (Zone 5), an earlier study of unlinked nuclear markers revealed that this hybrid zone was reducing the introgression of the chromosomal markers more than genic markers (allozyme and microsatellite) and therefore cline width might be controlled by the reduction in fitness of hybrids resulting from the actual chromosome rearrangement (Morgan-Richards et al. 2000). In contrast, chromosome hybrid zones in other orthopteran species are not thought to

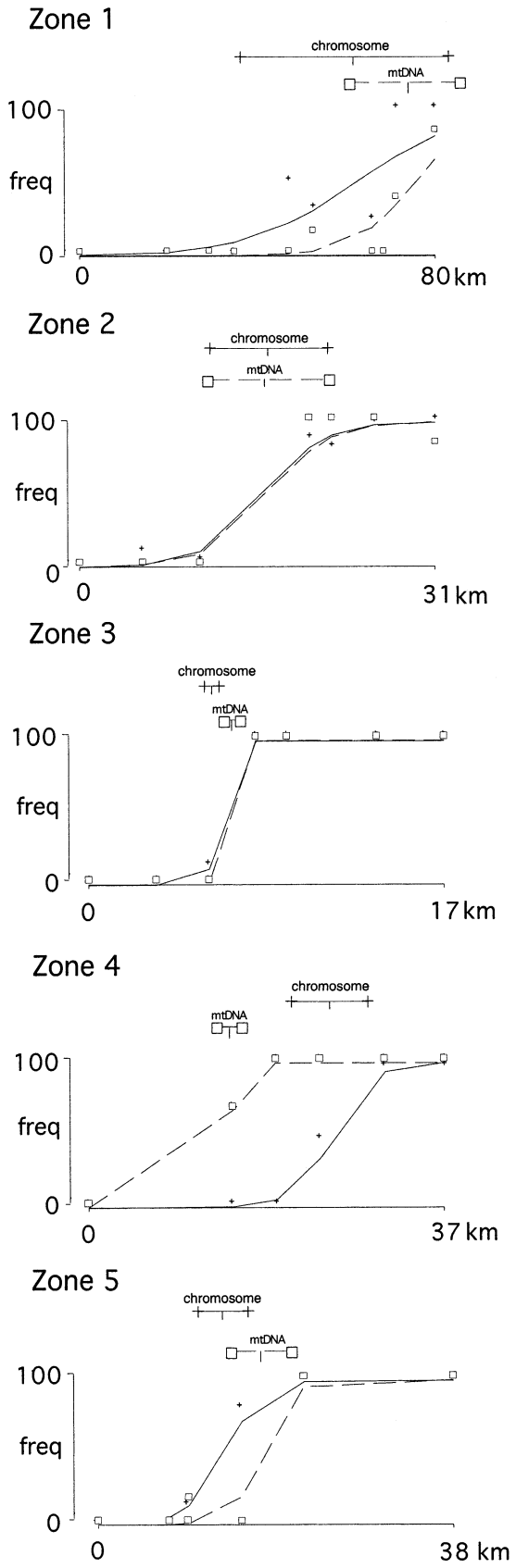

FIG. 3. Character clines across five hybrid zones of the weta *Hemideina thoracica*. Chromosome markers are indicated with crosses, mtDNA markers with open squares. Centers and widths of clines indicated above curves. Note that scales differ for the five zones.

have their widths determined by reduction in fitness of hybrids resulting from chromosome rearrangements (Shaw 1981; Harrison and Rand 1989; Butlin 1998). For example, the width of the hybrid zone between the Moreton and Torresian races of *Caledia captiva* on the eastern coast of Australia does not appear to be determined by the chromosome rearrangements even though evidence for karyotype selection has been found (Groeters and Shaw 1996). Although hybrid zone width may not be determined by the chromosome rearrangements in a number of studies, the chromosome clines themselves do seem to be influenced by chromosome heterozygote disadvantage. For example, in the Mexican lizard *Sceloporus grammicus*, narrow chromosome clines are apparently maintained by genomewide forces, whereas other nuclear markers have much wider clines (Marshall and Sites 2001). The hybrid zone between *Mus domesticus* and *M. musculus* in Denmark is maintained by NORII centromeric incompatibilities; selection detected against Rb fusions does not reduce gene flow, although it may maintain the chromosome cline (Fel-Clair et al. 1998). Within taxa there is some evidence that the width of chromosome clines varies according to the degree of karyotype differences but there are few chromosomally polymorphic taxa in which more than two independent zones have been studied. Both shrews and mice have been studied for multiple hybrid zones and a correlation between zone width and karyotypic differentiation seems to follow for races that belong to the same genetic group (Searle 1993; Searle and Wójcik 1998). In the grasshopper *C. captiva*, three hybrid zones suggest that cline width is determined by the level of genetic divergence rather than chromosome differentiation (Shaw et al. 1993). However, in pocket gophers, hybrid zone width is determined by patchiness of the available habitat rather than genetic (allozyme and chromosomal) or ecological differentiation of the two taxa (Patton 1993). In a comparison of four sunflower hybrid zones, Buerkle and Rieseberg (2001) concluded that intrinsic forces predominate in determining hybrid zone dynamics and boundaries.

