

favor an increase or a decrease in reproductive isolation depending on the relative fitness advantages to the individual, and the initial allelic variation existing within the populations. Gene flow is likely to reduce genetic distinctiveness and thus increase competitive interactions, while diverging selection would favor traits that reduce competition. However, an equilibrium might develop where different factors have opposing outcomes, resulting

each hind tibia, were recorded for each individual. Tubercles (small protuberances) in the position usually occupied by prolateral spine IV in *H. crassidens* and *H. trewicki* were recorded as half spines.

Evidence for the divergence of traits in regions of sympatry was sought by comparing the size of adults from allopatric and sympatric populations. Character displacement theory predicts greater differentiation in sympatry than in allopatry. Length of the left hind tibia was measured using electronic callipers accurate to 0.01 mm, as an indicator of overall body size (Minards et al. 2014). ANOVA was performed with Tukey's test to check for significant differences between population means using Minitab 16 Statistical software. The size comparison used a sample of 65 adult females, because male tree wētā can mature at three different instars whereas females mature only at the tenth (Kelly and Adams 2010; Minards et al. 2014).

Cytogenetics

Wētā were karyotyped as previously described (McKean et al. 2015). Both species pairs contain differences in the relative sizes of their chromosomes allowing differentiation of the three species (McKean et al. 2015). The karyotypes of F_1 hybrids were predicted based on parental karyotypes and compared to karyotypes obtained from putative hybrids. For the Mohi species pair (*H. thoracica* and *H. trewicki*), total chromosome number is the same, so F_1

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	<i>H. crassidens</i>					<i>H. thoracica</i>				<i>H. trewicki</i>
								▼▼	▼▼	▼▼
	174				5	1	6	1		
	176							1		
	177	4			8	16				
	17					3		4	1	
	182						14			
	184					1				
	187		10	2						
35	224	7	6	16	3					
	227		1	6						
	228			2						
	230			3						
	231	3								
	233	2		1	1					1

PCR products were sequenced using a capillary ABI3730 Genetic Analyzer (Applied Biosystems Foster City, CA, USA). DNA sequences were visualized and aligned in Geneious v6.1.7 (<http://www.geneious.com>; Kearse et al. 2012). For mtDNA haplotypes we used the integer neighbor-joining method (French et al. 2013) with reticulation tolerance set to zero. This allowed

unequivocal assignment of each haplotype to species clusters. Variation at nuclear loci was visualized using minimum spanning networks (Bandelt et al. 1999). Networks were generated using PopART (Population Analysis with Reticulate Trees; Leigh and Bryant 2015). We used Fisher's exact test to determine whether the hybrid mitochondrial haplotypes suggested a species bias of

mothers. Sequences are available at http://evolves.massey.ac.nz/DNA_Toolkit.htm.

Microsatellite loci

Sixteen microsatellite primer pairs developed for South Island species of tree wētā were trialled. Six loci that

indicate backcross hybrids. In contrast, putative hybrid wētā had the karyotype expected in F₁ hybrids between their respective parent species (Fig. 3). Female hybrids (n = 4) did not provide mitotic cells as they all lacked ovarian material usually used for cytogenetic preparations.

Mitochondrial DNA sequences

New mitochondrial COI sequences (645 bp) were obtained from 43 wētā and 12 haplotypes were identified (Table 2). These data supplemented previously haplotyped wētā individuals (Bulgarella et al. 2014). Haplotype clusters corresponded with the three species and two distinct *H. crassidens* lineages (Fig. 4A). There was no evidence of mitochondrial haplotype sharing among the three species. Eight out of nine putative *H. thoracica* and *H. crassidens* hybrids had a *H. crassidens* haplotype, from which it can be inferred that they had an *H. crassidens* mother. Only one putative hybrid had a *H. thoracica* mtDNA haplotype. This observation differs from expectations of equal likelihood of the two parent taxa being the mother (Fisher's exact test; P = 0.039). Haplotype data are available <http://evolves.massey.ac.nz/Text%20Files/DNA%20Toolkit.htm>.