The width of the five chromosome clines in five independent hybrid zones of *H. thoracica* varied considerably. As these clines all occur within the same morphological species we argue that their widths differ because of differences in the disadvantage suffered by hybrids rather than variable dispersal abilities of weta. The greater the karyotypic differences distinguishing the races, the lower the predicted fitness of the hybrids (Searle 1993; Spirito 1998) and the narrower the expected cline. This prediction was not met: the zones with the greatest proportion of their genome involved in multiple rearrangements (Zones 1 and 2) had wider clines than Zones 3, 4, and 5, in which single rearrangements involving small chromosomes differentiated the races. Where the least disadvantage due to meiotic disruption was predicted for hybrids, the narrowest clines for chromosome and mtDNA markers were measured (Zone 3; Table 4).

This variation in cline width may be because one or both of our assumptions are wrong: (1) the races differ in traits other than their karyotypes, or (2) there is habitat variation. Any differentiation among chromosomal races that reduces their compatibility (intrinsic), or could allow ecological selection (extrinsic), could affect cline width. Alternatively,

TABLE 4. Predicted relative zone widths based on degree of karyotype differentiation between chromosomal races at each zone and the observed relative cline widths for five independent hybrid zones of *Hemideina thoracica.*

Zone	Chromosomal races	Predicted relative zone width	Width of observed chromosome cline	Width of observed mtDNA cline	Clines	
					Concordant	Coincident
1	17′ and 19	narrow	very wide	wide	no	no
2	23 and 19	narrow	medium	medium	yes	yes
3	19 and 17	wide	narrow	narrow	yes	yes
4	17 and 15′	medium	medium	narrow	no	no
5	15 and 17	medium	medium	medium	yes	no

there may be problems with the data. The possible explanations suggested here are not mutually exclusive.

Chromosomes.—(1) Additional undetected chromosome rearrangements might differentiate the races where clines were narrower than expected. At four of the five zones, studies of meiosis in heterozygous males (which can detect rearrangements not visible by plain-staining) were possible and no evidence of such rearrangements was seen. At the narrowest zone (3), no male chromosomal heterozygotes were found. (2) Hybridization might result in an increased chromosomal mutation rate, which could disguise heterozygous weta and reduce the estimates of cline width (Shaw et al. 1983; Naveira and Fontdevila 1985; Morgan-Richards 1995). For example, where karyotypes differ in the number of pairs of small autosomes (Zones 1 and 3), duplication of unpaired autosomes in hybrids could produce parental-type karyotypes. (3) Zones could be wider than expected because of staggering of chromosome clines (Searle 1993; Gorlov and Tsurusaki. 2000). Zones 1 and 2 each involve two chromosome rearrangements in which the clines could be forced apart by selection against double heterozygotes. No evidence was found that the chromosome clines were staggered at Zone 1, in which single heterozygotes were very rare, but this could not be tested for Zone 2 because sampling was not possible in the center of the zone.

Width of zones.—The width of the hybrid zones might be determined by genetic factors not involving chromosome rearrangements (Shaw 1981). For example, shrew hybrid zones between races that belong to different karyotypic groups are much narrower than their degree of chromosome rearrangement would predict, and narrower than zones that involve similar numbers of Rb translocations between races that are genetically and morphologically more similar (Searle and Wójcik 1998). Similar cline estimates for both chromosomal and mitochondrial markers support the idea that the width of the hybrid zones in this species is determined by genomewide forces and not just chromosome heterozygote disadvantage. In addition, genetic changes that cause inviability may have occurred within chromosome rearrangements and could be held together by suppression of recombination (Noor et al. 2001; Rieseberg 2001). This model of chromosome-induced reproductive isolation is more likely to operate where inversions differentiate races because inversions are the only rearrangement commonly associated with crossover suppression. Only the chromosome races at the widest hybrid zone (Zone 1) of *H. thoracica* have been hypothesized to be differentiated by a chromosome inversion.

Age of the zones.—(1) A tension zone is maintained by a balance between hybrid disadvantage and dispersal, and the zone width should be constant once equilibrium is established. Where there is little or no selection against hybrids, shallow clines are expected. In such cases, cline width depends largely on dispersal rate and time since contact. Given enough time, the two populations might be expected to merge. The chromosome races involved in Zones 1 and 2 (17′-karyotype, 19-karyotype and 23-karyotype) may have been in contact (on and off) since the beginning of the Pleistocene (Morgan-Richards et al. 2001a); sufficient time to completely mix if there were no selection against hybrids. Zones 1 and 2 are thought to have formed at the same time yet they differ in width by 37 km (chromosome cline) and 16 km (mtDNA cline). At Zone 5, on the other hand, the current contact between the two races can be no older than the last Taupo eruption, 2000 years ago (Morgan-Richards et al. 2000). Although chromosome and mitochondrial clines at Zones 1 and 2 are significantly wider than at Zone 5 (42 km, 20 km, 5 km, and 4 km, respectively), weta are able to move at least 80 m per generation (Morgan-Richards et al. 2000), so it would take only 1050 years to close this kind of gap. These results suggest that differential age of zones is unlikely to be a major explanation for the variable cline widths observed. (2) Selection may have resulted in reduced hybrid disadvantage for hybrids (opposite to reinforcement) so that the older zones in northern New Zealand (Zones 1 and 2) have reduced disadvantage compared to younger zones (Howard 1993; Butlin 1995). Selection may be expected to reduce the frequency of alleles that cause karyotypic heterozygotes to suffer a high frequency of meiotic anomalies (Shaw 1981) and select for reproductive compensation traits that counteract reduced output of gametes or embryo loss (Searle 1993). The widest zones (1 and 2) are probably the oldest of the zones studied here (Morgan-Richards et al. 2001a) but they differ significantly in their widths. (3) Differences in time of origin or available quantitative genetic variation could have promoted reinforcement at some zones and not others. Reinforcement suffers from major theoretical problems (Spencer et al. 1986; Sanderson 1989), and evidence for it has been questioned (Butlin 1995). However, recent empirical (Noor 1995; Saetre et al. 1997) and theoretical (Cain et al. 1999) results suggest that it should not be completely discarded as a possible evolutionary mechanism. In addition, premating isolation between races may have arisen without reinforcement because of, for example, divergent sexual selection or mate-finding strategies. In our case, weta have a simple stridulatory mechanism that differs little among species (Field 1978). Interspecific matings are common between sympatric and parapatric pairs of the genus (Morgan-Richards et al. 2001b) so the opportunities for premating isolation may be limited. In

this study, no evidence of assortative mating was found, because samples that were polymorphic for karyotypes did not deviate from Hardy-Weinberg expectations.

Geographical barriers.—Geographical barriers such as the Waitangi River (Zone 3) may reduce dispersal and narrow the zone. However, rivers also occur at Zone 4, but do not seem to influence cline shape. Shrew chromosome hybrid zones are sometimes affected by rivers and sometimes not (Searle and Wójcik 1998).

Habitat.—Habitat may differ among the zones, which could alter dispersal rate. Pocket gophers form either unimodal or bimodal hybrid zones independent of the degree of genetic (allozymic and chromosomal) and ecological differentiation between parents, but associated with habitat patchiness (Patton 1993). All five zones studied are within 600 km of each other in lowland forest. Although there are no known changes in habitat over the zones it is possible human habitat modification has disturbed ecotones. For example, Zone 1 has something of the appearance of a mosaic hybrid zone with two monomorphic samples collected less than 3 km apart and large polymorphic samples at least 18 km apart. This may be caused by recent habitat fragmentation and recolonization into newly vegetated patches.

Sampling.—(1) Sampling was limited by availability of suitable habitat. For example, fire destroyed weta habitat in the center of Zone 2 three years before this study began. However, similar intensity of sampling from each hybrid zone should assure that width estimates are comparable. Only where polymorphic samples are absent will the scale of the sampling be problematic, as has been noted for mtDNA at Zone 3. (2) Transects might not be exactly perpendicular to the zone of contact between any pair of chromosomal races. Any transect not fully perpendicular to the zone of contact would tend to increase estimates of cline width. Zones 1 and 2 occur on fairly narrow strips of land formed from sand that connect old islands. Contact between weta races is therefore restricted to a narrow region. Although our transects might not be exactly perpendicular with the hybrid zones, the narrowness of the peninsulas physically limits how far out they could be. For example, the Far North Peninsula (Zone 1) is less than 15 km wide (east–west) but the chromosome cline measured down the peninsula (north–south) is 47 km wide. Therefore our cline width estimates cannot be much greater than actual cline widths. For Zones 3, 4, and 5, where it would be easy to make a nonperpendicular transect, cline estimates are all relatively narrow. Therefore, nonperpendicular transects cannot explain the failure of cline widths to meet our predictions.