Nuclear loci

We obtained unambiguous sequences for each locus from 105 wētā

(Morgan-Richards 1995; Trewick and Morgan-Richards 2004). At $K = 3$ (second highest support) the three species separated. The sympatric populations comprised two species' genotype clusters as identified by phenotype with low levels of introgression inferred from assignment prob-

exchange; $F_{ST} = 0.606$ (Mohi) and 0.665 (Kahuterawa). The distribution of alleles at two loci provided evidence of potential introgression between species in Kahuterawa (HR12, HR35; Table 2). For example the sample of Kahuterawa *H. thoracica* had two alleles that were also found in *H. crassidens* but were not observed in allopatric population samples of *H. thoracica* (alleles 242, 250; HR35 locus; Table 2). Our estimates of gene flow between the species pairs were very low, and not distinguishable from zero as inferred with BayesAss v3.0 (assuming neutrality; Fig. 5).

Discussion

The tree wētā *Hemideina thoracica* meets and mates with two different related species and the long-term outcome of these two zones of interspecific hybridization will be influenced by the rate of gene flow. Our data shows that at Mohi *H. thoracica* and *H. trewicki* differ in size.

Hemideina thoracica adult females have longer hind tibia than *H. trewicki*, and longer tibia than conspecifics at Kahuterawa. In addition, the absence of adult *H. thoracica* during sampling at Mohi suggests a difference in developmental timing. Both these traits (size and maturation timing) have the potential to contribute to reproductive isolation but are unlikely to prevent all mating (Gwynne and Jamieson 1998). Confirmation of an F_1 hybrid collected from the wild demonstrates that these two

low, as fixed differences within our samples were found at two nuclear loci, mitochondrial haplotype and karyotype. The parent species appear to be retaining separate identities in sympatry, so a bimodal hybrid zone appears to be the best description for the contact of *H. thoracica* and *H. crassidens* at Kahuterawa. It is likely these species are differentiated enough to maintain their own evolutionary trajectories in sympatry, but exchange of adaptive alleles cannot be ruled out (Jiggins and Mallet 2000). The similarity of phenotype of *H. thoracica* and *H. crassidens* is likely to result in stronger interspecific competition than between *H. thoracica* and *H. trewicki*. This finding is concordant with the competitive exclusion hypothesis for *H. thoracica* and *H. crassidens* based on distribution data, environmental modeling and genetic structure (Bulgarella et al. 2014). *Hemideina thoracica* has probably displaced *H. crassidens* during the current interglacial as the range of *H. thoracica* has expanded south (Trewick and Morgan-Richards 1995; Bulgarella et al. 2014).

None of the three species of tree wētā appeared to have complete premating barriers to reproduction. Few F_1 hybrids were detected but this could be due purely to postmating (such as sperm competition) or postzygotic selection (failure to hatch). Adults of *H. thoracica* have been observed in the same daytime refuge cavities as adults of both *H. crassidens* and *H. trewicki* (Trewick and Morgan-Richards 1995, 2000; pers. obs). Even the difference in the timing of maturity between *H. thoracica* and *H. trewicki* is not enough to prevent some first generation hybrids being produced. Bimodal hybrid zones are typically associated with strong pre-mating barriers (Jiggins and Mallet 2000; and references therein), although the bimodal hybrid zone between two species of chrysomelidae beetles is an exception (Peterson et al. 2005). Further studies involving female mate choice are warranted in order to determine the relative roles of pre- and postmating barriers that result in so few hybrids in natural populations. Given the karyotype differences (McKean et al. 2015) chromosomal and other genetic constraints are likely to be involved in limiting F_1 fertility.

Although the sample of *H. thoracica* × *H. crassidens*

Conflict of Interest

None declared.

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