Comparing mtDNA and Chromosome Clines within Zones

If the change in karyotype seen at each of the five zones results from the meeting of populations that are genetically distinct then mtDNA would be expected to show a stepped cline also. Variation in mtDNA was detected at all five zones, but at Zones 1 and 2 sequence variation within chromosomal races was greater than variation between races (Fig. 2). It is possible that the deeper differentiation reveals an original interracial pattern, now obscured by introgression. However, such an interpretation would widen clines at Zones 1 and 2, only strengthening our findings. In these cases we think that the distribution of the deeper genetic divergence is more likely to imply paraphyly or ancient introgression, as described for a mouse hybrid zone in Denmark and Germany (Ferris et al. 1983, Vanlerberghe et al. 1988), rather than current gene flow.

There have been many studies looking at cytoplasmic markers (mitochondrial and chloroplast) over hybrid zones. Many studies have found that mtDNA or cpDNA clines are wider than that of nuclear markers (Barton and Hewitt 1985), and cytoplasmic clines with different centers have been observed (e.g., Young 1996; Gill 1997; Ruedi et al. 1997). At least as frequently, however, cytoplasmic markers have been found to have clines that are coincident and concordant with the clines of nuclear markers, including sex-linked markers (e.g., Young 1996; Kirby et al. 1997; Highton 1998; Sattler and Braun 2000; Brumfield et al. 2002; Dasmahapatra et al. 2002; Munclinger et al. 2002). Frequently, mtDNA clines are indeed narrower than those for nuclear loci (Sattler and Braun 2000; Evans 2001; Marshall and Sites 2001; Bensch et al. 2002, Munclinger et al. 2002). In some cases this may reflect Haldane's Rule, when the female is the heterogametic sex, and in other cases, female philopatry and male dispersal.

At each of the five zones in this study, two clines (chromosomal and mtDNA) were measured and a comparison revealed a variety of concordance and coincidence among zones (Table 3, Fig. 3). At Zones 1 and 4, chromosome and mtDNA clines differ significantly in their widths and centers. At Zone 5 the two clines are concordant but their centers differ. At Zones 2 and 3 the clines are both coincident and concordant (although sampling limited the resolution of the mtDNA cline at Zone 3). Although at three zones the clines are not exactly coincident and at two zones the clines are not exactly concordant, there is a general match between relative cline widths at four zones. The exception is Zone 4 (chromosome cline of 8.1 km and mtDNA cline of 1.7 km) in which, although the support limits for the cline widths are nonoverlapping, the slopes of these two clines are very similar (Fig. 3). Our estimates of zone width are similar whether measured by chromosome or mtDNA. This suggests that genomewide forces are determining zone width rather than just selection against chromosome heterozygotes. However, cline comparisons are not strictly analogous because mtDNA haplotypes were grouped by common descent into two clades for the cline analysis including many uncommon haplotypes that were restricted to single sites. In contrast, none of the chromosomal markers were restricted to single sites, because only one or two rearrangements were detected per transect. This means that the gene flow detected for mtDNA is, at least in part, historical rather than ongoing. For example, at Zone 1 only two of the 18 mtDNA haplotypes were found at more than one site, although one haplotype (1g) was found at six sites over 51 km. This difference between the markers makes the finding that mtDNA has introgressed no farther than the chromosomal markers even more striking.

In the five weta hybrid zones studied here, mtDNA clines are either narrower (Zones 1 and 4) or no wider (Zones 2, 3, 5) than the chromosome clines. There are four possible explanations for why the mitochondrial cline is narrower than the chromosome clines: (1) the mitochondrial genome is un-

der stronger selection than the chromosome rearrangement; (2) mitochondrial introgression is prevented by asymmetric assortative mating; (3) there is sex-specific inviability; or (4) there are dispersal differences in the two sexes. The last explanation, that female weta may not disperse as far as males, is not in conflict with the little that is known of weta behavior. Adult male *Hemideina* defend cavities in trees where adult females shelter during the day. A male without a large cavity may travel farther to find one than would a female, and males may travel at night to mate with females that are feeding (Moller 1985, Field and Jarman 2001). However, if males do disperse farther than females, one would expect to see the same lack of nuclear and mitochondrial cline concordance at each weta hybrid zone. It is possible that weta behavior varies with density and/or habitat. None of the four explanations for why mtDNA clines are narrower than chromosome clines can yet be excluded.

Character clines with different centers suggest that there has been asymmetric gene flow, which could be caused by movement of the whole hybrid zone (e.g., *C. captiva*; Shaw et al. 1993) or asymmetric mating (Barton 1993). Nothing is known of the mating success of the various races of *H. thoracica*, so this possibility is worthy of further study. In addition, movement of the zones is possible for those zones without coincidence (Zones 1, 4, and 5), although we have no corroborating evidence for this. In chromosome hybrid zones of pocket gophers, female choice of large males seems to explain the asymmetrical mating between taxa and the introgression of mitochondrial DNA into only one of the two hybridizing taxa (Patton 1993). Tree weta have male-male competition for access to tree cavities and thus access to the females that use these daytime shelters, so there is potential for asymmetric mating if the races differ in body size. A size cline from smaller *H. thoracica* in northern New Zealand to larger weta in the south (M. Morgan-Richards, pers. obs.) has not been studied across the hybrid zones. In two of the three zones in which the clines have different centers (Zones 1 and 5), the mtDNA introgresses into the southern chromosome race, as would be expected if south–north size variation resulted in asymmetrical mating success.

Many hybrid zones have frequency clines that are not much wider than the dispersal ability of the organism involved (Barton and Hewitt 1985). For *H. thoracica*, dispersal has been estimated at a minimum of 80–110 m per generation (Morgan-Richards et al. 2000). At Zone 3, chromosome and mtDNA markers have frequency clines that are as narrow as this measure of dispersal. Zones 2, 4, and 5 have cline widths two orders of magnitude greater than dispersal, but not very different to cline widths given for other species with similar estimates of dispersal ability such as the grasshopper *Chorthippus parallelus* and the toad *Bombina bombina*/*variegata* (Barton and Hewitt 1985; Butlin 1998). However, the widths of chromosome and mtDNA clines at Zone 1 are almost three orders of magnitude greater than dispersal. It seems unlikely that dispersal is in fact much higher for these races in particular. Alternatively, the zone may have once lain on an ecotone that has since been disrupted by human modification, leading to widespread mixing.

Conclusions

We found that the proportion of the chromosome material involved in rearrangements at each zone fails to predict relative chromosome cline width. We also estimated that at five independent hybrid zones within *H. thoracica* mtDNA clines are either narrower or no wider than the chromosome clines. These two observations may be explained by chromosome markers at the widest of zones studied (Zones 1 and 2) being very close to neutral. It is implausible that at the wide zones the chromosome clines are as large as the weta's potential dispersal ability in the time since the races made contact. Cline width would therefore be determined by a variety of other characters not yet measured. In contrast, at one of the narrowest zones (5), clines of nuclear markers were wider than the chromosome clines, suggesting that chromosome markers might be limiting chromosome introgression through this zone (Morgan-Richards et al. 2000). Thus, even within a single species, the type and size of chromosome rearrangements cannot be used to predict fitness of hybrids. The two zones (1 and 2) in the north that we think both formed in the early Pleistocene differ significantly in their width. Loss of habitat may make it impossible to distinguish between possible explanations for their variation confounded by the likely repeated contact and isolation of the races involved during the sea level changes of the Pleistocene. In contrast, younger zones farther south may make excellent sites for future investigations. The narrow, concordant, coincident clines at Waitangi River (Zone 3) suggest that hybrid disadvantage may be limiting gene flow. Whether the karyotype differences that distinguish the races contribute to hybrid disadvantage at this zone remains to be seen.

Most hybrid zone studies involve one, (rarely) two (Flanagan et al. 1999), or four (Buerkle and Rieseberg 2001) transects across a single hybrid zone. A notable exception is a review of 14 well-studied shrew hybrid zones (Searle and Wójcik 1998). Because *H. thoracica* has many chromosomal races it was possible to compare independent zones within a single species. Even with just two markers studied for each of the five hybrid zones, an unexpected level of variation was found among zones. Barton and Hewitt (1985) concluded that most zones are similar in width to the dispersal ability of the animal involved. In our study, with a constant level of dispersal, we found zones that range in width by two orders of magnitude, a similar degree of variation to that observed in shrew hybrid zones. This disparity suggests fitness differences have a much greater role in determining zone width than how far weta and shrews can run, walk, and jump each generation. How true this is for other species may be worth investigation. As is the case with shrew chromosomal hybrid zones, only when the level of genetic divergence between races is similar is it possible to predict the relative fitness disadvantage that chromosome rearrangements will confer on hybrids.

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